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IN ISSUE:

RBM20 gene variants associated with left atrial dilatation in patients with old myocardial infarction and heart failure with reduced ejection fraction

Spectrum of desmosomal gene variations in patients with arrhythmogenic right ventricular cardiomyopathy

Spectrum of mutations and their phenotypic manifestations in children and adults with long QT syndrome

Molecular genetic features of the development of restrictive cardiomyopathy in Russian children

Variant of the *FLNC* gene nucleotide sequence in a family with different phenotypic manifestations of left ventricular non-compaction

Clinical features of post-COVID-19 period. Results of the international register "Dynamic analysis of comorbidities in SARS-CoV-2 survivors (AKTIV SARS-CoV-2)". Data from 6-month follow-up

IN FOCUS:

Genetics in cardiology

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Dear readers!

We would like to bring to your attention the next issue of the Russian Journal of Cardiology, dedicated to the genetic determinants of cardiovascular diseases. The content of this issue clearly reflects the advances in the field of biomedical research in Russia and, in particular, in the field of molecular and genetic research in cardiology. The presented topics in this issue largely relate to monogenic and inherited cardiovascular diseases. Today, domestic research in this area is fully consistent with the modern vector of international studies and is carried out with the use of leading genetic technologies — new-generation sequencing, genetic engineering and high-tech cell technologies. The original studies in the issue reflect many foreign innovative trends in cardiology and are presented in accordance with the most modern guidelines. The interpretation of new genetic variants of uncertain significance, the optimal use of cell culture models, the concept and definition of the role of polymorphic variants as modifiers of disease course and prognosis and the possibility of their inclusion in modern risk stratification scales.

The wide geography of the presented studies is noteworthy. So, there are studies from various regions of Russia and neighboring countries, many of which are collaborated. This indicates the development of cooperation within various cardiology communities of the country, the formation of joint research principles and the joint use of resource base and clinical data. Without this approach in the study of rare pathologies, which, in many respects, are the majority of hereditary myocardial diseases, effective cardiogenetics research is impossible. Another feature of this issue is the inclusion of articles on the diagnosis and course of congenital heart diseases in the pediatric group. Such continuity and interaction of pediatric and adult cardiology in this field can create a good basis for developing domestic registers and generally available databases with the possibility of long-term prospective follow-up of family cases.

We are confident that this issue of the journal, mainly devoted to genetic research in cardiology, will be interesting and useful for the practical and research work of cardiologists, therapists, arrhythmologists and pediatricians.

Anna A. Kostareva, Doctor of Medical Science

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ORIGINAL ARTICLES
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RBM20 gene variants associated with left atrial dilatation in patients with old myocardial infarction and heart failure with reduced ejection fraction

Vakhrushev Yu. A., Kuular A. A., Lebedeva V. K., Kozyreva A. A., Kostareva A. A., Sitnikova M. Yu., Lyasnikova E. A.

Aim. To study the prevalence of *RBM20* gene polymorphisms and their relationship with the structural and functional left atrial (LA) characteristics in patients with coronary artery disease and heart failure with reduced ejection fraction (HFrEF).

Material and methods. The study included 138 men aged 55,8±6,6 years with prior myocardial infarction ≥12 months ago and HFrEF (class II-IV heart failure, left ventricular ejection fraction (Simpson's methods), 25,1±7,2%). The control group consisted of 384 healthy donors. Genotyping of two *RBM20* polymorphic variants (rs942077 and rs35141404) was performed by real-time polymerase chain reaction.

Results. The prevalence of *RBM20* polymorphisms did not differ in the HFrEF cohort and the control group. The GA rs35141404 genotype was more common among patients with a less pronounced increase in LA volume index (LAVI) (p=0,034). The minor A allele rs35141404 was associated with a protective effect on severe LA remodeling. However, this association did not reach the level of significance.

Conclusion. For the rs942077 and rs35141404 polymorphic variants of the *RBM20* gene, no significant associations were found with the LA size and atrial fibrillation presence in patients with HFrEF and old myocardial infarction. There was a tendency towards the association of the A allele and the GA rs35141404 genotype with a protective effect on LA remodeling. The data obtained confirm the need for further search for genotype-phenotype relationships of a wider population of patients with heart failure and coronary artery disease.

Keywords: heart failure with reduced ejection fraction, left atrium, polymorphic variants, *RBM20*.

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The influence of polymorphic genetic variants on development and course of various cardiovascular diseases has been noted and actively studied for many years, but the volume of study in this area has increased significantly after development of new methods of molecular biology and genetics. The application of genome-wide studies and the use of next-generation sequencing allowed to detect new determinants associated with the risk of development and adverse outcome of chronic heart failure (CHF) of various etiologies. At the same time, the influence of genetic factors on some structural and functional parameters of the cardiac muscle in CHF development and myocardial remodeling remained poorly studied.

In recent years, many studies have been published analyzing the association of various genetic determinants with myocardial structural parameters and cardiac chamber sizes. The greatest number of works was devoted to the search of genotype-phenotypic associations in development of pathological myocardial remodeling of predominantly dilated phenotype. At the same time, single works were devoted to the analysis of genetic variants associated with structural atrial remodeling. Thus, in 2010, the relationship between genetic variants of polymorphic loci in the genes *NTN1*, *MYH10*, *COX10* and *MYOCD* with left atrium (LA) size was demonstrated [1]. In 2015, Mints Y, et al. confirmed the association of the polymorphism rs10033464, located near the gene *PITX2* in locus 4q25, with LA dilatation development [2]. The gene *PITX2* encodes the protein Pitx2, which is a transcription factor that is a member of the RIEG/Pitx family of homeodomain transcription factors that play an essential role in embryonic development. It is important to note that this transcription factor undergoes alternative splicing, resulting in four isoforms (Pitx2A, Pitx2B, Pitx2C, PitxD) in the body and is connected to LA formation and structural and morphological features of pulmonary venous mouths [3]. This transcription factor is expressed asymmetrically during different stages of cardiac development and plays an integral role in cardiogenesis, and insufficiency of its function leads to development of structural and electrophysiological abnormalities in atrium [4].

Another foreign study demonstrated the association of the above-described polymorphism rs10033464 with early recurrence of atrial fibrillation (AF) after cardioversion and larger LV size in a meta-analysis of 7034 patients with this rhythm disorder [5]. The mechanism of structural and functional interconnections of the LA tissue remains incompletely understood. Atrium dilation is thought to cause activation of signal-dependent extracellular kinase Erk1/ERk2, causing further progression of atrium tissue fibrosis, formation of re-entry foci and contributing to arrhythmogenesis [6]. There is also an inverse relationship: LA dilation can occur due to AF in patients without its primary expansion. Currently, the search for genotype-phenotypic associations with regard to structural and functional remodeling of cardiac chambers is actively continuing [5, 6].

The development of dilatational remodeling is associated with multiple genetic determinants of myocardial structural proteins, including the gene *RBM20*, pathogenic variants in which lead to development of arrhythmogenic cardiomyopathy in aggressive form [7]. The gene *RBM20* encodes the protein RBM20, which is a transcriptional splicing factor for many genes expressed in cardiac muscle and involved in maintaining sarcomere structure, diastolic function and ion transport, such as: *TTN*, *CaMKII*, *CACNA1C*, *LDB3*, *LMO7*, *FHOD3*, *PDLIM3*, *RTN4*, *TRDN*, *OBSCN*, *RYR2* [7, 8]. The main target of *RBM20* is the gene *TTN* encoding the giant protein taytin, which acts as a spring that provides sarcomere stiffness and plays an important role in passive relaxation of the cardiomyocyte [9]. It is noteworthy that 2 above-mentioned genes, *TTN* and *RBM20*, are predominantly associated with the development of dilated cardiomyopathy (DCM), and in the last 3 years much attention has been paid to studying the role of their genetic variants in development of arrhythmic events in patients with CHF and DCM, including AF, both in inherited forms of cardiomyopathies and in the general population [1, 9].

It seems relevant to study the relationship of genetic determinants, such as polymorphic variants of the gene *RBM20* (rs942077 and rs35141404), with structural and functional characteristics of LA in patients with heart failure (HF) with low ejection fraction (HFrEF) of predominantly non-monogenic nature.

Material and methods

This study is a single-center study including 138 men with coronary heart disease, postinfarction cardiosclerosis, and stable HFrEF of II-IV functional class, aged 40-68 years, on standard drug therapy, who were treated at the V.A. Almazov Federal State Medical Research Center in 2012-2018. The control group was represented by practically healthy people who constituted the donor control base of the V.A. Almazov Scientific Research Center (384 people), comparable in age to the studied cohort. The criteria for non-inclusion in the study were: primary and postmyocardial DCM, hypertrophic cardiomyopathy, hemodynamically significant organic heart valve lesions, cardiac chamber dilata-

tion due to accumulation disease, secondary arterial hypertension (AH), extensive cardiac surgery or percutaneous coronary intervention or valvuloplasty, and electrophysiology intervention within 12 months before randomization, acute or decompensated HF less than 3 months prior to inclusion.

The study was approved by the ethical committee of the V. A. Almazov Scientific Research Center and was conducted in accordance with good clinical practice and the ethical standards of the Declaration of Helsinki. All respondents signed an informed consent for the necessary examination methods.

Patients' status was assessed, and routine laboratory and instrumental diagnostic methods were performed. Echocardiography was performed according to the standard protocol of the center. The LA volume (LAV) was indexed to the body surface area (BSA) and the degree of severity of growth (in meters). Normative values of the LA size: LAV (ml)/BSA (m^2) and LAV (ml)/(height, $m)^2$ specific for gender was determined in accordance with the recommendations of the American and European associations of echocardiographic (ASE/EAE) — 2015 for echocardiography in adults and the European communities on cardiology and hypertension (ESC/ESH) — 2018 for treatment of hypertensive patients (⩽40 mm, ⩽34 ml/BSA $(m)^2$ and ⩽18,5 ml/(height in $m)^2$, respectively) [10, 11]. The ejection fraction (EF) of the left ventricle (LV) was calculated using the Simpson method. The study included patients with LV EF <35%.

Characteristics of patients. The main characteristics of the cohort under study are given in Table 1. The average age of patients was 55,8±6,6 years. Most respondents had undergone myocardial revascularization and had HFrEF of functional class II. AH, diabetes mellitus and obesity were observed in 96%, 17%, and 23% of cases, respectively. Implanted devices, including permanent pacemaker, implanted cardioverter defibrillator, cardiac resynchronization therapy device, cardiac contractility modulator had 60% of patients. According to the anamnesis and programming results, AF (constant, persistent or paroxysmal form) was registered in 27% of cases. LV EF was 25,1±7,2%. An increase in LA volumes was detected in more than 90% of cases.

To study polymorphic variants of *RBM20* (rs942077 and rs35141404), DNA was isolated from whole blood using the FlexiGene DNA Kit (Catalog No.51206). These polymorphic variants were identified by real-time polymerase chain reaction using allele-specific primers from Applied Biosystems on an Applied Biosystems 7500 RealTimePCRSystem amplifier and Syntol reagent kit.

Statistical processing was performed using the Statistica 10 package. Data are presented as: mean ± standard deviation (M±SD) in case of normal distribution, medians (Me) and 25% (Q25) and 75% (Q75) quartiles, frequencies, and percentages of the total number of follow-up n (%). Continuous values with a normal distribution were compared with each other using a t-test. The nonparametric Mann-Whitney test for independent samples was used to compare continuous values with a distribution other than normal. Independent categorical data were compared using Fisher's two-sided test and chi-square (χ^2) test with Yates continuity correction at statistical significance p<0,05.

Table 1

Characteristics of patients

Indicator	n=138
Gender (men), n (%)	138 (100%)
Age, years, M±SD	55,8±6,6
Minimum/maximum range, years	40-68
Q-myocardial infarction, n (%)	138 (100%)
Q-myocardial infarction of anterior LV wall, n (%)	131 (95%)
Myocardial revascularization, n (%)	94 (68%)
Arterial hypertension, n (%)	132 (96%)
Duration of arterial hypertension, years	11,6±10,6
Obesity, n (%)	32 (23%)
Diabetes mellitus, n (%)	23 (17%)
Atrial fibrillation, n (%)	36 (26%)
PCP/ICD/CPT, n (%)	9 (7%)/56 (41%)
Cardiac contractility modulator, n (%)	18 (13%)
Functional class of CHF II/III/IV, n (%)	94 (68%)/36 (26%)/ 8 (6%)
LV ejection fraction (Simpson), M±SD	25,1±7,2%
Minimum/maximum range, %	14-35%
End-diastolic volume of LV, ml, M±SD	249,8±78,8
End-systolic volume of LV, ml, M±SD	185,0±65,4
LA size, mm M±SD	49,8±6,6
LA volume/(height, $m)^2$, ml/m^2, M±SD	36,5±11,8
LA volume/BSA, ml/m^2, M±SD	55,8±17,5

Note: the values are given in %, absolute values (indicated in parentheses), as the average value ± standard deviation.

Abbreviations: LA — left atrium, LV — left ventricle, BSA — body surface area, PCP — permanent cardiac pacing, ICD — implanted cardioverter defibrillator, CRT — cardioresynchronizing therapy, CHF — chronic heart failure.

Results

The occurrence of alleles and genotypes of the polymorphic variants of *RBM20* (rs942077 and rs35141404) in the presented sample of patients with HFrEF did not differ compared with the control group (Table 2). There were also no differences in

Table 2

Distribution of genotypes and alleles of polymorphic variants *RBM20* (rs942077 and rs35141404) in the study groups

Genotypes and alleles of polymorphic variants		CHF group, n=138	Control group, n=384	Distribution according to dbSNP
rs942077	CC	83% (114)	79% (304)	79%
	CG	17% (24)	19% (72)	17%
	GG	0% (0)	2% (8)	4%
	C	91% (252)	88,5% (680)	87%
	G	14% (24)	11,5% (88)	13%
rs35141404	GG	75,4% (104)	80,4% (309)	77%
	GA	23,2% (32)	18,8% (72)	22%
	AA	1,4% (2)	0,8% (3)	1%
	G	86,9% (240)	89,8% (690)	86%
	A	13,1% (36)	10,2% (78)	14%

Note: the values are given in %, absolute values.

Table 3

Distribution of genotypes and alleles of polymorphic variants rs942077 and rs35141404 of the gene *RBM20* in groups of patients with HFrEF, depending on the presence of AF

Genotypes and alleles of polymorphic variants		Group without AF, n=102	Group with AF, n=36	All patients, n=138	Control group, n=384
rs942077	CC	80% (82)	86% (31)	83% (114)	79% (304)
	CG	20% (20)	14% (5)	17% (24)	19% (72)
	GG	0% (0)	0% (0)	0% (0)	2% (8)
	C	90% (184)	93% (67)	91% (252)	88,5% (680)
	G	10% (20)	7% (5)	14% (24)	11,5% (88)
rs35141404	GG	76,5% (78)	72,2% (26)	75,4% (104)	80,4% (309)
	GA	22,5% (23)	25% (9)	23,2% (32)	18,8% (72)
	AA	1% (1)	2,7% (1)	1,4% (2)	0,8% (3)
	G	88% (179)	85% (61)	87% (240)	90% (690)
	A	12% (25)	15% (11)	13% (36)	10% (78)

Note: the values are given in %, absolute values.
Abbreviation: AF — atrial fibrillation.

the prevalence of the studied *RBM20* gene polymorphisms depending on the presence of AF (Table 3).

Given the high detectability of LA dilatation in this cohort of patients, the analysis of the relationship between SNP rs942077 and rs35141404 of the gene *RBM20* with the degree of gender-specific increase in LA size according to ASE/EAE recommendations was carried out [11]. As a result of this analysis, no statistically significant difference in the frequency of genotypes and alleles of *RBM20* polymorphic variants was obtained (Table 4).

It is known that among different parameters of LA there is more prognostic value with regard to adverse cardiovascular outcomes in the index of LAV/BSA regardless of the presence of AF, HF and its etiology [12]. The values of LAV index (LAVI) of >50 ml/m^2 and >40 ml/m^2 had the same predictive ability with LV EF in relation to hospitalizations due to HF and mortality in patients with coronary heart disease [13]. Therefore, we attempted to analyze the prevalence of the studied *RBM20* SNPs in patients with varying degrees of LAVI enlargement.

When analyzing alleles and genotypes rs942077 and rs35141404 *RBM20* in the study groups, depending on the presence of severe LA dilatation according to LAVI indicator, significant differences in the representation of the GA genotype rs35141404 were detected. In patients with LAVI <40 ml/m^2, corresponding to less pronounced LA dilatation according to ASE/EAE classification, was dominated by GA genotype and allele A rs35141404 compared with patients with LAVI 50 ml/m^2 (38%:16%, p=0,034% and 19%:11%, p>0,05). At the same time,

Table 4

Distribution of genotypes and alleles of polymorphic variants rs942077 and rs35141404 of the gene *RBM20* in groups of patients with HFrEF depending on the size of LA

Genotypes and alleles of polymorphic variants		Subgroups of patients			
		LA* ⩽40 mm, n=11	LA 41-51 mm, n=79	LA* ⩽40 mm, n=11	All patients, n=138
rs942077	CC	72,7% (8)	86% (68)	77% (37)	83% (114)
	CG	27,3% (3)	14% (11)	22% (11)	17% (24)
	C	86,4% (19)	93% (147)	88,5% (85)	91% (252)
	G	13,6% (3)	7% (11)	11,5% (11)	14% (24)
rs35141404	GG	72,7% (8)	75,9% (60)	77% (37)	75,4% (104)
	GA	27,3% (3)	22,8% (18)	21% (10)	23,2% (32)
	AA	0% (0)	1,3% (1)	2% (1)	1,4% (2)
	G	86% (19)	87,3% (138)	87,5% (84)	87% (240)
	A	14% (3)	12,7% (20)	12,5% (12)	13% (36)

Note: the values are given in %, absolute values, * — left atrium, antero-posterior size in parasternal position.
Abbreviation: LA — left atrium.

Table 5

Distribution of genotypes and alleles of polymorphic variants rs942077 and rs35141404 of the gene *RBM20* in groups of patients with HFrEF depending on LAVI

Genotypes and alleles of polymorphic variants		Group with LAVI <40 ml/m², n=21	Group with LAVI ⩾40 ml/m², n=96	Group with LAVI 50 ml/m², n=75	All patients, n=138
rs942077	CC	81% (17)	81,3% (78)	80% (59)	83% (114)
	CG	19% (4)	18,7% (18)	21% (16)	17% (24)
	GG	0% (0)	0% (0)	0% (0)	0% (0)
	C	90% (38)	91% (174)	89% (134)	91% (252)
	G	10% (4)	9% (18)	11% (16)	14% (24)
rs35141404	GG	62% (13)	79% (76)	81% (61)	75,4% (104)
	GA	38% (8)	19% (18)	16% (12)	23,2% (32)
	0,034				
	AA	0% (0)	2% (2)	3% (2)	1,4% (2)
	G	81% (34)	88,5% (170)	89% (134)	87% (240)
	A	19% (8)	11,5% (22)	11% (16)	13% (36)

Note: the values are given in %, absolute values.
Abbreviation: LAVI — left atrium volume index, reduced to body surface area.

there was a higher prevalence of GG genotype among patient groups with LAVI ⩾40 ml/m² and >50 ml/m² when the threshold of significance was not reached (79%:62% and 81%:62%, respectively, all p>0,05). Patients with LAVI <40 ml/m² had less pronounced clinical manifestations of CHF and greater LV EF compared with patients in the reference group with LAVI >50 ml/m². AF was observed more frequently among the contingent with more pronounced LA dilatation (p>0,05). Having said so, the subgroups of patients did not differ in age, body mass index, AH frequency and its duration, prevalence of diabetes mellitus, obesity (all p>0,05). Data on subgroups of patients are given in Tables 5 and 6.

Discussion

Currently, much attention is paid to the study of molecular determinants associated with the development of various HF phenotypes and myocardial remodeling, the so-called molecular epidemiology of CHF, in which an important place is given to genetic predictors [14].

The gene *RBM20* regulates the splicing of many cytoskeletal protein genes, including *TTN*, *CAMK2D*, *LDB3*, *LMO7*, *PDLIM3*, *RTN4*, and *RYR2*, and is also connected to sarcomere assembly, ion transport and posttranslational splicing of a number of calcium signaling and calcium homeostasis protein genes [7]. The listed functions of the gene *RBM20* largely

Table 6

Clinical characteristics of groups of patients with HFrEF depending on LAVI

Indicator	Group with LAVI <40 ml/m², n=21	Group with LAVI 50 ml/m², n=75
Age, years, Me [Q25;Q75]	56 [53;62]	57 [53;62]
AH, % (n)	76 (16)	68 (50)
AH duration, years, Me [Q25;Q75]	14 [0;15]	11 [0;18]
BMI, kg/m², Me [Q25;Q75]	27,1 [20,9;29,4]	25,8 [20,1;28,1]
Obesity, % (n)	24 (5)	21 (16)
Diabetes mellitus, % (n)	43 (9)	27 (20)
AF, % (n)	19 (4)	35 (26)
LV FV, %, Me [Q25;Q75]	32 [30;34]	27 [21;31]*
CHF FC, Me [Q25;Q75]	2 [2;2]	2 [2;3]*

Note: the values are given in %, absolute values (indicated in parentheses), in the form of median and quartiles; * — p<0,01.
Abbreviations: AH — arterial hypertension, BMI — body mass index, LAVI — left atrium volume index, LV EF — left ventricular ejection fraction, FC — functional class, AF — atrial fibrillation, CHF — chronic heart failure.

determine the high frequency of arrhythmological events in patients carrying pathogenic variants of *RBM20* [1]. In this regard, it can be assumed the presence of genotype-phenotypic associations of polymorphic variants of this gene with regard to structural remodeling, in particular, of atrium tissue in CHF of noninherited genesis.

In our study, the occurrence of genotypes and alleles of polymorphic variants rs942077 and rs35141404 of the gene *RBM20* in patients in the narrow phenotypic HFrEF group with postinfarct cardiosclerosis did not differ from data obtained in controls and dbSNP genetic database data, but SNP rs35141404 was associated with LA dilatation. The GA rs35141404 genotype was significantly more common in patients with a less pronounced increase in LAVI. A similar association was seen for the prevalence of minor allele A (rs35141404), although the significance threshold was not reached. It is important to note that the group of patients with the highest occurrence of this genotype and allele did not differ in age and cardiometabolic factors contributing to LA dilatation from the reference group with more pronounced LA remodeling and, naturally, a higher incidence of AF, which largely agrees with the work of Refaat MM, et al. (2012) [15]. When studying the associations of SNP rs942077 and rs35141404 of the gene *RBM20* with arrhythmic events and outcomes in a sample of patients with HFrEF of coronary etiology and DCM, the authors demonstrated the association of rs35141404 polymorphism of the gene *RBM20* with AF regardless of the CHF causative factor. It is worth noting that in this work, similar to the data we obtained, it was the rs35141404 A allele that was associated with a protective effect in relation to the risk of developing AF (odds ratio 0,59, 95% confidence interval 0,40-0,84, p=0,006) [15].

The results of several recent studies demonstrate the association of rare, shortening variants of the gene *TTN* (*TTNtv*) with AF, CHF and lower LV EF in patients even without the diagnosis of DCM [16]. Taking into account these data and the key role of *RBM20* in the *TTN* splicing process, further investigation of the molecular mechanisms of pathogenesis and structural and functional myocardial remodeling in CHF of ischemic etiology associated with *RBM20* and shortening variants and/or pathogenic SNPs of the tetin gene seems relevant, providing a potential opportunity to develop a personalized medical approach.

Study limitations. An important limitation of this study was the relatively small sample size of the study group (138 patients), the presence of possible additional causal factors potentially influencing myocardial remodeling, including the ongoing drug and non-drug therapy, which may have an effect on the LA dilatation development. Only male patients were included in the work, while the control group had no gender restrictions. The above predetermines the cohort expansion of the studied patient population and the application of multivariate statistical methods for a more complete understanding of genotype-phenotypic associations.

Conclusion

The polymorphic variant and rs35141404 of the gene *RBM20* is associated with the severity of LA dilatation in patients with HFrEF and pronounced postinfarction myocardial remodeling. Our preli-

minary data support the need to further search for genotype-phenotypic associations in the broader population of patients with CHF of coronary etiology in the focus of personalized medicine.

Relationships and Activities. The study was performed at the expense and within the framework of the state assignment of the V.A. Almazov Scientific Research Center Reg. No. AAAA19-119070490034-4.

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Spectrum of desmosomal gene variations in patients with arrhythmogenic right ventricular cardiomyopathy

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Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a hereditary myocardial disease with a high risk of sudden cardiac death. The most common genetic forms of the disease are associated with desmosomal gene mutations.
Aim. To study the prevalence of desmosomal forms of ARVC and to analyze variations in the *PKP2*, *DSG2*, *DSP*, *DSC2* and *JUP* genes in a sample of Russian patients with ARVC.
Material and methods. Included patients with ARVC underwent resting electrocardiography (ECG), 24-hour Holter ECG monitoring, echocardiography, chest x-ray, myocardial biopsy (if indicated), contrast-enhanced cardiac magnetic resonance imaging. All patients underwent medical genetic counseling. Mutations in the *PKP2*, *DSG2*, *DSP*, *DSC2*, and *JUP* genes was detected using high-throughput sequencing on the IonTorrent platform, followed by Sanger sequencing of uncovered gene regions. The pathogenicity of identified genetic variations was assessed according to modern guidelines.
Results. ARVC was established in 80 Russian unrelated patients. More than half of the probands (57%) in the study sample had definite diagnosis of ARVC, while 30% and 13% — borderline and possible ARVC, respectively. A positive family history of heart disease and/or SCD was noted in 30%. Genetic variants of pathogenicity class IV-V were detected in 15 (18,75%) probands in the *PKP2*, *DSG2*, *DSP* genes. The detection of genetic variants of pathogenicity class IV-V was different in the subgroups of patients with varying degrees of diagnosis reliability: 13 probands (28,3%) in the subgroup with definite ARVC and 2 probands (8,3%) in the subgroup with borderline ARVC. No genotype-positive probands were found in the subgroup with possible ARVC. Variations of unknown clinical significance were found in 13 (16,25%) probands.
Conclusion. The diagnostic yield of the desmosomal genes *PKP2*, *DSG2*, *DSP*, *DSC2*, and *JUP* was 19% with initial diagnosis of ARVC. The detection of mutations was significantly higher in patients with definite ARVC and severe disease manifestations.

Keywords: arrhythmogenic right ventricular cardiomyopathy, ARVC, medical genetic counseling, desmosomes.

Relationships and Activities: none.

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Arrhythmogenic right ventricular cardiomyopathy (ARVC) (OMIM #107970) — a genetically determined myocardial disease with a high risk of sudden cardiac death (SCD), characterized by fibrous and/or fatty replacement of cardiomyocytes mainly of the right ventricle, frequent ventricular arrhythmias leading to the heart failure development [1].

According to European and American studies, ARVC occurs in the population with a frequency from 1:2000 to 1:5000 people [2-4], but no large-scale epidemiological studies have been conducted in Russia. In different ethnic groups, ARVC causes 10-25% of SCD cases in young people aged 17-40 years [5-8]. ARVC is characterized by autosomal dominant type of inheritance with incomplete penetrance. Cases of autosomal recessive type of inheritance (Naxos syndrome, Carvajal syndrome) are known [9-11].

Historically, ARVC has been considered "desmosomal disease" because most cases of the disease are associated with potentially pathogenic variants in the genes encoding desmosomal proteins: transmembrane desmosomal cadherins (desmocollin, desmoglein) and adaptor proteins (desmoplakin, plakophilin, plakoglobin). However, potentially pathogenic variants have now been identified in the genes encoding area composita (cell adhesion proteins also associated with desmosomes) [12, 13].

Desmosomes are complex protein structures of the cell membrane that ensure structural and functional integrity of cells in various types of tissues, including in myocardium. Desmosomes are most represented in cells and tissues of organs that are exposed to frequent mechanical effects: skin, heart, salivary glands, thyroid gland, stomach, liver, pancreas, intestine, gallbladder, uterus, epithelial cells of nephrons [14].

It was hypothesized that disruption of desmosome assembly leads to the release and translocation of placoglobin protein into the nucleus, where it acts as a competitor of β-catenin and suppresses the canonical Wnt-signaling pathway. This leads to increased expression of adipogenesis and fibrogenesis genes and, thus, to the dominance of adipogenesis over myogenesis [15]. In addition, the role of glycogen synthase kinase 3 β (GSK3b), a suppressor of Wnt-signaling pathway, whose suppression led to prevention or delay of arrhythmogenic cardiomyopathy development in cellular and mouse models of the disease was shown [16].

Recent studies support the concept of a close functional relationship between the desmosome and the $Na_v1.5$ sodium channel protein. This is confirmed by experiments in which $Na_v1.5$ is co-deposited with protein N-cadherin [17], as well as the results of super-resolution microscopy demonstrating the presence of "adhesion/excitation" nodes formed by aggregates of $Na_v1.5$ and N-cadherin [18].

The goal of our study was to investigate the representation of desmosomal forms of the disease and analyze the spectrum of genetic variants in the genes *PKP2*, *DSG2*, *DSP*, *DSC2*, and *JUP* in a sample of Russian patients with ARVC.

Material and methods

The study included 80 probands with a referring diagnosis of ARVC, established on the basis of diagnostic criteria of ARVC 2010 in specialized cardiology and cardiosurgical institutions [19]. Voluntary written informed consent was obtained from all adult patients to participate in the study and further use of the data for scientific purposes. For minors, the consent was signed by a parent or legal guardian.

Clinical and instrumental examination was performed at the place of initial diagnosis and included resting electrocardiogram, 24-hour Holter electrocardiogram monitoring, transthoracic echocardiography, chest radiography, myocardial biopsy (when indicated), contrast-enhanced magnetic resonance imaging of heart. The diagnosis reliability was assessed using ARVC 2010 diagnostic criteria [19] prior to DNA diagnosis.

All patients underwent medical genetic counseling (primary and repeated consultations). The average period of dynamic observation was 73 months (minimum — 7 months, maximum — 11 years). The genetic study was performed in accordance with the protocol approved by the Local Ethics Committee of the FSBSI of the Russian Surgery Research Center n.a. academician B.V. Petrovsky (Protocol No. 135), and with the norms of the Helsinki Declaration (1964), and its subsequent revisions.

The search for mutations in the "desmosomal" genes *PKP2*, *DSG2*, *DSP*, *DSC2*, and *JUP* within the target gene panel (Appendix 1) was performed by high-throughput sequencing on the IonTorrent platform (device: Ion PGM™ System) (Thermo Fisher Scientific, USA) followed by direct capillary Sanger sequencing of uncovered gene regions. Verification of the genetic variants identified by the NGS method and cascade family screening for relatives of probands with genetic variants of pathogenicity classes IV-V were also performed by direct Sanger sequencing.

The pathogenicity of the identified genetic variants was evaluated *in silico* according to the guidelines for the interpretation of genetic variants [20-22]. Each identified genetic variant was assigned a pathogenicity class I to V according to the guidelines [20, 21]. Only genetic variants of classes V (pathogenic), IV (probably pathogenic), III (variant with unknown clinical significance) of pathogenicity

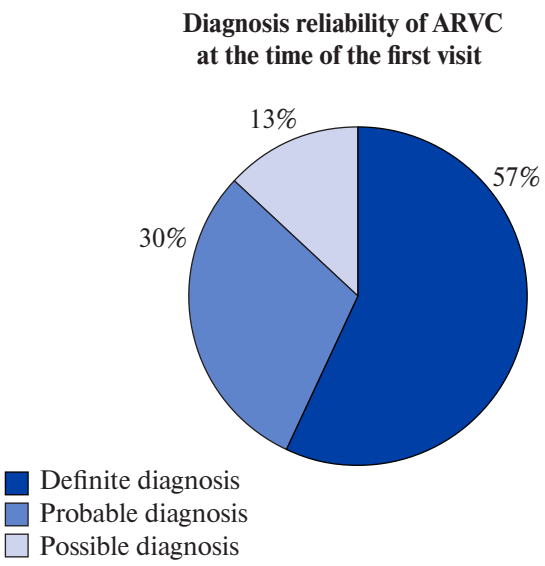

Figure 1. The severity of ARVC clinical signs and reliability of diagnoses in probands of the examined group.
Abbreviation: ARVC — arrhythmogenic right ventricular cardiomyopathy.

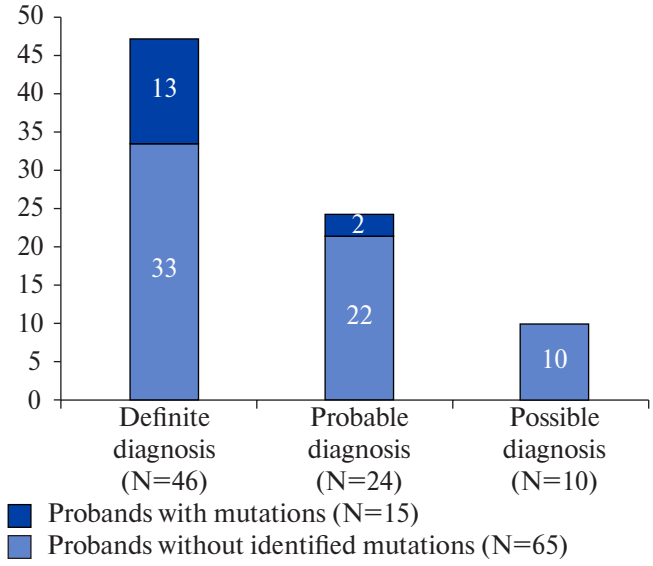

Figure 2. Detectability of genetic variants of pathogenicity classes IV-V in desmosomal genes in samples of patients with reliable, probable, and possible diagnoses of ARVC.

Appendix 1

Reference sequence numbers (NCBI) of genes cDNA *PKP2*, *DSG2*, *DSP*, *DSC2*, *JUP*, *TMEM43*, *LMNA*, *DES*, *TGFB3*, *PLN*, *SCN5A*, *CTNNA3*, *EMD*, *CRYAB*, *LDB3*, *FLNC* included in the studied panel of genes

No.	Gene	cDNA Isoform (NCBI RefSeq)
1	*PKP2*	NM_004572.3
2	*DSG2*	NM_001943.5
3	*DSP*	NM_004415.4
4	*DSC2*	NM_024422.6
5	*JUP*	NM_001352773.1
6	*TMEM43*	NM_024334.3
7	*LMNA*	NM_170707.4
8	*DES*	NM_001927.4
9	*TGFB3*	NM_003239.4
10	*PLN*	NM_002667.5
11	*SCN5A*	NM_198056.2
12	*CTNNA3*	NM_013266.4
13	*EMD*	NM_000117.3
14	*CRYAB*	NM_001289807.1
15	*LDB3*	NM_007078.3
16	*FLNC*	NM_001458.4

were included in the final DNA diagnostic report given to patients and subsequent analysis.

For pathogenic (V) and probably pathogenic (IV) genetic variants, we used the historical term "mutations" later in the paper.

Quantitative indicators are presented as an average ± SD.

Results

Medical genetic counseling and genetic screening were performed in 80 probands (men 36) with a referring diagnosis of ARVC, which was established in specialized cardiology and cardiac surgery centers. The average age of patients at the time of applying for DNA diagnostics was 38,7±14,4 years.

The reliability of clinical diagnosis of ARVC, established before DNA diagnosis, was assessed on the basis of diagnostic criteria of ARVC 2010 [19]. Most of all in the study sample were patients with a reliable diagnosis (N=46; average age 40,7±15,1 years; 24 M). Probands with probable (N=24; average age 35,0±12,5 years; 11 M) and possible (N=10; average age 37,8±14,4 years; 1 M) diagnoses of ARVC were 30% and 13% of the sample, respectively (Figure 1).

A family history of a demonstrably aggravated primary heart disease and/or SCD was noted in 24 (30%) families. In 20 probands, cardiomyopathies were detected in first-degree relatives, and in 4 families — also in relatives of the second or more degree of relationship. In two families, the sudden death of a relative of a young age (up to 40 years old) was noted. In addition, in two families with a burdened family history, the death of a relative in infancy (up to 1 year) was noted by unknown cause. According to 21 probands, they were the only patients among known relatives; therefore, we estimate the frequency of sporadic cases of ARVC in

Table 1

Spectrum of class IV-V pathogenicity genetic variants detected in patients with ARVC in genes encoding desmosome proteins

Gene	Nucleotide replacement	Protein change	Frequency (gnomAD)	Pathogenicity class	Number of probands
PKP2	c.336+1G>T		n/a	V	1
PKP2	c.962_965del	p.Val321Alafs*30	n/a	IV	1
PKP2	c.1523_1538del	p.Asn508Thrfs*7	n/a	IV	2
PKP2	c.1613G>A	p.W538*	0,00001591	IV	1
DSG2	c.146G>A	p.R49H	0,000004008	IV	1
DSG2	c.523+1G>A		0,000004201	V	1
DSG2	c.581C>T > (in homozygous state)	p.S194L (in homozygous state)	0,00002807	IV	2
DSP	c.1141-2A>G		0,000003981	V	1
DSP	c.1542dupT	p.Pro515Serfs*13	n/a	IV	1
DSP	c.1846C>T	p.Gln616*	n/a	IV	1
DSP	c.2130+1G>A		n/a	V	1
DSP	c.2672dup	p.Y891*	n/a	IV	1
DSP	c.3583delinsAATATAGT	p.Val1195Asnfs*8	n/a	IV	1

Abbreviation: n/a — no data.

Table 2

Spectrum of class III pathogenicity genetic variants detected in patients with ARVC in genes encoding desmosome proteins

No.	Gene	Nucleotide replacement	Protein change	Frequency (gnomAD)	Pathogenicity class	Number of probands
1	*PKP2*	c.1576A>G	p.T526A	0,0001202	III	1
2	*PKP2*	c.1745T>C	p.L582P	n/a	III	1
3	*DSG2*	c.733A>C	p.N245H	0,00003183	III	1
4	*DSP*	c.273+5G>A		0,00028	III	1
5	*DSP*	c.1349C>T	p.P450L	0,00001769	III	1
6	*DSP*	c.2622C>G	p.I874M	0,00004248	III	1
7	*DSP*	c.3600T>G	p.N1200K	0,00007083	III	1
8	*DSP*	c.4018C>T	p.R1340C	0,00004389	III	1
9	*DSP*	c.7856T>C	p.I2619T	n/a	III	1
10	*DSC2*	c.601G>A	p.V201I	0,00001193	III	1
11	*DSC2*	c.1436G>A	p.R479H	0,000007077	III	1
12	*JUP*	c.884_886del	p.Leu295_Ala296delinsPro	n/a	III	1
13	*JUP*	c.1916A>G	p.E639G	n/a	III	1

Abbreviation: n/a — no data.

Russian patients to be at least 26%. In the remaining families (35 probands, 44%), there was insufficient information on the health status of relatives (including one of the parents) to conclude that the disease was familial or sporadic.

We analyzed the spectrum of detected genetic variants in the desmosomal genes *PKP2*, *DSG2*, *DSP*, *DSC2*, and *JUP* in the examined group of patients (n=80) (Table 1). Variants with high pathogenicity classes (IV-V) were detected in 15 probands, which amounted to 18,75% of the whole examined group of patients (Table 1). Half of the identified genetic variants were identified for the first time.

Most of the mutations were detected in the heterozygous state, connected to autosomal dominant type of inheritance. The largest number of mutations (n=6) was detected in the gene *DSP*. 4 mutations in 5 probands were detected in the gene *PKP2*, 3 mutations in 4 probands — in the gene *DSG2*. Deletion of C.1523_1538del in the gene *PKP2* was detected by us in two unrelated probands. The missense mutation p.S194L in the homozygous state

in the gene *DSG2* was also detected in two unrelated probands.

No variants with high pathogenicity class were detected in the genes *DSC2* and *JUP*, which allows to consider these genetic forms of ARVC quite rare in the group of Russian patients.

We also analyzed the detection of genetic variants of pathogenicity classes IV-V in desmosomal genes separately in subgroups of patients with different degrees of confidence in the diagnosis of ARVC, assessed only on the basis of clinical manifestations of the disease, before DNA diagnosis (Figure 2).

Pathogenic and probably pathogenic genetic variants in desmosomal genes predominated in the subgroup of probands with a reliable diagnosis of ARVC, including probands carrying >1 potentially pathogenic genetic variant (13 probands; 28,3% in the subgroup) (Figure 2). In the subgroup of probable probands, mutations were detected in 2 probands (8,3% in the subgroup). No genotype-positive patients were detected among probands with a minimal set of diagnostic features (diagnosis of ARVC is possible) (Figure 2).

We also detected 13 rare genetic variants that were assigned to class III of pathogenicity (variants of unknown clinical significance (hereinafter — VUS) based on the ACMG2015 and ROMG criteria [20, 21] (Table 2). These variants were detected in 13 probands (16,25%). Three of these probands also had detected pathogenic/probably pathogenic genetic variants and 10 (12,5%) probands had only variants of unknown clinical significance. The largest number of variants with unknown clinical significance was detected in the gene *DSP*, which also dominated by the number of identified mutations. Unfortunately, the finding status of class III of pathogenicity does not allow any convincing comparative analysis of the clinical picture in patients who are carriers of these variants.

Discussion

Initially, ARVC was considered a "desmosis disease", however, taking into account the new diagnostic capabilities of DNA diagnostics, descriptions of mutations in other genes showed the genetic heterogeneity of the disease. It can be assumed that desmosomal genetic variants cause a more frequent pronounced ARVC phenotype, while mutations in other genes lead to a spectrum of diseases, including other types of cardiomyopathies and ARVC phenocopies.

The phenotype of "desmosomal" ARVC is associated with damage to both the right and left ventricle, in some cases skin manifestations of the disease are possible, for example, with Naxos syndrome [13].

To date, NGS sequencing of target gene panels and full-exome sequencing are the main approaches to DNA diagnosis of ARVC [2]. Despite increasing opportunities for genetic testing, the molecular cause of the disease remains undetectable in approximately 50% of patients, and some of the findings represent genetic variants of unknown clinical significance [2]. Whole-genome sequencing is considered, but it does not lead to a significant increase in the detectability of mutations, so the cost/efficiency ratio of this approach remains suboptimal.

According to current guidelines [19, 23], identification of a pathogenic/probably pathogenic genetic variant associated with ARVC and/or arrhythmogenic left ventricular cardiomyopathy phenotypes is a great diagnostic criterion for the disease. Therefore, one of the main purposes of this study is to clarify the ARVC diagnosis in proband. But only in 2 (2,5% of the examined cohort) probands with a probable diagnosis of ARVC, we detected variants of pathogenicity classes IV-V by DNA diagnosis, and managed to raise the diagnosis status to a reliable one. The data from our study show that the highest detectability of mutations is observed in probands with an extensive clinical picture of the disease, for which the appearance of an additional large criterion does not change the level of diagnosis reliability. This may give the impression of a decrease in the relevance of DNA diagnostics for patients with a reliable ARVC diagnosis. However, the importance of positive genetic test results remains high for family members of proband (1st and 2nd degree relatives as well as more distant relatives) and for the health care system as a whole. All current recommendations emphasize the need for regular dynamic monitoring and instrumental examination for first- and second-degree relatives if there is already a patient diagnosed with ARVC in the family. The start date of dynamic monitoring of relatives coincides with the diagnosis of proband. However, stipulated timeframe for leaving dynamic observation is not specified. Given the incomplete penetrance of mutations and different timing of manifestation, dynamic monitoring is assumed to be lifelong. This means that even in the absence of manifestations of the disease, relatives must be examined regularly, which is a time-consuming, psychological, and often financial burden on the family as well as on the health care system. In a number of European countries, such as Sweden, the role of cascade family screening and the identification of *genotype-negative* relatives who do not need a program of dynamic surveillance is considered a priority goal, more important for the

health care system than confirming the diagnosis in the proband himself [24].

In our group of patients, mutations were found in the genes *PKP2*, *DSG2* and *DSP*. The types of ARVC caused by mutations in these genes are among the most frequent in all ethnic groups except the inhabitants of Naxos Island (Greece) [9]. Usually, the greatest number of mutations are detected in the plakophilin gene (*PKP2*) — 20-46% [2]. In European countries, carriers of mutations in the *PKP2* gene account for up to 70% of carriers of mutations in desmosome genes [25]. In the desmoglein gene (*DSG2*), 3-20% of mutations are detected in European patients [2]. In Asian countries, the frequency of mutations in the gene *DSG2* is higher than in European countries: 15,8% in Japan [26] vs 4% in the Netherlands [25].

Mutation frequencies have also been determined for the desmoplakin (*DSP*) genes at 3-20% and desmocollin (*DSC2*) at 1-15% [2]. In our study, the most mutations were detected in the gene *DSP* rather than in *PKP2*, which distinguishes our sample of patients from the European samples.

Phenotype of patients with mutation in the gene *DSP* has its own peculiarities: these patients significantly more often have biventricular ARVC, develop LV systolic dysfunction (40%) and chronic heart failure (13%), as compared to patients with mutation in the gene *PKP2* [27, 28]. In addition, there is evidence that mutations in the gene *DSP* are associated with myocarditis accession [29], which is confirmed by our own data [30, 31].

Mutations in the gene *JUP* are rare in all ethnic groups, and their detection rate is not reliably known. In our study, we also failed to identify any variant in this gene that is reliably associated with the disease.

"Founder effect" is known for some mutations in ARVC, for example, for the mutation p.C796R in the gene *PKP2* found in 9 unrelated patients, all of Dutch origin [32]. None of the "European" mutations with the founder effect were found in Russian patients. The vast majority of mutations occurred only once and were unique to the family. Only 2 mutations occurred more than once: the mutation p.S194L in the homozygous state in the gene *DSG2* was found in two families of Caucasian origin, and the mutation s.1523_1538del in the heterozygous state in the gene *PKP2* was also found in two unrelated probands. To date, there is no information on relationship of the two probands carrying the same mutation, but it cannot be ruled out that both probands may have a common ancestor.

In our study, 15 mutations and 10 variants with unknown clinical significance were detected in the desmosome genes. The ratio "mutation:VUS" was 1:0,86. Thus, about 1/3 of the patients had at least one identified rare desmosomal variant. However, the *diagnostic* efficacy of the genetic study performed was only 19%, because only variants of pathogenicity class IV-V have a reasonable contribution to the diagnosis. Interestingly, in our sample of patients, probands carrying desmosomal variants of pathogenicity classes III-V were twice as numerous in the subgroup with a reliable diagnosis of ARVC (41,3%) compared with the subgroups with probable (16,6%) and possible (20,0%) diagnoses. In our opinion, this is an indirect confirmation that many variants qualified as VUS have pathogenetic significance, but insufficient study of molecular pathogenesis does not allow using these data correctly for diagnostic purposes.

In our laboratory, there is a rule to put class III pathogenicity variants in genes reliably associated with ARVC in the final report. Therefore, much attention is paid to correct medical-genetic counseling of families where such variants have been detected. In the medical consultation protocol, the phrase "The detection of pathogenicity class III variants cannot be used to confirm or exclude any diagnosis, or to justify the prescription, modification or cancellation of previously prescribed treatment or examinations" is routinely entered into the medical consultation protocol. Direct indication of this avoids undesirable iatrogenic influences on the family and relatives, and avoids the appointment of unwarranted instrumental examinations. However, putting these options in the conclusion has an important aspect — it opens to the family an opportunity to reinterpret the identified options. Genetic and basic studies are actively developing; data on the genetic nature of diseases and the functional significance of individual genetic variants are accumulating and being updated very rapidly. The need for periodic re-analysis and repeated contact with patients when new data are obtained regarding the genetic variants identified in them has been repeatedly emphasized [33, 34]. The optimal frequency of re-contacts and re-interpretation of such options is still a matter of discussion, but most experts recommend re-evaluating the value of the options 1-2 years after initial conclusion issuance. Therefore, proper medical genetic counseling of such families includes discussion of the significance, possibility, frequency, and procedure for re-interpretation of variants with unknown clinical significance.

Conclusion

In the molecular genetic study of desmosomal genes *PKP2*, *DSG2*, *DSP*, *DSC2* and *JUP* in patients with a guiding diagnosis of ARVC, the diagnostic

yield was 19%. At the same time, the detectability of mutations depended on severity of clinical signs of the disease, and was greatest among patients with the highest reliability of diagnosis. The detectability of variants with unknown clinical significance was also high, the ratio "mutation:VUS" was almost 1:1. Although these genetic variants are currently *not clinically used* and do not allow any actions to be taken, we consider it possible to make them into conclusions on the DNA diagnostics results. However, this tactic is justified only if patients receive appropriate medical-genetic counseling and they can seek reinterpretation of these variants after 1-2 years.

Given the central role of desmosomes in the process of cell adhesion, further functional studies of mutant proteins can shed light not only on clarification of molecular pathogenesis of the disease, but also contribute to the correct reinterpretation of genetic variants.

Relationships and Activities: none.

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Spectrum of mutations and their phenotypic manifestations in children and adults with long QT syndrome

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Aim. To determine the spectrum of mutations in the genes responsible for the long QT syndrome (LQTS) and study their phenotypic manifestations in patients with LQTS in different age groups.

Material and methods. The study included 35 unrelated probands with a clinical diagnosis of LQTS: 23 adults (8 men) and 12 children (9 boys). There were following clinical features: syncope — 54%, positive family history for SCD — 29%, implanted cardioverter defibrillator (ICD) — 46%. All participants underwent 12-lead electrocardiography (ECG), 24-hour Holter monitoring, genealogical analysis, echocardiography and cardiac MRI. The genetic study was performed by next-generation sequencing (NGS) using the MiSeq system (Illumina). The quantitative comparison of two unrelated groups was carried out using the nonparametric Mann-Whitney U-test. The differences were considered significant at $p<0,05$.

Results. In the examined group of 35 probands, 23 genetic variants of pathogenicity class IV and V (hereinafter referred to as) were identified. The molecular genetic variant of the disease was verified in 66% of probands. At the same time, the detection of mutations in the group with early manifestation (children) was significantly higher: 83% (10 out of 12 children) vs 57 % in adults (13 out of 23). Rare genetic variants of uncertain significance (VUS, class III pathogenicity) were detected in 4 probands (11%).

In the groups of children and adults with LQT1, LQT2 and LQT3, the sex distribution deviated from the 1:1 ratio. Among children, two-thirds were boys, among adults — the same proportion was represented by women. Disease manifestation time, QTc duration and adverse events risk depended on the genetic type of LQTS, intragenic localization of mutations and sex. In children, all 4 missense mutations in the *KCNQ1* gene were located in transmembrane domain, and in adults, 4 mutations were in the transmembrane domain and three — in the C-terminal domain of the protein. LQT1 in boys was characterized by early manifestation, while QTc did not exceed 500 ms and there were no adverse outcomes. Two women out of 7 adults with LQT1 with mutations in the transmembrane domain had na ICD (QTc >520 ms). All patients with LQT2 (4 children, 4 adults) had QTc >500 ms. At the same time, 2 children and 3 women had an ICD. LQT3 was diagnosed only in the children subgroup (2 boys, with QTc of 510 ms and QTc of 610 ms); one of them died suddenly despite beta-blocker therapy. Four adult patients, carriers of class III pathogenicity variants, had QTc <500 ms and delayed disease manifestation (after 30 years). Three of them had episodes of clinical death with subsequent resuscitation and implantation of cardioverter defibrillator.

Conclusion. The average diagnostic efficiency of mutation identification using NGS in patients with clinically manifest LQTS was 66%. At the same time, mutations were more common in the children's group. In genotype-positive probands, the risk of adverse outcomes correlated with sex, age and the genetic variant of disease. The greatest number of adverse outcomes was observed in carriers of mutations in both *KCNH2* (LQT2) and *SCN5A* (LQT3) genes. Variants with unknown clinical significance were identified in 4 probands (11%), which potentially allowed to confirm the diagnosis after functional tests.

Keywords: long QT interval syndrome, spectrum of mutations, *KCNQ1* gene, *KCNH2* gene, *SCN5A* gene, *CACNA1C* gene, *ANK2* gene, risk stratification.

Relationships and Activities: none.

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Long QT syndrome (LQTS) is a genetically determined disease characterized by an increase in QT interval on an electrocardiogram (ECG), a high risk of developing life-threatening ventricular tachyarrhythmias, syncopal conditions and sudden cardiac death (SCD) at a young age. LQTS is mainly caused by functional changes in potassium, sodium and calcium ion channels, which are most often caused by defects in the genes encoding the pore-forming α-subunits and regulatory β-subunits of ion channels, as well as some other proteins. The prevalence of clinically pronounced LQTS is at least 1 per 2 thousand people [1].

Modern approaches to the diagnosis of LQTS, risk assessment of SCD and choice of treatment tactics in patients with such pathology are largely based on information on molecular genetic nature of the disease. Determination of the genetic cause allows to minimize genotype-specific triggers of life-threatening arrhythmias [2], to exclude drugs that prolong the QT interval, and to individualize treatment and prescribe optimal antiarrhythmic drugs for a particular type of LQTS [1]. The clinical significance of genetic testing in patients with LQTS is also confirmed by the fact that asymptomatic mutation carriers without preventive measures and appropriate therapy have a significantly increased risk of serious cardiac events by the age of 40 [3]. To date, algorithms for risk stratification and management of patients with different genetic variants of LQTS have been proposed, which significantly help in clinical practice [2-5]. At the same time, it has been shown that the prognosis of the disease depends on the sex of the patient and changes with age: the most malignant course of the syndrome and increased risk of SCD is more often observed in adult women, and among children — in boys [6, 7]. However, in our opinion, little attention has been paid to the question of modulating effect of sexual and age characteristics on phenotypic manifestation of specific mutations, depending on their intragenic localization. In this regard, the goal of our study was to determine the spectrum and prevalence of mutations, as well as to study their phenotypic manifestations in patients with LQTS of different age groups.

Material and methods

The study included 35 unrelated probands diagnosed with LQTS based on the ESC Guidelines 2015 [8] and the modified Schwartz PJ, et al. (2011) [9]. The adult group included 23 patients over 18 years of age (median 31 [21; 35]; 8 men) seen at the National Scientific and Practical Center "Cardiology". The group of children is represented by 12 probands (median age 7 [5; 9] years, 9 boys) who were treated at the National Scientific and Practical Center "Pediatric Surgery". Prior to enrollment, written informed consent was obtained from all adult study participants and parents/guardians of probands from the children's group. The study was carried out in accordance with the Good Clinical Practice standards and the principles of the Helsinki Declaration.

The clinical and instrumental study included ECG registration in 12 leads, daily ECG monitoring, genealogical history collection with ECG assessment of all family members and detection of SCD cases. To exclude myocardial structural abnormalities, echocardiographic examination was carried out according to the current recommendations.

The search for mutations in the coding sequences of genes connected to the development of channelopathy and other hereditary heart rhythm disorders was carried out by high-throughput sequencing (NGS) on the genetic analyzer MiSeq (Illumina). Sample preparation of samples was carried out using a TruSight™ Cardio Sequencing Panel (Illumina) set, which includes 174 genes associated with diseases of the cardiovascular system. Verification of the identified mutations was performed by direct Sanger sequencing. The sequencing results were annotated using the ANNOVAR software [10]. The pathogenicity of new and previously described genetic variants was assessed according to the recommendations of the American College of Medical Genetics (ACMG2015) [11]. Pathogenic (Class V) and probably pathogenic (class IV) genetic variants were considered to be diagnostically significant. Variants with uncertain clinical significance (VUS, class III) in genes associated with inherited rhythm disorders were also included in the data analysis [12].

Table 1

Genetic variants of pathogenicity class IV and V identified in Belarusian patients with LQTS

Code	Gender	Age[a], years	Gene (exon)	Replacement in DNA, rs	Replacement in protein	Pathogenicity class	Critical events
	Children's patients						
682	M	7	*KCNQ1*	c.IVS96+1G>A rs762814879	Мутация сплайсинга	V	SCD in family
2H	M	6	*KCNQ1* (5)	c.394C>T rs1994722719	p.Arg132Cys	V	–
602	F	3	*KCNQ1* (7)	c.641C>T rs12720459	p.Ala214Val	V	Syncope in women in family
5H	M	9	*KCNQ1* (9)	c.1233delA	p.Lys411Asnfs*8	V*	–
4H	M	10	*KCNH2* (7)	c.1496T>G rs794728370	p.Leu499Arg	V	SCD in family
1H	M	11	*KCNH2* (7)	c.1682C>T rs121912504	p.Ala561Val	V	–
6H	M	7	*KCNH2* (7)	c.1868C>T rs199472950	p.Thr623Ile	V**	VT/ICD
684	F	12	*KCNH2* (7)	c.1928G>A	p.Cys643Tyr	V*	Syncope, SCD in family, resuscitation, ICD
3H	M	6	*SCN5A* (10)	c.1231G>A rs72549410	p.Val411Met	V**	–
722	M	12	*SCN5A* (28)	c.4931G>A rs28937316	p.Arg1644His	V	–
	Adult patients						
566	F	24	*KCNQ1* (5)	c.379G>A rs120074179	p.Val127Met	V	Syncope, SCD in family, VT/ICD
609	F	12	*KCNQ1* (6)	c.535G>C rs120074181	p.Gly179Arg	V	VT/VF, resuscitation, ICD
656	F	35	*KCNQ1* (7)	c.592G>A rs199472756	p.Gly198Arg	V	–
713	M	21	*KCNQ1* (7)	c.641C>T rs12720459	p.Ala214Val	V	Syncope
639	F	25	*KCNQ1* (12)	c.1555C>T rs199472787	p.Arg519Cys	IV	Syncope
640	F	18	*KCNQ1* (13)	c.1621G>A rs199472796	p.Val541Ile	IV	–
635	M	19	*KCNQ1* (16)	c.1999G>A rs776119582	p.Val667Met	IV	–
564	F	24	*KCNH2* (3)	c.371T>A	p.Met124Lys	V*	Syncope, VT/ICD
655	F	34	*KCNH2* (6)	c.1424A>G rs199472907	p.Tyr475Cys	V	Syncope, SCD in family, ICD
589	F	35	*KCNH2* (8)	c.2131A>G rs199473532	p.Ile711Val	IV	Syncope
720	F	43	*KCNH2* (12)	c.2775dupG rs794728455	p.Pro926AlafsX14	V	Syncope, SCD in family, ICD
628	F	14	*CACNA1C* (14)	c.2053C>T	p.Arg685Trp	IV*	–
610	F	31	*CACNA1C* (19)	c.2573G>A rs786205753	p.Arg858His	V	VT/ICD

Note: [a] — age of manifestation; * — new, previously undescribed mutation, ** — *de novo* mutation; m — male, f — female; IV and V — pathogenicity class of genetic variant according to ACMG2015 criteria.

Abbreviations: SCD — sudden cardiac death, DNA — deoxyribonucleic acid, ICD — implantable cardioverter-defibrillator, VT — ventricular tachycardia, VF — ventricular fibrillation.

Table 2

Variants with unspecified clinical significance (class III pathogenicity according to ACMG2015 criteria) identified in group of adult patients

Code	Gender	Age[a], years	Gene (exon)	Replacement in DNA, rs	Replacement in protein	Additional option (Class III)	Critical events
613	F	39	*CACNA1C* (8)	c.1186G>A rs762756712	p.Val396Ile	*KCNH2*: c.49A>T (p.Arg17Trp)*	VT/VF/ICD
607	M	33	*CACNA1C* (40)	c.4942G>A rs370432385	p.Ala1648Thr	*SCN3B*: c.260C>G (p.Pro87Arg), rs371050389 *DSG2*: c.1442T>C (p.Ile481Thr), rs371854289	VT/VE/RFA
543	M	45	*ANK2* (14)	c.1397C>T rs786205722	p.Thr466Met	*SNTA1*: c.787G>T (p.Ala263Ser), rs15057653	VT/AF, ICD
586	M	33	*ANK2* (38)	c. 9161C>G rs139007578	p.Ala3054Gly	*KCNE1*: c.253G>A (p.Asp85Asn), rs1805128	VT/VF, ICD, storms

Note: [a] — age of manifestation; * — new, previously undescribed mutation; m — male, f — female.

Abbreviations: DNA — deoxyribonucleic acid, ICD — implantable cardioverter-defibrillator, VT — ventricular tachycardia, VE — ventricular extrasystole, RFA — radio frequency ablation, VF — ventricular fibrillation, AF — atrial fibrillation.

Two unrelated groups were compared for quantitative characteristics using the nonparametric Mann-Whitney U-criterion. The differences were considered statistically significant at $p<0,05$.

Results and discussion

The paper presents a comparative analysis of the spectrum of mutations between groups of adults and children diagnosed with LQTS, as well as the study of some clinical indicators, including adverse events and outcomes, in patients with different genetic types of LQTS, taking into account gender and age characteristics. The clinical characteristics of the general group were as follows: syncopal conditions were registered in 54% of patients, 29% had a family history of SCD, 46% had an implanted cardioverter defibrillator (ICD) (in 88% of cases — due to SCD or cardiac arrest with successful resuscitation, in 12% — for the purpose of primary prevention of SCD).

During genotyping of 35 probands with a clinical diagnosis of LQTS 23 genetic variant classes IV and V of pathogenicity (next mutation) patients were identified in 4 genes in 23 (66%) patients: *KCNQ1* (LQT1) — 11 mutations, *KCNH2* (LQT2) — 8 mutations, *SCN5A* (LQT3) — 2 mutations, *CACNA1C* (LQT8) — 2 mutations (Table 1). In 10 (83%) of 12 children, mutations were in 3 major genes: in *KCNQ1* — 4 mutations, in *KCNH2* — 4 mutations, in *SCN5A* — 2 mutations. In the adult group, 13 mutations were detected in 3 genes: *KCNQ1* — 7 mutations, *KCNH2* — 4 mutations, *CACNA1C* — 2 mutations (Table 1).

In addition, 4 rare variants (VUS) with a frequency of 0,00001 were identified in the examined group of adult patients, of which 2 were in the gene *CACNA1C* encoding the α-subunit of the calcium channel, and 2 were in the gene *ANK2* responsible for ankyrin synthesis (Table 2).

In these patients, additional genetic variants of VUS were also identified in genes associated with rhythm disturbance and encoding subunits of potassium (*KCNH2*, *KCNE1*) and sodium (*SCN3B*) ion channels, and some other proteins (*SNTA1*, *DSG2*) (Table 2).

Among the examined probands, the proportion of genotype-positive patients with mutations of classes IV and V was 66% (23 out of 35). Comparative analysis of this index between groups of children (10 of 12) and adults (13 of 23) showed that the detection of diagnostically relevant mutations in the group with early manifestation (children) was higher (83%) than in adults (57%), and for major genes *KCNQ1*, *KCNH2*, *SCN5A*, the difference was statistically significant (83% vs. 48%, $p<0,05$). A combination of several VUS in genes associated with LQTS and other hereditary arrhythmogenic diseases was detected in 4 patients (11%).

The distribution of 27 genetic variants of classes III-V (Tables 1, 2) identified in the general group of probands is shown in Figure 1. In the gene *KCNQ1*, (LQT1) was 11 (41%) pathogenic mutations in the gene *KCNH2* (LQT2) — 8 (30%), in the gene *SCN5A* (LQT3) — 2 (7%). 4 (15%) nucleotide variants (VUS class III pathogenicity), 2 of which were localized in the gene *CACNA1C* and 2 — in the gene *ANK2,* combined with VUS in other genes.

A comparative analysis of mutation distribution depending on the age of patients showed significant differences between groups of children and adults. In children, all 10 identified mutations were con-

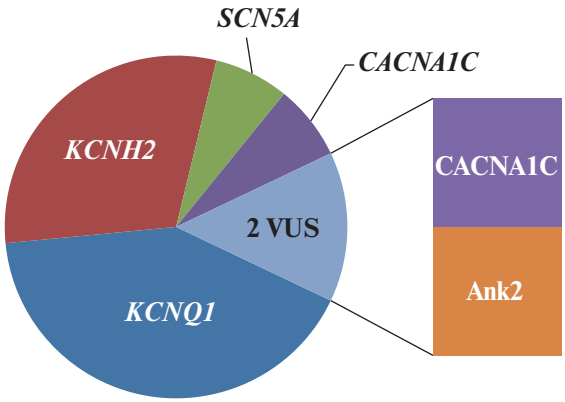

Figure 1. Distribution of genetic variants of pathogenicity classes III-V in the general group of patients with LQTS.

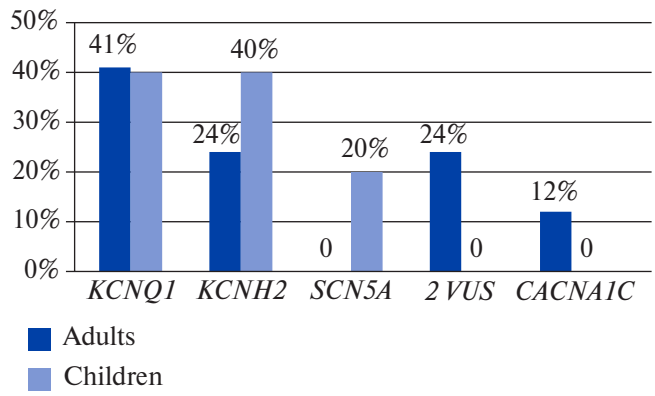

Figure 2. Comparative analysis of the distribution of genetic variants of pathogenicity classes III-V between adults and children.

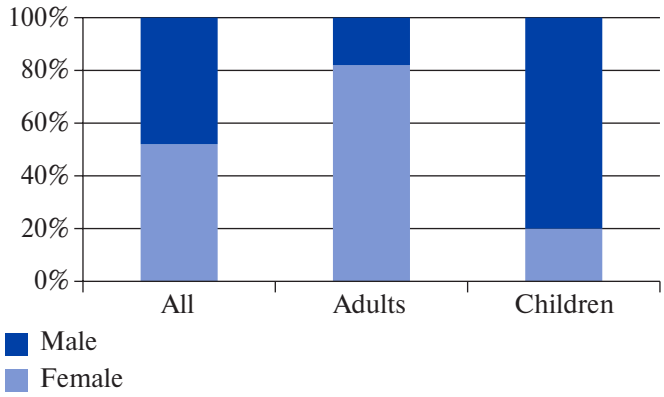

Figure 3. Gender distribution among patients with LQTS in the general group and depending on age.

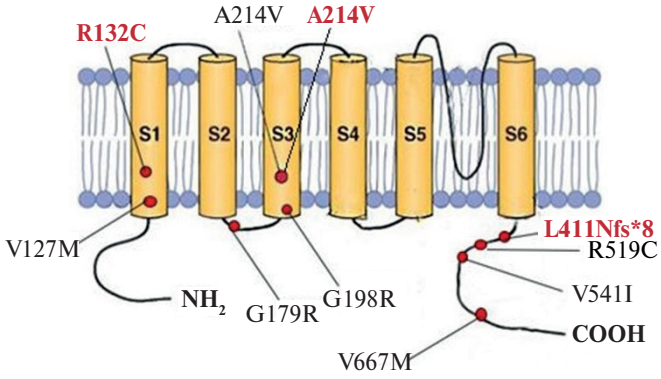

Figure 4. Mutations in the *KCNQ1* (LQT1) gene: mutations in children are marked in red font.

centrated in three major genes (*KCNQ1*, *KCNH2* and *SCN5A*), whereas in adults the proportion of mutations in these genes was only 65% (11 out of 17). The proportion of mutations in the gene *KCNH2* in the group of children was 2 times greater (40%) than in adult probands (22%), while the proportion of mutations in the gene *KCNQ1* was the same in both samples (Figure 2).

Mutations in the gene *SCN5A* were found exclusively in a group of children: in 2 out of 10 patients (Table 1, Figure 2). 2 pathogenic mutations of classes IV and V in the gene *CACNA1C* were found in 2 adult probands, one of which was not previously described. In the group of adult patients, a combination of several VUS in different genes was also detected (24%) (Table 1, Figure 2).

A study of the gender distribution in different age groups of patients with a genetically confirmed diagnosis of LQT1-LQT3 detected the following differences: among adult probands, 82% (9 out of 11 patients) were women, and in the group of children, 80% (8 out of 10 patients) were boys. It should be noted that the gender distribution in the general group was 1:1 (Figure 3).

The observed shifts in the sex distribution indicate that this characteristic has a significant impact on the age of manifestation of the clinical manifestations of LQTS of the first three types. Our findings confirm the results of other studies, which also noted the predominance of males in the younger age group and the predominance of females in the older age group [6].

Gene *KCNQ1* (LQT1)

Table 1 and Figure 4 show the mutations found in Belarusian patients in the *KCNQ1* gene encoding the α-subunit of the potential-dependent potassium channel (K_V7.1) responsible for the slow flow of positively charged potassium ions from cells.

82% of mutations in the general group were missense mutations, while this type of mutation was observed in all adults, and in children — only in half of the cases. The remaining genetic variants in the group of children were represented by a mutation

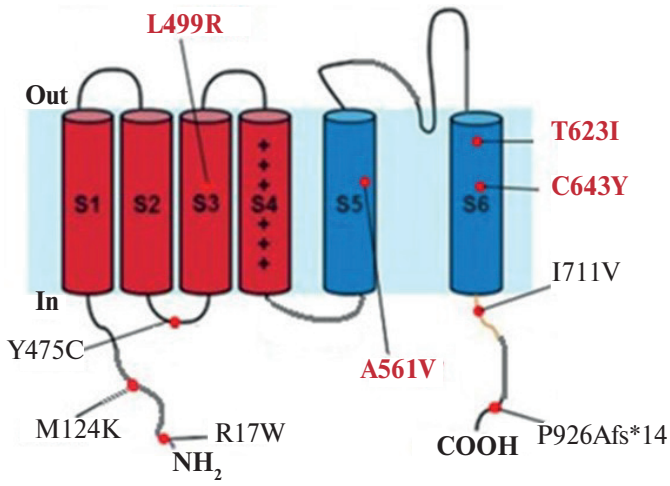

Figure 5. Genetic variants in the *KCNH2* (LQT2) gene: mutations in children are marked in red font.

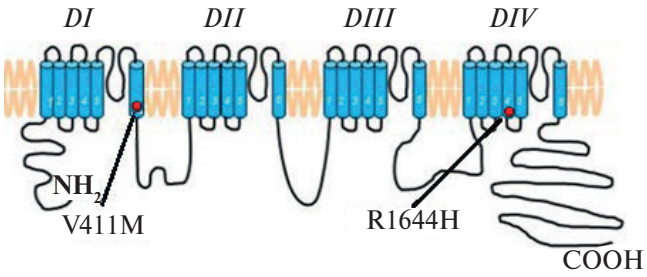

Figure 6. Mutations in *SCN5A* (LQT3).

IVS96+1G>A in the second intron leading to splicing failure and a new deletion of c.1233delA (p.Lys411Asnfs*8) in exon 9 with a frameshift and formation of a premature stop codon.

57% of missense mutations in the gene *KCNQ1* in adults and all missense mutations in children were concentrated in exons 5-7 of the gene *KCNQ1*, corresponding to the 1st (S1) and 3rd (S3) segments of the α-subunit transmembrane domain. 43% of mutations in adult patients were concentrated in exons 12, 13, and 16 encoding the C-terminal region of the protein (Table 1, Figure 4).

The QTc value in children with LQT1 ranged from 447 to 528 ms, the clinical course was characterized by the absence of syncope, but all probands had a family history of the disease, with female relatives. The QTc interval duration in adults with LQT1 varied from 450-630 ms. Severe form of the disease was observed only in 2 women (29%) with pathogenic mutations in the 5th (p.VAL127MET) and 6th exons (p.gly179arg) of the gene *KCNQ1*. These patients had QTc values >520 ms, recurrent syncope, and ventricular tachycardia (VT)/ventricular fibrillation (VF), followed by successful resuscitation and ICD implantation. It should be noted that QTc value >500 ms was observed in all adults with mutations in exons 5-7 of the gene *KCNQ1* regardless of gender, but adverse events were observed in women over 24 years of age with QTc >520 and mutations in exons 5 and 6.

All mutations were unique, except for p.Ala214Val localized in S3, which was found in 2 unrelated probands (a 30-year-old male with 6-year-old manifestation, recurrent syncope, nonsustained ventricular tachycardia (NVT), and QTc =630 ms, and a 3-year-old girl with QTc =505 ms).

Gene *KCNH2* (LQT2)

Mutations in the gene *KCNH2* (LQT2) encoding the α-subunit of rapidly activating potential-dependent potassium channel (K_V11.1) are presented in Table 1 and Figure 5. Mutations in this gene, as well as in the gene *KCNQ1*, lead to a decrease in the repolarizing current from cell and an increase in the action potential duration due to QT prolongation.

7 out of 8 (88%) genetic variants were missense mutations, and duplication leading to the appearance of a premature stop codon was also detected.

All mutations were unique and were concentrated in exons 6-8 in 75% of cases. 23% of the mutations were located in the N- and C-terminal regions and were detected in the group of adults (Figure 5).

All 4 pediatric patients with LQT2 had mutations in exon 7, which corresponds to the 5-6 segments of the transmembrane domain that directly form the pore channel region. Three of the probands were boys, which indirectly indicates an early phenotypic manifestation of these mutations (before age 11) primarily in males. QTc >500 ms was observed in all children and adults with LQT2, regardless of the mutation localization. For 50% of children and 75% of adults, ICD implantation was required. 50% of children and adults have a family history of CHF. Risk factors for adverse events among children with LQT2 were mutation localization in exon 7 of the gene *KCNH2*, and in adult patients — female sex and age older than 28 years.

Gene *SCN5A* (LQT3)

Mutations in the gene *SCN5A* encoding the α-subunit of potential-dependent sodium channel (Na_V1.5) and responsible for development of LQTS of the third type (LQT3), were detected only in

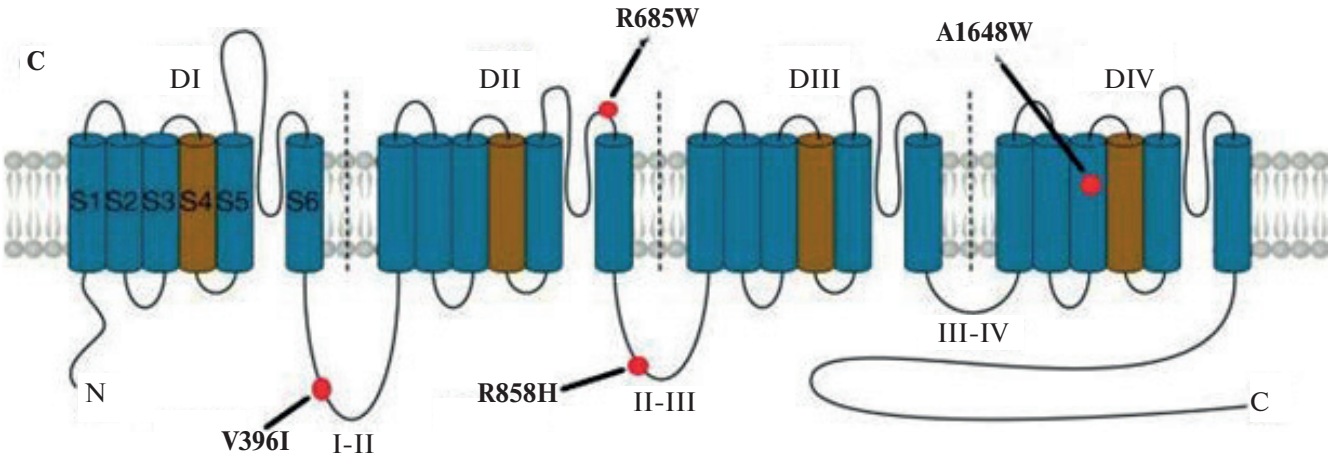

Figure 7. Genetic variants in the gene *CACNA1C* (LQT8).

a group of children — in 2 probands (Table 1). The most dangerous of the identified genetic variants was the *de novo* pathogenic mutation c.1231G>A in the gene *c.1231G>A*, which leads to the replacement of an amino acid in transmembrane region of DI-S6 sodium channel (p.VAL411MET, rs72549410) (Figure 6). In the boy, the disease was manifested by syncopal states lasting 5-7 seconds, the first of which occurred at the age of 3, and the value of QTc =595 ms (max. QTc =616 ms). Despite therapy with beta-blockers, the boy had SCD in his sleep against the background of viral infection at age of 9.

The p.VAL411MET mutation was described earlier as having arisen *de novo* in three unrelated cases, which indicates its special malignancy and the "hot spot" of mutations. Functional studies have shown that it leads to hyperactivation of sodium channel and prolonged repolarization of action potential.

In the second patient with LQT3, the pathogenic mutation c.4931G>A affected the DIV-S4 region of sodium channel protein (p.ARG1644HIS, rs28937316) (Figure 6) and phenotypically manifested at the age of 12 with a slight QTc prolongation (up to 490 ms). This variant has been reported previously in several patients with LQT3. At p.ARG1644HIS, a stable internal current of Nations is shown, however, this nucleotide variant may be less serious than other changes in this gene. The boy's mother's father and her 34-year-old cousin were diagnosed with LQTS; in the patient's mother, the presence of the p.Arg1644His mutation was not accompanied by phenotypic manifestations. There was no SCD in the family.

A comparative analysis of clinical parameters between patients with different genetic types of LQTS showed that the QTc value was independent of gender and age and was mainly determined by the genetic type of LQTS, as well as by intragenic mutation localization in patients with LQT1. Thus, all probands with LQT2 and LQT3 showed an increase in QTc >500 ms, and in patients with LQT1, only carriers of mutations in exon 5-7 had QTc >500 ms. The probability of adverse outcomes correlated with QTc value and was higher in LQT2 and LQT3 patients compared with LQT1 patients. At the same time, in adult patients with LQT1, the risk factors were female, QTc >520 and mutation localization in exons 5 and 6. All adult patients with LQT2 requiring ICD insertion were also female, and in the pediatric group of patients with LQT2 and LQT3, events requiring ICD implantation were more often recorded in boys.

A combination of several VUS was found in 4 adult patients, while in 2 probands one of the variants of class III pathogenicity was in the gene *CACNA1C* (Figure 7), in 2 — in the gene *ANK2*. All patients with several genetic variants in the genes associated with this pathology had a severe disease course.

Gene *CACNA1C*

The gene *CACNA1C* encodes the α-subunit of the potential-dependent calcium channel ($Ca_V1.2$), generating L-type calcium currents. This gene has previously been associated predominantly with Timothy syndrome, which occurs as multiple organ dysfunction, including webbing of fingers and toes, congenital heart defects, immunodeficiency, hypoglycemia, cognitive impairment and autism [13]. Recent studies have found more and more evidence of its importance in the development of isolated autosomal dominant LQTS without extracardiac features [14], SQTS [15], as well as seizure states, including epilepsy [16].

The most severe disease pattern with QTc interval prolongation up to 500 ms, syncopal episodes, development of VT/VF with successful resuscitation and ICD implantation was observed in the patient with p.Val396Ile substitution in exon 8 of gene *CACNA1C* (Table 2, Figure 7) combined with a new

substitution p.Arg17Trp (c.49A>T) in gene *KCNH2* (Table 2, Figure 5).

A patient with p.ALA1648THR replacement in exon 40 of the gene *CACNA1C* in combination with rare variants in the genes *SCN3B* and *DSG2* had frequent episodes of NVT and malignant ventricular extrasystole, which required radiofrequency ablation of ectopic foci. A pronounced clinical picture of the disease with QTc prolongation >500 ms, recurrent syncope, cardiac arrest episode and successful resuscitation with ICD implantation was observed in a patient with a pathogenic mutation (class V) of p.Arg858His in exon 19 of the gene *CACNA1C* (Table 1, Figure 7). Over the course of 8 years, the patient developed pirouette-type polymorphic VTs (TdP) three times, which were stopped by ICD.

In a patient with a new, pathogenic by *in silico* predictors, p.Arg685Trp mutation in exon 14 of the gene *CACNA1C* (Table 1, Figure 7), the disease course was more mild, without syncope and significant episodes of ventricular tachyarrhythmias, controlled by drug therapy.

Gene *ANK2*

VUS substitutions in 15 and 38 exons of the gene *ANK2,* previously associated with the development of type 4 LQTS, in combination with additional variants in the genes *SNTA1* and *KCNE1* were detected in 2 unrelated male probands (Table 2) [17]. The gene *ANK2* encodes an adapter protein from the ankyrin family involved in localization and stabilization of membrane ion carriers and ion channels. Not so long ago, it was established that mutations in the gene *ANK2* lead to other rhythm disorders, including sinus node weakness syndrome, atrial fibrillation, as well as life-threatening ventricular tachyarrhythmias with a high risk of SCD. All this phenotypic variety of cardiac rhythm and conduction abnormalities is united, for the time being, in "ankyrin-B syndrome" [18].

Despite the absence of a burdened family history and QT prolongation on the ECG series (375 and 440 ms), both patients had recurrent syncopal conditions, VT/VF development, requiring resuscitation and ICD implantation. A 43-year-old proband with substitutions in the genes *ANK2* and *SNTA1* developed polymorphic VT/VF, which was stopped by ICD three times during the 8-year follow-up, and ICD replacement was carried out three times. In recent years, episodes of syncopal states and repeated ICD triggers leading to resuscitation measures have not been repeated.

All patients with multiple VUS were characterized by a slight prolongation of the QTc interval falling into the "gray zone", but had rhythm disturbances and a high risk of SCD, the same, in fact, as patients with mutations in the gene *KCNH2* (LQT2). Thus, during the follow-up period, 3 of 4 (75,0%) patients with multiple genetic variants and 3 of 4 patients with LQT2 had VT/FV with ICD implantation, whereas patients with LQT1 only had life-threatening arrhythmic events in 25,0% of cases, as mentioned above.

A distinctive feature of the group of adult patients with multiple VUS was some predominance of men, whereas the clinical manifestations of LQT1 and LQT2 were observed mainly in female patients.

Study limitation. The results of this study should be considered in light of several limitations, the main one being the relatively small patient sample due to the low prevalence of LQTS. An increase in the number of groups under consideration would allow to obtain more accurate results. Nevertheless, the presented data are in good agreement with those already described in the literature. The second limitation concerns assumptions about diagnostic significance of combination of several VUS in genes associated with malignant arrhythmias in patients with LQTS. Conducting family cascade screening of first-degree relatives of such probands in the future will help assess the diagnostic reliability of the identified variants.

Conclusion

To date, despite huge leaps in understanding of the LQTS pathogenesis due to identification of molecular genetic causes, there are still gaps in knowledge about phenotypic manifestations of genetic defects, including the role of age and sex factors in clinical manifestation of LQTS symptoms is not fully understood. Prognosis and risk stratification of SCD in patients with LQTS is mainly based on QT interval prolongation on ECG, history of syncopal episodes due to torsade de pointes VT or cardiac arrest, and cases of SCD in blood relatives. An independent prognostic factor for development of adverse outcomes is the presence of pathogenic mutations in the genes associated with this pathology.

Comparative analysis of SCD predictors (age of manifestation, syncope, cardiac arrest, type and localization of mutation) in groups of children and adults of Belarusian patients with LQTS of the first three types confirms a significant modulating effect of age and gender on phenotypic realization of the disease. During the study, it was found that in the groups of younger and older probands the sex distribution deviated from the ratio of 1:1 and was exactly the opposite — among children, two-thirds were boys, among adults — women. Based on this fact, it can be assumed that the age of manifestation of clinical manifestations of LQTS of the first three types significantly depends on patient gender. In males, signs of the disease appear in childhood more often than in women.

The results of our study showed that the spectrum of mutations also has age-related features. In the group of children with LQTS, all identified mutations were concentrated in three major genes: 40% each in the genes *KCNQ1*, *KCNH2* and 20% in the gene *SCN5A*. In adult probands, the proportion of mutations in these genes was only 65%: 41% in the gene *KCNQ1* and 24% in the gene *KCNH2*. There were no mutations in the gene *SCN5A* in this group. In 4 adult patients, a combination of several VUS was found in the genes associated with rhythm disturbances, while in 2 probands, one of the variants was in the gene *CACNA1C* and in 2 other probands — in the gene *ANK2*.

In the course of studying the association between age of manifestation and intragenic localization of mutations, it was revealed that in children all missense mutations in the gene *KCNQ1* were concentrated in exons 5-7 corresponding to transmembrane domain region of α-subunit, while in adults almost half of the mutations resulted in amino acid substitutions in the C-terminal region of the protein. Based on these data, it can be assumed that mutations in the gene *KCNQ1* affecting the C-terminal region of the protein have a later phenotypic manifestation. Relating to LQT2, all pediatric patients with this type of LQTS had mutations in exon 7 that predominantly disrupted the protein in pore region. This fact indirectly indicates an early phenotypic manifestation of these mutations, at least in males. All patients with multiple VUS substitutions had a severe course of the disease, but the disease manifestation in these probands was statistically significantly later than in carriers of mutations in the major genes (*KCNQ1*, *KCNH2*, and *SCN5A*).

Due to the above, monitoring of the main clinical and electrophysiological markers of SCD risk in prolonged QT interval syndrome should preferably be performed taking into account age and gender. This will optimize treatment and avoid adverse outcomes of this type of canalopathy, as well as timely prevention of life-threatening arrhythmias in persons with latent and nonsyncopal forms of the disease taking into account these parameters. A comparative approach to study of LQTS phenotypic manifestations, taking into account the age of patients, allows a better assessment of the prognostic significance of identified mutations.

Relationships and Activities: none.

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Molecular genetic features of the development of restrictive cardiomyopathy in Russian children

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Aim. To identify the proportion of restrictive cardiomyopathy (RCM), as well as cardiomyopathy (CMP) with a restrictive type of hemodynamics among all cases of genetic CMP and to determine the relative frequencies and spectrum of nucleotide variants in Russian children with RCM, as well as to search for phenogenotypic correlations.

Material and methods. The study included 689 children with CMPs. All children underwent a molecular genetic testing of the target regions of 419 genes responsible for various cardiomyopathies and channelopathies using the method of massively parallel sequencing (MPS).

Results. In 668 (97,0%) children, pathogenic, likely pathogenic nucleotide variants, as well as nucleotide variants with unknown clinical significance, were identified. Of these, 45 (6,7%) patients were selected to determine the molecular genetic characteristics of RCM, 20 of whom had clinical symptoms and morphofunctional structure of RCMP (3,0%), while the remaining 25 (3,7%) children were diagnosed with another CMP type with a restrictive type of hemodynamics. In total, these patients had 41 nucleotide variants in 15 different genes, while 19 (46,3%) variants were pathogenic, 12 (29,3%) — likely pathogenic, 10 (24,4%) — uncertain clinical significance. Pathogenic and likely pathogenic variants were identified in a total of 38 (84,4%) patients, while in 19 (42,2%) patients, the pathogenic variants described earlier were found. The most common genetic marker of RCM in Russian children was *TNNI3* gene mutations. In total, they were identified in 12 (25%) children: with RCP — 8 (40%) patients; with CMP with a restrictive type of hemodynamics — 4 (16%) patients. At the same time, the most common mutation of the *TNNI3* gene was the nucleotide variant c.575G>A, leading to the amino acid variant p.R192H, described earlier in patients with RCM and identified by us in three (15%) unrelated children with RCM. In addition, a significant difference was found between the averaged values of N-terminal pro-brain natriuretic peptide in patients with mutations in the *MYH7* and *TNNI3* genes (0,0039, p<0,05), as well as between the peak flow gradient values in children with mutations in *TNNI3* and *FLNC* genes (0,0016, p<0,05), *TNNI3* and *MYH7* genes (0,039, p<0,05).

Conclusion. The results of this study indicate a significant genetic heterogeneity of RCM in Russian children and the need for further research aimed at finding genotype-phenotype associations in order to predict the course of the disease and select the proper therapy.

Keywords: restrictive cardiomyopathy, genetics, mutations, children, DNA sequencing.

Relationships and Activities. The work was carried out within the state assignment № AAAA-A19-119012590190-6.

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Restrictive cardiomyopathy (RCMP) is one of the rarest forms of cardiomyopathy (CMP), ranging from 2,6% [1] to 5% in European countries [2], in Australia, RCMP accounts for 2,5% of all CMPs [3], according to US data, RCMP and other non-dilatative or hypertrophic types account for 3% of CMPs in children [3]. In Russia, there are currently no accurate data on the prevalence of genetically associated RCMP in children.

RCMP is defined as a condition of the heart with restrictive ventricular physiology in the presence of normal or reduced diastolic volume (one or both ventricles), normal or reduced systolic volume and normal ventricular wall thickness [4]. The ejection fraction usually exceeds 50%. RCMP is also characterized by mild to moderate tricuspid and mitral valve regurgitation, as well as the development of bicuspid dilatation due to chronic elevation of atrial pressure, which along with blood stasis in pulmonary veins and pleural effusion can lead to moderate or severe cardiomegaly [5]. Children with CRPS are characterized by rapid progression of the disease, as well as high mortality: when diagnosing at the age of 10 years or older, the 5-year survival rate is 64%, whereas 50% of patients in the younger cohort have a fatal case within 2 years after diagnosing [6]. Death can occur suddenly as a result of cardiac arrhythmias, thromboembolism, or therapy-resistant congestive heart failure (HF). At the moment, heart transplantation is the only effective method in RCMP treatment.

RCMP causes include hereditary and non-hereditary factors, which are divided into infiltrative, non-infiltrative, endomyocardial and storage diseases [7]. Most RCMP cases are acquired. Among the genetic RCMP causes, mutations in genes encoding sarcomeric subunits are predominantly found: troponin I (*TNNI3*), troponin T (*TNNT2* gene), troponin (*TNNC1* gene), tropomyosin (*TPM1* gene) and the β-myosin heavy chain (*MYH7*). The continuous operation of all sarcomeres in each cardiomyocyte of heart muscle is fundamental importance for the contractile function of heart and is based on balanced interaction of sarcomeric proteins. Even one dysfunctional sarcomeric protein alters protein-protein interactions, causing disturbances in the sarcomere structure and dynamics, leading to contractile dysfunction, CMP and further HF. In addition, the cause of RCMP development may be mutations of genes encoding non-sarcomeric proteins, as well as proteins connected to sarcomeres [8]. Most mutations of these genes are inherited by autosomal dominant type [7]. Some scientists believe that RCMP is the consequence of mutation combinations of genes encoding sarcomeric and cytoskeletal proteins [9]. Others believe that *de novo* mutations are accompanied by very rapid disease progression and poor prognosis in children with RCMP [10, 11].

In our country, the first scientific studies devoted to the study of RCMP features in children were published by Serbin V. I. and his students in 1999. In these works, for the first time in Russia, the RCMP phenotype was characterized using the example of 19 children aged 2,5 to 15 years with primary myocardial RCMP [12]. Summarizing the follow-up analysis results, it was noted that RCMP in children at the early stages of its development proceeds little or asymptomatically. The disease intelligence is connected to the appearance of clinical signs of congestive HF. The difficulties in diagnosing this form of CMP are also caused by the scantiness of acoustic symptomatology, unsharp increase in percussion and radiological dimensions of heart. Electro- and echocardiography (EchoCG) occupy a key place in RCMP diagnosis. Sharp dilatation of both atria, caused by their marked overload due to significant disturbance of ventricular relaxation and difficulty of atrial emptying, comes into the picture. A study of the genetic causes of RCMP in 35 patients of various age groups was also performed in our country, which showed that pathogenic and probably pathogenic variants were detected in 74% of cases of idiopathic RCMP, 20% of which were caused by *FLNC* gene mutations [4]. Separately, the role of *FLNC* gene mutations in RCMP development was assessed by a group of researchers headed by Kiselyov A. [13]. According to the authors, mutations in the *FLNC* gene have been connected to neuromuscular diseases for a long time, and only recently have their associations with RCMP and hypertrophic CMP (HCMP) been discovered. The study describes new clinical phenotypes of filaminopathies in 4 pediatric patients with early manifestation of RCMP in combination with myopathy [14]. Continuing own studies of genetically determined CMPs [15], studying the molecular genetic features of RCMP in Russian children and their correlations with clinical picture of the disease was decided.

Material and methods

The study included 689 children of various ages with cardiomyopathy. The study protocol was approved by the independent local ethics committee at the Federal State Autonomous Institution "National Medical Research Center for Children's Health".

All children underwent molecular genetic study of the target regions of 419 genes responsible for the development of various CMPs and channelopathy by mass parallel sequencing. From among them,

patients with RCMP were selected, as well as children with an initially different CMP phenotype with restrictive hemodynamics, who were diagnosed with a hypertrophic CMP phenotype and/or non-compact left ventricular myocardium (NLVM) at the onset of disease with subsequent phenotype and hemodynamics transformation. Consents to the study were obtained from all parents. The gender and age of the children (at the time of their last discharge from the Federal State Autonomous Institution "National Medical Research Center for Children's Health"), the presence of hereditary factors, the concentration of biomarker of N-terminal propeptide of brain natriuretic hormone of B-type (NT-proBNP), EchoCG parameters, including the maximum gradient of blood flow (PGr max) on pulmonary artery valve were assessed.

Genomic DNA was isolated using a set of DNA Blood Mini Kit reagents (QIAGEN, Germany), at the QIAQUBE automatic station (QIAGEN, Germany). The quality and quantity of DNA were assessed using an NP80 nanophotometer (Implen, Germany) and a Qubit 3.0 fluorimeter of new generation (Invitrogen, USA).

Mass parallel sequencing was carried out on a MiSeq sequencer (Illumina, USA). Biotinylated SeqCap EZ samples (Roche, USA) were used for targeted enrichment. The total size of the panel, which included the coding and adjacent regions of 419 genes, was 1498000 pairs of nucleotides; the average reading depth was at least 150X with an average reading length of 300 nucleotides.

The detected variants were searched and annotated using the programs Alamut Batch and Alamut Focus (Interactive Biosoftware, France). All undescribed nucleotide variants were investigated using the built-in bioinformatic modules SIFT, PolyPhen2, MutationTaster, FATHMM and MetaLR in the program Alamut Visual (Interactive Biosoftware, France). The pathogenicity of nucleotide variants not previously described was determined using the Human DNA Sequence Data Interpretation Guide [15]. The GenBank Access database was used as a reference data base for nucleotide sequences. The HGVS recommendations were used for nomenclature of detected genome variants.

Validation of the detected nucleotide variants was carried out by Sanger sequencing on an automatic DNA sequencer ABI 3500 (Thermo Fisher Scientific, USA) using a set of reagents BigDye® Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, USA) in accordance with the manufacturer's protocols and recommendations. DNA fragments were amplified on a ProFlex thermal cycler (Thermo Fisher Scientific, USA) in 20 μl of Amplitaq Gold 360 reaction mixture (Thermo Fisher Scientific, USA) containing 500 nmol of primers and 20 ng of genomic DNA. PCR conditions: 95° C/3 min — 1 cycle; 94° C/10 sec, 54-66° C/30 sec, 72° C/15 sec — 34-40 cycles; 72° C/40 sec — 1 cycle.

Statistical processing of the obtained results was carried out in the Statistica 10.0 package (StatSoft, IBM, USA). The Mann-Whitney p-test was used for quantitative data.

Results and discussion

To determine the molecular and genetic features of RCMP in Russian children, 45 (6,7%) out of 668 pediatric patients with genetically determined CMPs were selected, 20 of whom had clinical symptoms and morphofunctional structure of RCMP (3,0%), and the remaining 25 (3,7%) had a different CMP phenotype with a restrictive type of hemodynamics. Boys accounted for 53,3% (24 people), girls — 46,7% (21 people). Among children with RCMP, boys predominated (12 boys/60%), whereas in patients with CMP with restrictive type of hemodynamics, both sexes were distributed approximately equally with a small predominance of girls (13 girls/52%).

The average age of 45 enrolled patients with RCMP and CMP with restrictive hemodynamics at the time of the last discharge from the Federal State Autonomous Institution "National Medical Research Center for Children's Health" was 112 months (9 years 4 months), the median age was 107 months (8 years 11 months) (Figure 1).

Among children with RCMP, patients of the age category from 7 to 13 years prevailed (45% — 9 children), while their average age was 97 months (8 years 1 month), and the median was 93 months (7 years 9 months). Predominant age category in children with CMP with restrictive hemodynamics was not found, their average age was 124 months (10 years 4 months), and the median was 133 months (11 years 1 month).

The anamnesis was taken into account for hereditary history of cardio-vascular system, such as CMP, congenital heart defects, myocardial infarction, and stroke. Cases of sudden death in childhood and young age were taken into account. According to the obtained data, the biological relatives of patients had pathology of the circulatory system in 44,4% of cases (20 people), in 37,8% (17 people) — heredity was not burdened and in 17,8% (8 people) — it was not possible to collect accurate data (Figure 2).

It should be noted that burdened heredity was more common in children with CMP and restrictive hemodynamics than in patients with RCMP, and amounted to 52% (13 people) and 35% (7 people), respectively.

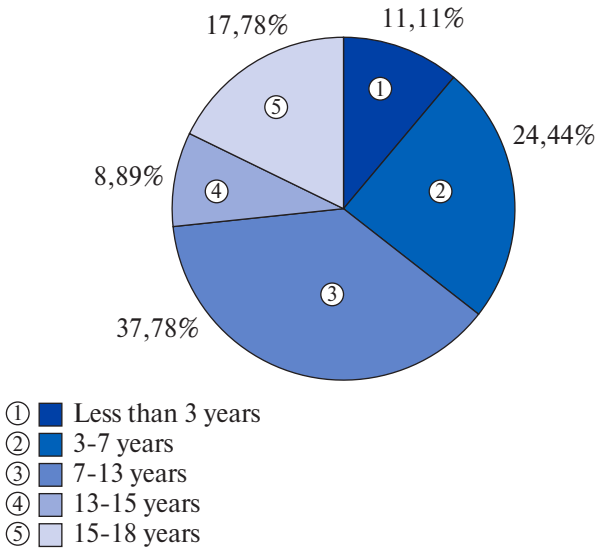

Figure 1. Distribution of patients by age at the time of the last discharge from the Federal State Autonomous Institution "National Medical Research Center for Children's Health".

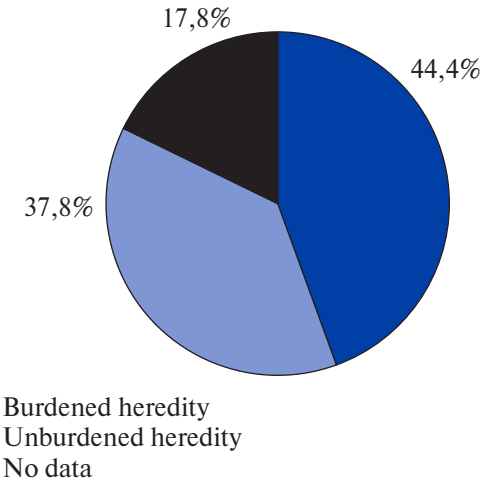

Figure 2. Distribution of patients by presence of hereditary pathology in biological parents.

A total of 41 nucleotide variants in 15 different genes were detected in children with RCMP and CMP with restrictive hemodynamic type, while 19 (46,3%) variants were pathogenic, 12 (29,3%) were probably pathogenic, and 10 (24,4%) variants could not be pathogenic. Pathogenic and probably pathogenic variants were detected in total in 38 (84,4%) patients, while mutations described earlier were detected in 19 (42,2%) patients.

In patients with RCMP, 18 nucleotide variants were detected in 11 different genes (*TPM1*, *MYH7*, *DES*, *TNNI3*, *LMNA*, *FHL1*, *TBX20*, *DSG2*, *VCL*, *FLNC*, *MYL2*). In patients with CMP and restrictive hemodynamics, 25 nucleotide variants were detected in 11 different genes (*TPM1*, *MYH7*, *DES*, *TNNI3*, *TBX20*, *FLNC*, *MYL2*, *TNNT2*, *DSP*, *JUP*, *MYBPC3*). Mutations in the *TPM1*, *MYH7*, *DES*, *TNNI3*, *TBX20*, *FLNC*, and *MYL2* genes were detected in both groups. Mutations in the genes *LMNA*, *FHL1*, *DSG2*, *VCL* were unique for patients with true RCMP, and mutations in the genes *TNNT2*, *DSP*, *JUP*, *MYBPC3* were unique for patients with CMP and restrictive type of hemodynamics.

In children with RCMP, the proportion of pathogenic nucleotide variants was 77,8% (14 nucleotide variants), while in patients with CMP with restrictive hemodynamics – 36% (9 nucleotide variants). Mutations of the gene *TNNI3* were the most common cause of genetically determined RCMP and CMP with a restrictive type of hemodynamics. They were detected in 12 (25%) of the children and presented 8 different nucleotide variants, the most frequent mutation of the gene *TNNI3* was nucleotide variant c.575G>A resulting in the amino acid variant p.R192H, previously described in patients with RCMP [16], we found three unrelated children with RCMP.

In 9 children (20%), 8 different nucleotide variants in the gene *MYH7* were found, in 6 children, 5 different nucleotide variants in the gene *FLNC* were found, in 8 children — 8 different variants: equally in the genes *TPM1* and *DES*, in 2 children — 2 nucleotide variants in the gene *TNNT2*, in two other children — the nucleotide variant c.484G> A, p.G162R of the gene *MYL2*, two nucleotide variants in two children in the gene *TBX20* and one nucleotide variant was found in the genes *MYBPC3*, *DSG2*, *FHL1*, *JUP*, *LMNA*, *DSP* and *VCL* (Table 1) [6, 8, 9, 11, 13-26].

Two mutations in the gene *TNNI3* were detected by us twice.

• Pathogenic nucleotide variant c.509G>A, p.R170Q, described by Kaski JP, et al. in patients with RCMP [27] was found in a boy (patient No. 25) with CMP with a restrictive type of hemodynamics, diagnosed at the age of 99 months (8 years 3 months), and in a girl (patient No. 26) with RCMP, who debuted at the age of 74 months (6 years 2 months). At the same time, the boy's parents were not examined for the carrier state of this mutation, and the girl's heredity is not tainted.

• Pathogenic nucleotide variant c.611G>A, p.R204H, described by Yang SW, et al. with RCMP [28], and Doolan A, et al. [29] with HCMP was detected in a boy (patient No. 33) with RCMP, diagnosed at the age of 175 months (14 years 7 months), and a girl (patient No. 34) with CMP with

a restrictive type of hemodynamics, with the onset of disease at the age of 79 months (6 years 7 months). At the same time, the boy's father suffers from RCMP, and the girl's mother suffers from Wolf-Parkinson-White syndrome.

Pathogenic nucleotide variant c.575G>A, p.R192H in the gene *TNNI3* described by Mogensen J, et al. with RCMP [30], Hayashi T, et al. with HCMP [31] and Fujino M, et al. [26] in patients with NLVM, was detected by us in three unrelated patients with CRMP: a boy (patient No. 29), who was diagnosed at 146 months of age (12 years 2 months of age), and two girls who were diagnosed at 75 months of age (6 years 3 months, patient No. 30) and 96 months (8 years old, patient No. 28). At the same time, one of the girls had a *de novo* mutation, the second had no heredity, and the boy could not collect an accurate anamnesis.

The likely pathogenic nucleotide variant c.7781G>T, G2594V in the gene *FLNC*, not previously described in the world literature, was detected by us in two unrelated girls, with onset of disease at 24 months (2 years, patient No. 10) and 150 months (12 years 6 months, patient No. 9), enrolled in the group with CMP with restrictive hemodynamic type. At the same time, one girl's heredity is not tainted, and the other does not have accurate anamnesis data (adopted child).

Pathogenic nucleotide variant c.2146G>A, p.G716R in the gene *MYH7*, described by Anan R, et al. with HCMP [20] and Hayashi T, et al. with RCMP [31], was detected by us twice. Mutations were found in two boys with CMP and restrictive type of hemodynamics, found at the age of 102 months (8 years 6 months, patient No. 17) and 171 months (14 years 3 months, patient No. 18). At the same time, both children's heredity is tainted patrilineally (in the first case, the father died from HCMP at the age of 23).

Pathogenic nucleotide variant c.484G>A, p.G162R in the gene *MYL2*, described by Olivotto I, et al. in patients with HCMP [21] was found in children of both sexes with RCMP, detected at the age of 8 months (patient No. 24) and 16 months (1 year 4 months, patient No. 23). At the same time, the mutation occurred *de novo* in the boy, and the girl could not collect an accurate anamnesis.

The remaining nucleotide variants were detected once, which indicates a significant genetic heterogeneity of Russian children with RCMP, as well as children with CMP and restrictive type of hemodynamics. All nucleotide variants, which, as we

Table 1

Mutations found in Russian children with RCMP and CMP with restrictive type of hemodynamics, anamnesis data, laboratory and instrumental studies

No.	Phenotype	Gene	Nucleotide, amino acid variant	Frequency, %	Bioinformatic analysis, description in the literature	A	NT-proBNP (pg/ml, average)	PGr max (mm Hg) Diagnosis	PGr max (mm Hg) Extract
1	RCMP	*DES*	c.1132A>G, p.K378E	n/a	UCS	1	668	2,75	3
2	RCMP	*DES*	c.1360C>T, p.R454W	n/a	P, RCMP [17], HCMP [18]	1	1266	n/a	4,97
3	RCMP	*DES*	c.1243C>T, p.R415W (homozygote)	0,0018	P, myopathy [18]	2	n/a	4	3
4	RGem	*DES*	c.218G>A, p.R73Q	n/a	UCS	1	5794	3	3
		DSP	c.4477_4480del, p.E1493Qfs*32	n/a	P				
5	RGem	*FHL1*	c.4del, p.A2Rfs*28	n/a	P	2	6084	4,87	4,87
6	RGem	*FLNC*	c.31G>A, p.G11S	0,006	UCS	2	4872	3	6
7	RGem	*FLNC*	c.3557C>T, p.A1186V	n/a	P, RCMP [13]	2	n/a	5,9	4,5
8	RCMP	*FLNC*	c.6826G>A, p.V2276M	n/a	UCS	2	632	3,56	5
		JUP	c.1916A>G, p.E639G	n/a	UCS				
9	RGem	*FLNC*	c.7781G>T, G2594V	n/a	PP	2	1190	3,53	6,9
10	RGem	*FLNC*	c.7781G>T, p.G2594V	n/a	PP	n/a	4521	5,76	4,49
11	RGem	*FLNC*	c.6772T>C, S2258P	n/a	UCS	1	907	5,48	4
12	RCMP	*LMNA*	c.1279C>G, p.R427G	n/a	P, cardiac muscular dystrophy [19]	1	1997	4,84	2,35
13	RGem	*MYBPC3*	c.716G>A, p.C239Y (homozygote)	n/a	PP	1	1365	3,41	2,55
14	RGem	*MYH7*	c.545C>T, p.A182V	n/a	PP	n/a	240	3,08	2,86

Table 1. Continuation

No.	Phenotype	Gene	Nucleotide, amino acid variant	Frequency, %	Bioinformatic analysis, description in the literature	A	NT-proBNP (pg/ml, average)	PGr max (mm Hg) Diagnosis	PGr max (mm Hg) Extract
15	RCMP	*MYH7*	c.746G>A, p.R249Q	n/a	P, HCMP [20], NLVM [19]	1	n/a	2,62	4
16	RGem	*MYH7*	c.1120G>A, p.E374K	n/a	PP	1	1140	5	10
17	RGem	*MYH7*	c.2146G>A, p.G716R	n/a	P, HCMP [8], RCMP [13]	1	924	7,08	8,45
18	RGem	*MYH7*	c.2146G>A, p.G716R	n/a	P, HCMP [8], RCMP [13]	1	1106	6,1	5
19	RGem	*MYH7*	c.2302G>A, p.V768R	n/a	P, HCMP [11], RCMP [14]	1	n/a	2,5	3
20	RGem	*MYH7*	c.4894G>A, p.A1632T	n/a	UCS	1	2613	6,71	8
21	RGem	*MYH7*	c.4045G>A, p.E1349K	n/a	PP	1	1537	3	3
22	RGem	*MYH7*	c.2203C>T, p.F735L	n/a	PP	2	4022	4	4
23	RCMP	*MYL2*	c.484G>A, p.G162R	n/a	P, HCMP [21]	2	5945	2,68	2,9
24	RCMP	*MYL2*	c.484G>A, p.G162R	n/a	P, HCMP [16]	n/a	n/a	4,93	5,5
25	RGem	*TNNI3*	c.509G>A, p.R170Q	n/a	P, HCMP [22]	n/a	2237	4	3
26	RCMP	*TNNI3*	c.509G>A, p.R170Q	n/a	P, HCMP [22]	2	2208	3,7	4
27	RCMP	*TNNI3*	c.571T>A, p.W191R	n/a	PP	2	2416	4	4
28	RCMP	*TNNI3*	c.575G>A, p.R192H	n/a	P, RCMP [12], HCMP [23], NLVM [24]	2	5380	3,24	1,59
29	RCMP	*TNNI3*	c.575G>A, p.R192H	n/a	P, RCMP [12], HCMP [23], NLVM [24]	н/д	5704	3	4
30	RCMP	*TNNI3*	c.575G>A, p.R192H	n/a	P, RCMP [12], HCMP [23], NLVM [24]	2	4370	3,24	3,24
31	RCMP	*TNNI3*	c.601G>T, E201*	n/a	P	2	2787	4	4
32	RCMP	*TNNI3*	c.610C>T, p.R204C	n/a	P, HCMP [9], RCMP [21]	1	1745	2,3	1,96
33	RCMP	*TNNI3*	c.611G>A, p.R204H	n/a	P, HCMP [6], RCMP [25]	1	5190	3	2
34	RGem	*TNNI3*	c.611G>A, p.R204H	n/a	P, HCMP [6], RCMP [25]	1	4841	4,08	4
35	RGem	*TNNI3*	c.617_619del, p.K206_F207delinsI	n/a	UCS	2	7219	2	2
36	RGem	*TNNI3*	c.499C>G, p.D167H	n/a	PP	n/a	4770	n/a	1,44
37	RGem	*TNNT2*	c.299T>C, p.I100T	n/a	PP	2	2680	2,1	3
38	RGem	*TNNT2*	c.421C>T, p.R141W	n/a	P, DCM [15], HCMP [26]	1	2331	4,65	4
39	RCMP	*TPM1*	c.76G>A, p.E26K	n/a	PP	2	1077	2,57	2,57
40	RGem	*TPM1*	c.187G>C, p.A63P	n/a	UCS	1	5492	0,82	1,7
41	RCMP	*TPM1*	c.218T>A, p.L73Q	n/a	PP	2	н/д	3,41	2,68
42	RCMP	*TPM1*	c.287A>T, p.E96V	n/a	PP	2	2408	4,46	8
43	RCMP	*VCL*	c.1708C>T, p.R570*	0,0004	P	1	415	4,22	5,85
44	RGem	*DSG2*	c.1088C>A, S363*	0,0004	P	1	1263	4,14	3
		TBX20	c.830_831dup, p.D278*	n/a	P				
45	RGem	*TBX20*	c.346C>G, p.L116V	n/a	UCS	n/a	n/a	n/a	4

Note: "A" — history: "1" — burdened heredity, "2" — unburdened heredity; "PGr max extract" — value at the time of last discharge from hospital, "max PGr Diagnosis" — value at the time of diagnosis, "n/a" — no data, "P" — pathogenic variant "PP" — probably pathogenic, "UCS" — variant of uncertain clinical significance. The population frequencies are given in accordance with the gnomAD database, version 2.1.1.

Abbreviations: HCMP — hypertrophic cardiomyopathy, NLVM — non-compact left ventricular myocardium, RCMP — restrictive cardiomyopathy, NT-proBNP — N-terminal propeptide of brain natriuretic hormone of B-type, PGr max — maximum blood flow gradient.

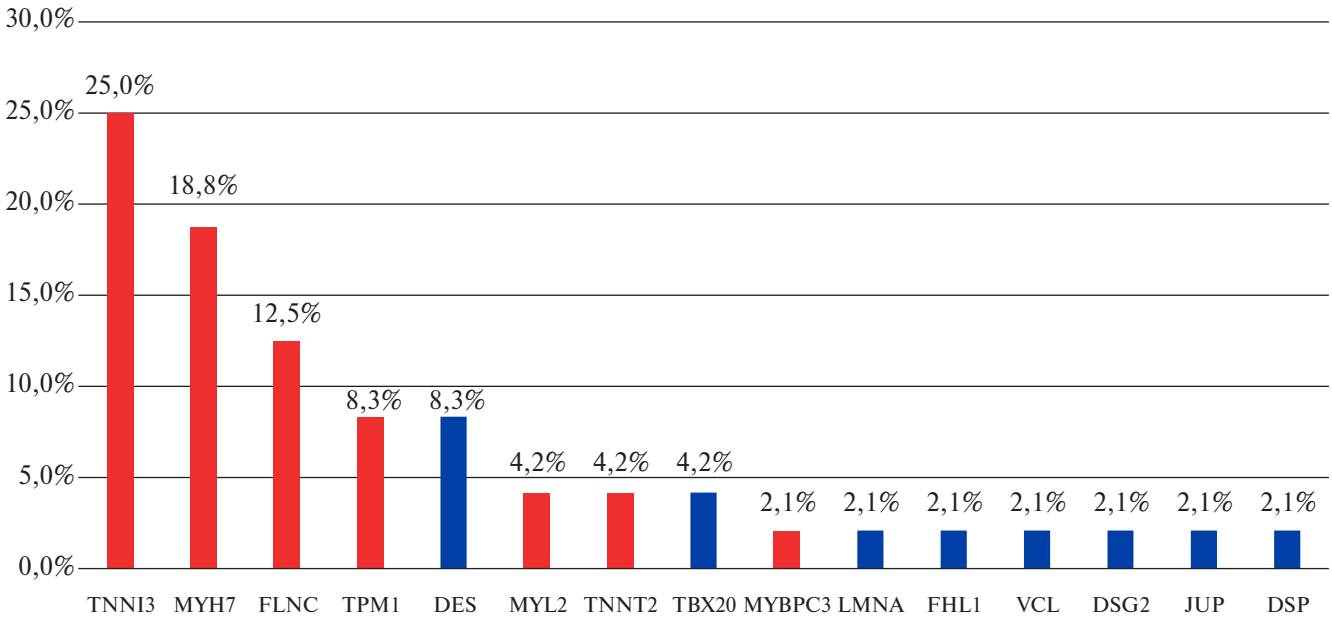

Figure 3. Percentage of genes containing mutations that caused RCMP and CMP with a restrictive type of hemodynamics in the examined Russian children.
Note: red — sarcomeric genes, blue — non-sarcomeric genes.

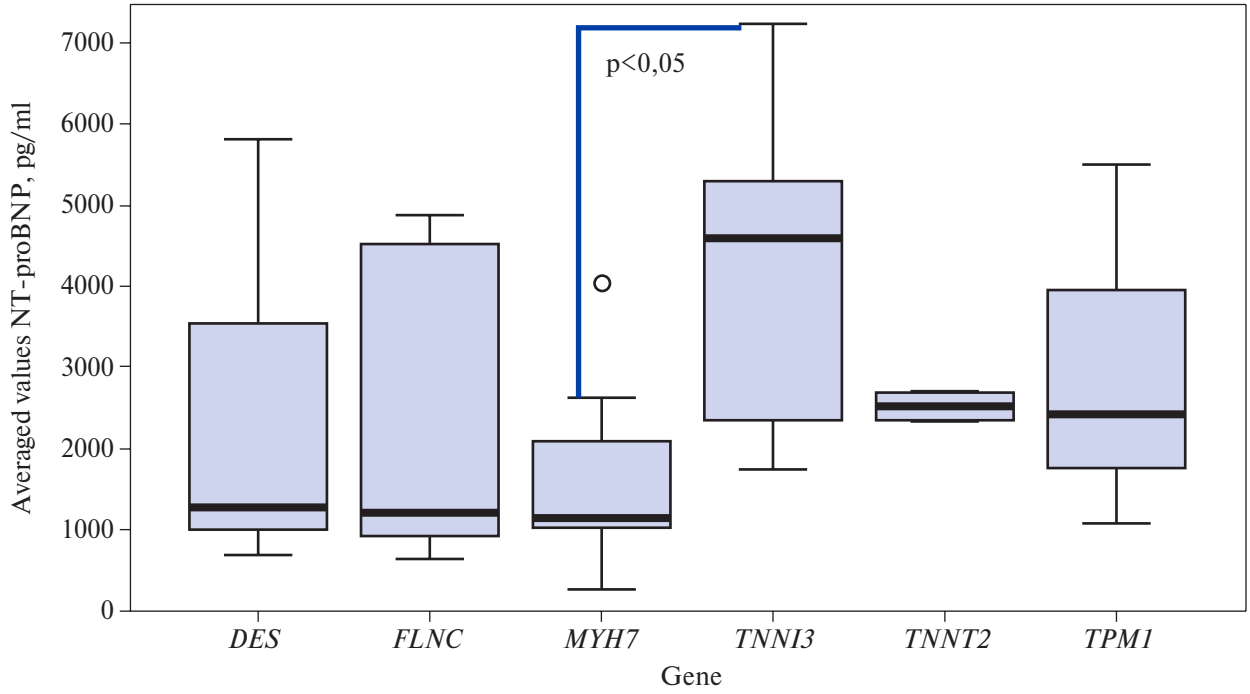

Figure 4. Distribution of the averaged values of NT-proBNP according to affected genes.

believe, may be the cause of CMP development in the examined children, were found in a heterozygous state with the exception of two variants. One of them, c.1243C>T, p.R415W detected in the gene *DES* the child (patient No. 3) with severe RCMP diagnosed at the age of 14 months (1 year 2 months old) was previously described only in a heterozygous state in patients with myopathy, manifested after the age of 40. The second, not previously described variant c.716G>A, p.C239Y, was detected in the gene *MYBPC3* in a child (patient No. 13) with a restrictive type of hemodynamics, who debuted with HCMP and NLVM at the age of 144 months (12 years) (Table 1).

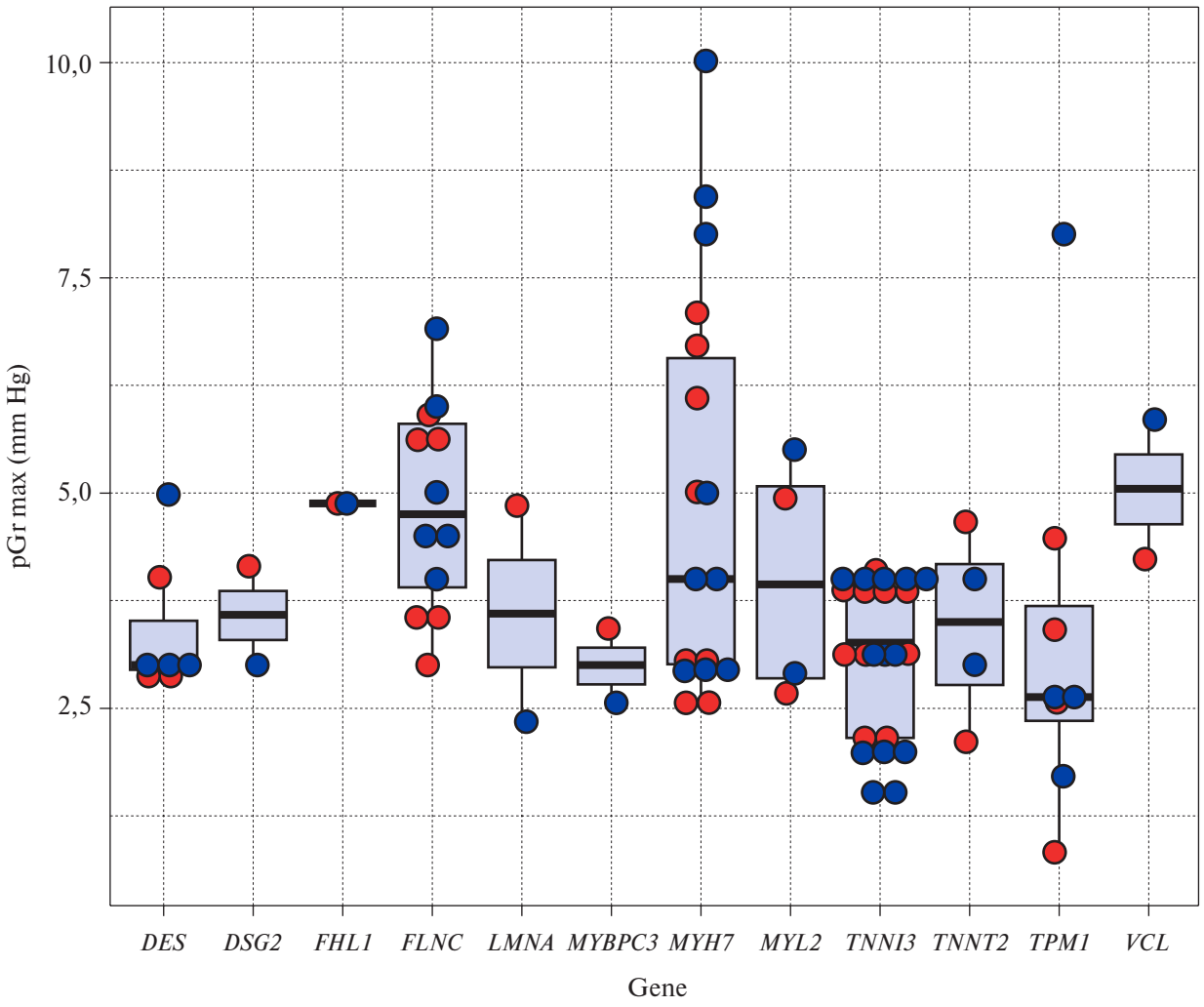

Figure 5. Distribution of values of the parameter PGr max (mm Hg) of blood flow on pulmonary artery valve in the general group of children with RCMP and children with CMP with restrictive hemodynamics.
Note: individual indicators of PGr max (mm Hg) are presented in the form of dots: red indicates the indicators related to the time of diagnosing of RCMP or CMP with a restrictive type of hemodynamics, blue indicates the value at the time of the last discharge from the Federal State Autonomous Institution "National Medical Research Center for Children's Health".
Abbreviations: CMP — cardiomyopathy, RCMP — restrictive cardiomyopathy, PGr max — maximum gradient of blood flow.

Among the 45 Russian patients examined by us, the predominance of mutations that caused the RCMP and CMC development with a restrictive type of hemodynamics is noticeable in sarcomeric genes (red color) in contrast to non-sarcomeric genes (blue color) (Figure 3).

In total, mutations of sarcomeric genes were detected in three quarters of the patients we examined, while non-sarcomeric ones were found in only a quarter. Foreign colleagues also report the prevalence of mutations of sarcomeric genes in patients with RCMP: *TNNI3*, *TNNT2*, *TNNC1*, *TPM1*, *TTN*, *MYH7*, *MYL2*, *MYBPC3* over non-sarcomeric ones: *MPN*, *DES*, *FLNC*, *LMNA*, *BAG3* [8].

Mutations in the gene *TNNI3* predominated in patients with RCMP (8/40,0%), while mutations in the gene *MYH7* (8/32,0%) predominantly occurred in children with CMP and restrictive hemodynamics. Similarly, to the data obtained by us, mutations in the gene *TNNI3* prevailed among Chinese with RCMP, in whom a significant number of *de novo* mutations were detected [17]. At the same time, mutations in the gene *FLNC* prevailed in other studies, in which the disease manifested at the age of less than 10 years [23]. Recent studies

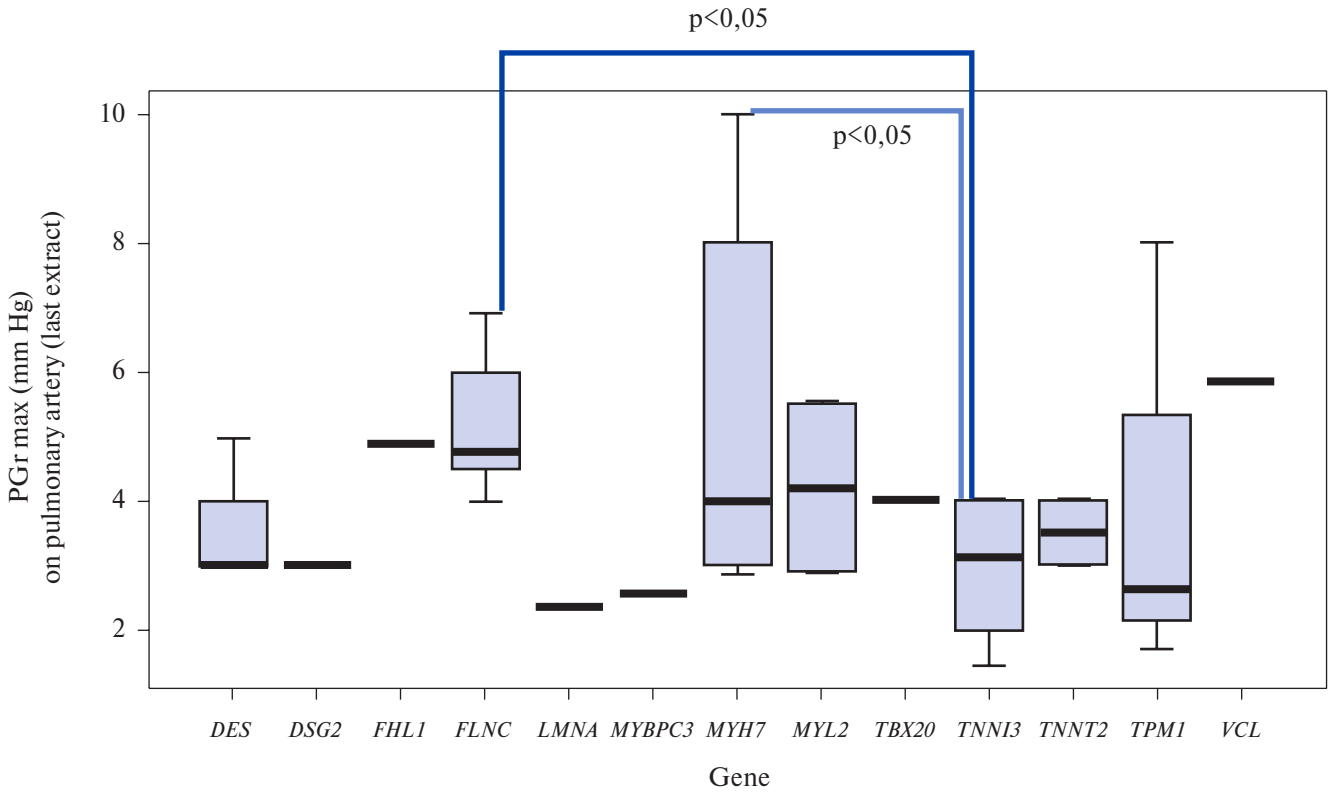

Figure 6. Distribution of registered values of pGr max (mm Hg) during the last discharge from the hospital in children with mutations in various genes.

suggest that gene *MYH7* mutations, predominantly found in patients with CMP worldwide [24], are not characteristic of patients with RCCM, as confirmed by our study, which detected only one (5%) child (patient No. 15) whose RCMP was due to the pathogenic c.746G>A, p.R249Q variant of the gene *MYH7* previously described in patients with HCMP [20] and NLVM [19] (Table 1).

An additional objective of the study was to try to link the clinical manifestations of the disease with mutation found in a particular gene. The average value of NT-proBNP biomarker concentration, which reflects severity of CH manifestations, was taken as a criterion of clinical diagnosis. Figure 4 shows 6 genes in which mutations were more frequent in patients whose medical histories had 3 or more reported NT-proBNP values measured at least six months apart between any two measurements.

The average concentrations of the NT-proBNP biomarker in patients with mutations in the following genes were: *DES* — 2645 pg/ml, *MYH7* — 1314 pg/ml, *TPM1* — 1553,67 pg/ml, *FLNC* — 2728,75 pg/ml, *TNNI3* — 4072 pg/ml, *TNNT2* — 2322,5 pg/ml. A significant difference was revealed between the mean values of NT-proBNP parameter for patients with gene *MYH7* and *TNNI3* mutations (0,0039, p<0,05) (Figure 4), which may indicate different severity of the disease course.

To date, the link between the level of biochemical indicator NT-proBNP and mutations in a particular gene has not been described in the world literature, so the work requires further study. Whereas foreign publications indicate almost unavoidable development of pulmonary hypertension in patients with RCMP, which can lead to such complications as arrhythmias and sudden cardiac death [8]. However, the literature does not contain indication on the relationship between the severity of RCMP with instrumental EchoCG indices, which was also done in this paper for the first time. Due to indications on obligatory development of pulmonary hypertension, a parameter reflecting the maximum blood flow gradient at the pulmonary artery valve (PGr max) was taken, which may indicate the level of pulmonary hypertension. The association of EchoCG data (PGr max parameter on the pulmonary artery valve) with the damage of a certain gene was analyzed (Figure 5).

Two values of PGr max blood flow at pulmonary artery valve were analyzed: at the diagnosing of RCMP or CMP with restrictive type of hemodynamics and at the time of the last hospital discharge. The first value reflects the state of small circulatory system at the diagnosing, the second value characterizes the state after some time and disease treatment. Both indicators jointly show the disease course in a certain period of time.

No statistically significant correlation was found between the values of PGr max parameter in mm Hg on pulmonary artery valve by EchoCG recorded at the diagnosing of RCCM with a particular gene lesion, but a statistically significant difference was found between the values of PGr max parameter (mm Hg) on pulmonary artery valve by EchoCG at the diagnosing the last discharge from the Department of Cardiology of the Federal State Autonomous Institution "National Medical Research Center for Children's Health" in the groups of patients with gene mutations *FLNC* and *TNNI3* (0,0016 inch, p<0,05), as well as genes *MYH7* and *TNNI3* (0,039, p<0,05) (Figure 6).

The obtained information may indicate a different degree of progression of pulmonary hypertension in patients with mutations in the genes *FLNC* and *TNNI3*, as well as in the genes *MYH7* and *TNNI3*. Thus, the severity of pulmonary hypertension increases in the following sequence: *TNNI3→MYH7→FLNC*, which may allow to recommend paying close attention to children with mutations in the described genes in the pediatrician's practice for therapy correction.

Conclusion

For the first time in Russia, the proportions of children with RCMP (3,0%), as well as with CMP with a restrictive type of hemodynamics (3,7%) among 668 cases of genetically determined CMPs examined by us, were described, and the relative frequencies and spectrum of mutations causing RCMP were determined. The most frequent genetic RCMP marker in Russian children are mutations of the gene *TNNI3* detected in 40% of cases, while the predominant pathogenic variant that caused the RCMP development is the c.575G>A, p.R192H mutation of the gene *TNNI3*, detected by us in 15% of cases. A significant difference between the mean values of the biomarker NT-proBNP concentration in patients with *MYH7* and *TNNI3* gene mutations (0,0039, p<0,05) may indicate different severity of the disease course. In addition, no significant differences between the values for PGr max blood flow across the pulmonary artery valve in children with *FLNC* and *TNNI3* gene mutations (0,0016 inch, p<0,05), *MYH7* and *TNNI3* (0,039, p<0,05) and with increasing severity of pulmonary hypertension in sequence: *TNNI3→MYH7→FLNC* can be a recommendation for treatment special attention to the data of molecular genetic diagnosis. The study results indicate the significant genetic heterogeneity of RCMP and the need for further studies aimed at finding associations of genotype and phenotype to predict the disease course and select the correct therapy.

Relationships and Activities. The work was carried out within the framework of the state task of the research institute AAAA19-119012590190-6.

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Variant of the *FLNC* gene nucleotide sequence in a family with different phenotypic manifestations of left ventricular non-compaction

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Left ventricular non-compaction is a heterogeneous heart disease with various phenotypic and clinical manifestations. The article presents the results of clinical, instrumental and molecular genetic investigations of a family with diagnosed left ventricular non-compaction (LVNC) with different clinical and phenotypic manifestations. As a result of a molecular genetic testing, all family members with the LVNC phenotype were found to have a likely pathogenic variant in the *FLNC* gene. Variants in this gene are associated with a number of cardiomyopathies: dilated, hypertrophic, and restrictive. In the international scientific literature, isolated clinical cases of LVNC development with variants of the *FLNC* gene nucleotide sequence are presented. In our work, we present a case report of LVNC with a variety of clinical manifestations within the same family.

Keywords: left ventricular non-compaction, atherosclerosis, heart failure, sudden cardiac death, familial forms, thromboembolism, stroke, filamin C, *FLNC*.

Relationships and Activities: none.

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Non-compact left ventricular myocardium (NLVM) — a heterogeneous heart disease characterized by the presence of bilayered myocardial structure and increased trabecularity [1]. Despite the relatively short history of study of this disease, it is obvious that its phenotypic manifestations are extremely diverse. Thus, the study by Towbin JA, et al. identified 8 NLVM phenotypes [1]. The study by Waning JI, et al. identified only 3 phenotypes of the disease and showed genetic diversity of NLVM [2]. Given the pronounced heterogeneity of NLVM, as well as the clinical course variability — the presence of both asymptomatic forms and forms with progressive heart failure (HF), life-threatening arrhythmias and thromboembolic complications, more and more studies are aimed to assess the genotype-phenotype correlation and to assess the disease prognosis [3]. A number of studies have shown a link between NLVM development and variants of nucleotide sequence in the gene *FLNC* [4]. Filamin C (FLNC) is a structural protein that has an actin binding domain and a C-terminal dimerization domain. Expression of filamin C is restricted to the transverse striated muscles and is localized around the Z-disk, sarcolemma, myotendinous junction, and intercalated discs. Its main role is to maintain the sarcomere structural integrity by cross-linking actin filaments and attaching sarcolemmal proteins to cytoskeleton. The main FLNC interacting elements are either part of the Z-disk (myotilin, myosenin, myopodin, and calcarcin) or sarcolemma-associated proteins (integrin β1, sarcoglycan delta). Proteases, such as calpain, can regulate the interaction between FLNC and sarcoglycans by cleaving the corresponding FLNC binding domains [5].

The gene *FLNC* corresponds to chromosome 7q32-35 and has two major transcripts, NM_001127487.2 and NM_001458.4. It consists of 49 coding exons [6]. The difference between two transcripts is the presence or absence of exon 31, which encodes the hinge region between Ig-like domains 15 and 16 [7]. Variants in the gene *FLNC* were initially described in patients with myofibrillar myopathy [8, 9], later, when analyzing a large cohort of patients with cardiomyopathies, the important role of *FLNC* in development of hypertrophic (HCMP) [10] and dilated (DCMP) cardiomyopathies was determined; a few cases of restrictive cardiomyopathy (RCMP) have also been described [11]. In this paper, we want to present a family with NLVM resulting from mutation in the gene *FLNC*, with different phenotypes of the disease and a rather late onset.

Material and methods

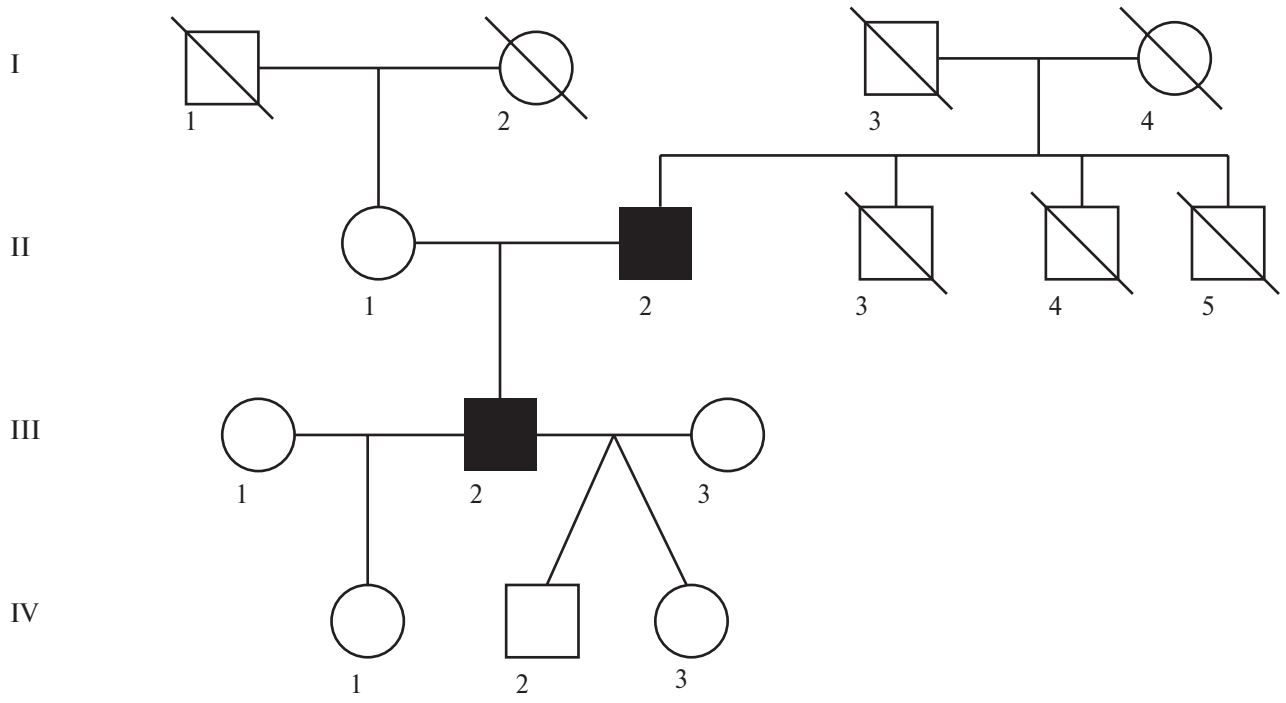
Based on the multicenter register "Non-compact myocardium", a family with a familial form of NLVM was selected (Figure 1). All participants signed an informed consent to participate in the study and to process personal data. The study design was approved by the Ethics Committee of the FSBI "National Medical Research Center for Therapy and Preventive Medicine" of the Ministry of Health of Russia. All participants underwent a clinical and instrumental examination according to the protocol described earlier [12]. The NLVM diagnosis was established based on the criteria of noncompact myocardium by echocardiography (EchoCG) [13] and magnetic resonance imaging (MRI) [14].

Deoxyribonucleic acid (DNA) was isolated using the QIAamp DNA Blood Mini Kit (Qiagen, Germany). DNA concentration was determined on Qubit 4.0 fluorimeter (Thermo Fisher Scientific, USA). The next generation sequencing was performed on Ion S5 device (Thermo Fisher Scientific, USA). Ampliseq libraries were prepared using Ion Chef (Thermo Fisher Scientific, USA) using a custom panel developed by Ion AmpliSeq Designer (Thermo Fisher Scientific, USA). The panel included the sequences of exons of 137 genes associated with NLVM and other types of cardiomyopathies [15]. As a result of sequencing and bioinformatic analysis, files in FASTQ and VCF formats were obtained. Genetic variants with frequencies in the gnomAD database of <1% were selected for clinical interpretation (https://gnomad.broadinstitute.org/) [16]. The variant pathogenicity was assessed in accordance with the ACMG/AMP 2015 recommendations [17]. Validation of the identified variant was performed by Sanger sequencing. The nucleotide sequence of polymerase chain reaction products was determined using an ABI PRISM BigDye Terminator v.3.1 reagent kit followed by analysis of reaction products on an Applied Biosystem 3500 DNA Analyzer (Thermo Fisher Scientific, USA). All sequencing steps were performed according to the manufacturers' protocols.

Results

Proband — a 47-year-old patient with normosthenic physique. Height: 187 cm. Weight: 90 kg. Body mass index: 35,7 kg/m^2.

At the age of 39, in 2013, he first noticed dyspnea on light exercise, general weakness, swollen legs. In January 2014, he had a short-term episode of loss of consciousness, for which he was hospitalized, where EchoCG revealed mitral regurgitation of the 4th degree, dilatation of the left heart, decreased left ventricular ejection fraction (LV EF) to 40%. In this connection, in July 2014, mitral valve prosthetics with a mechanical prosthesis and tricuspid valve plastic surgery according to De Vega were performed. In the early postoperative period, he suffered an ischemic stroke in the basin



I-1	Died at the age of 68, the cause is unknown
I-2	Died at the age of 72, the cause is unknown
I-3	Died at 56, cardiomyopathy, heart failure
I-4	Died at the age of 70, the cause is unknown
II-1	65 years old, disease none
II-2	69 years old, cardiomyopathy, heart failure, cardiac arrhythmias
II-3	Died at 64, cardiomyopathy, heart failure
II-4	Died at 65, myocardial infarction
II-5	Drowned at 2 years old
III-1	40 years old, not examined
III-2	Proband, 42 years old, cardiomyopathy, heart failure, cardiac arrhythmias
III-3	38 years old, not examined
IV-1	12 years old, not examined
IV-2	1 year old, disease none
IV-3	1 year old, disease none

Figure 1. Pedigree.

of the right middle cerebral artery. After surgical treatment, no significant improvement was noted, the phenomena of chronic HF (CHF) persisted against the background of multicomponent CHF therapy. Consulted by a cardiologist at the FSBI "National Medical Research Center for Therapy and Preventive Medicine" of the Ministry of Health of Russia, it is recommended to conduct an MRI of the heart. According to MRI (Figure 2): end-diastolic dimension (EDD) 8,1 cm, in the apex of the left ventricle thickness of compact layer is 5 mm, noncompact layer — 33 mm, in the area of apex and middle segments of anterior, lateral and lower walls the thickness of noncompact layer was 17 mm, compact 8 mm, DCM, noncompact myocardium, EF — 23%, akinesis myocardium of apex location and diffuse hypokinesis. A transmural area of contrast is detected in the medial anterolateral segment. In 2015, he was on inpatient treatment at the FSBI "National Medical Research Center for Therapy and Preventive Medicine": taking into account the presence of CHF, systolic dysfunction, signs of left leg complete bundle branch block (LLBBB) with a QRS complex width of 160 ms, a resynchronizing device with cardioverter defibrillator (CRT-d) function was implanted and HF therapy was adjusted. However, 6 months later, against the background of atrial flutter, his condition worsened in the

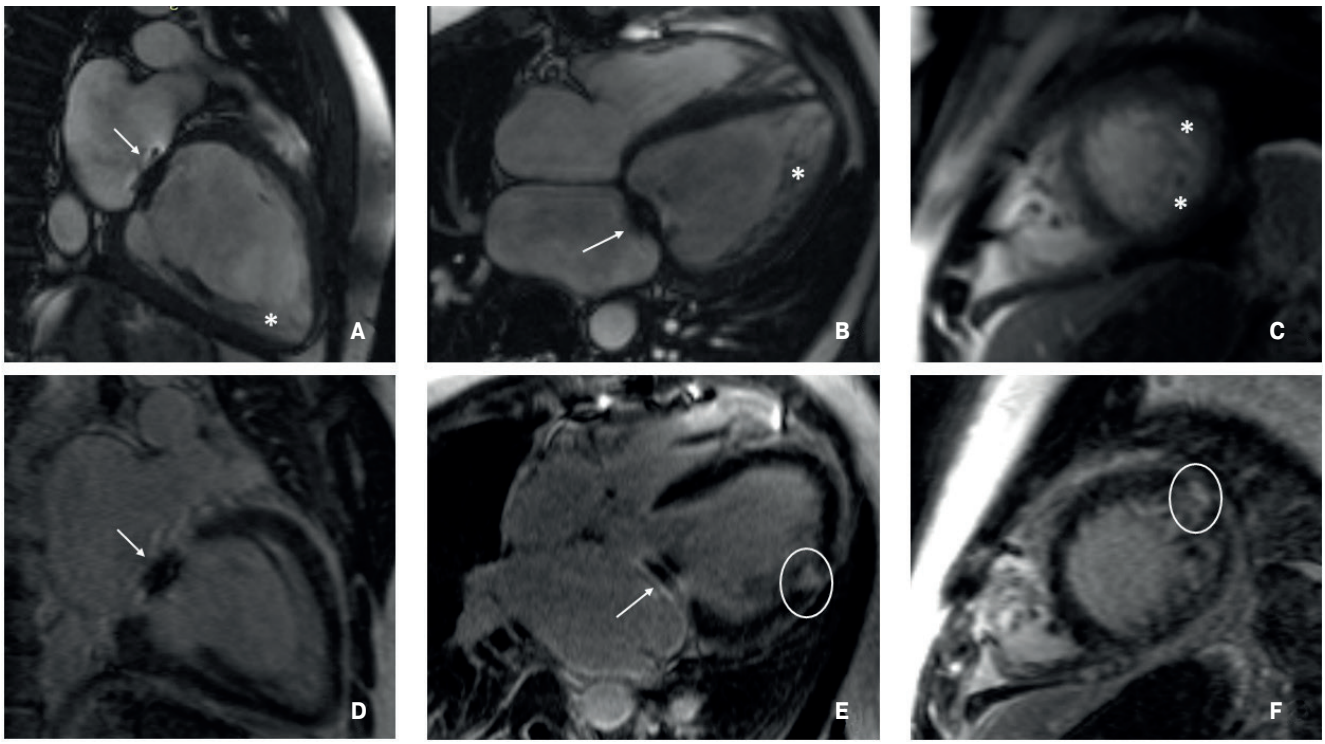


Figure 2. Cardiac MRI, **proband** in cine mode, SSFP-sequencing:
A — long axis, 2-chamber projection, **B** — long axis, 4-chamber projection, **C** — short axis at the level of apical segments.
Notes: the left chamber of the heart significantly expanded (indexed LV EDV — 167 ml/m^2 at the rate of up to 92 ml/m^2), LV EF — 23%; * — noncompact myocardial layer; an arrow indicates the artifact from prosthetic mitral valve; (**D**-**F**) — delayed contrast enhancement, IR sequence with the suppression of signal from myocardium.
A transmural area of contrast (circled) is detected in the medial anterolateral segment.
Abbreviations: EDV — end-diastolic volume, LV — the left ventricle, EF — ejection fraction.

form of decreased tolerance to physical exertion, edema appeared; therefore, the patient underwent radiofrequency ablation of the arrhythmogenic focus in October 2016. After the intervention, the condition is with positive dynamics. According to EchoCG data in 2016: left atrium (LA) — 7,3 cm, EDD — 7,7 cm, interventricular septum thickness (IVST) — 1,2 cm, EF — 42%. He felt satisfactory for a long time. In March 2020, deterioration of the condition due to decompensation of CHF, underwent inpatient treatment at the FSBI "National Medical Research Center for Therapy and Preventive Medicine". EchoCG data showed negative dynamics in the form of sharp increase of heart chambers and decrease of LV systolic function (LV EF — 25%). Holter electrocardiogram monitoring (daily monitoring of ECG) recorded 22 unstable paroxysms of ventricular tachycardia. Drug therapy was adjusted, including the addition of sacubitril/valsartan at a dose of 400 mg/day. At the dynamic examination in 2021, CHF phenomena were relatively compensated, however, according to EchoCG data, the tendency to myocardial remodeling persisted: LV EDD — 9,1 cm, LV EF — 22%, systolic pressure in the pulmonary artery — 58 mm Hg. Taking into account the worsening course, dapagliflozin at a dose of 10 mg was added to the therapy and a waiting list for heart transplantation was recommended.

Proband's father is 75 years old, of normosthenic build, height 178 cm, weight 74 kg.

From the age of 40, he noted an increase in blood pressure to 200/120 mm Hg, from the age of 52, had a constant form of atrial fibrillation (AF), CHF phenomena in the form of shortness of breath during exercise, edema of the lower extremities. Signs of focal changes were recorded on the electrocardiogram (ECG). In 2006, at the age of 60, an acute visual impairment occurred against the background of increased blood pressure up to 200/110 mm Hg, an acute violation of cerebral circulation was diagnosed. In August 2015, as part of a family screening, he underwent a follow-up examination at the FSBI "National Medical Research Center for Therapy and Preventive Medicine". In the tests: N-terminal brain natriuretic peptide 345 (0-125) pg/ml, hemoglobin 105 g/l, D-dimer 847 ng/ml (0-255), creatinine 153 mmol/l. On ECG: AF with a heart rate of 47-100, signs of LV

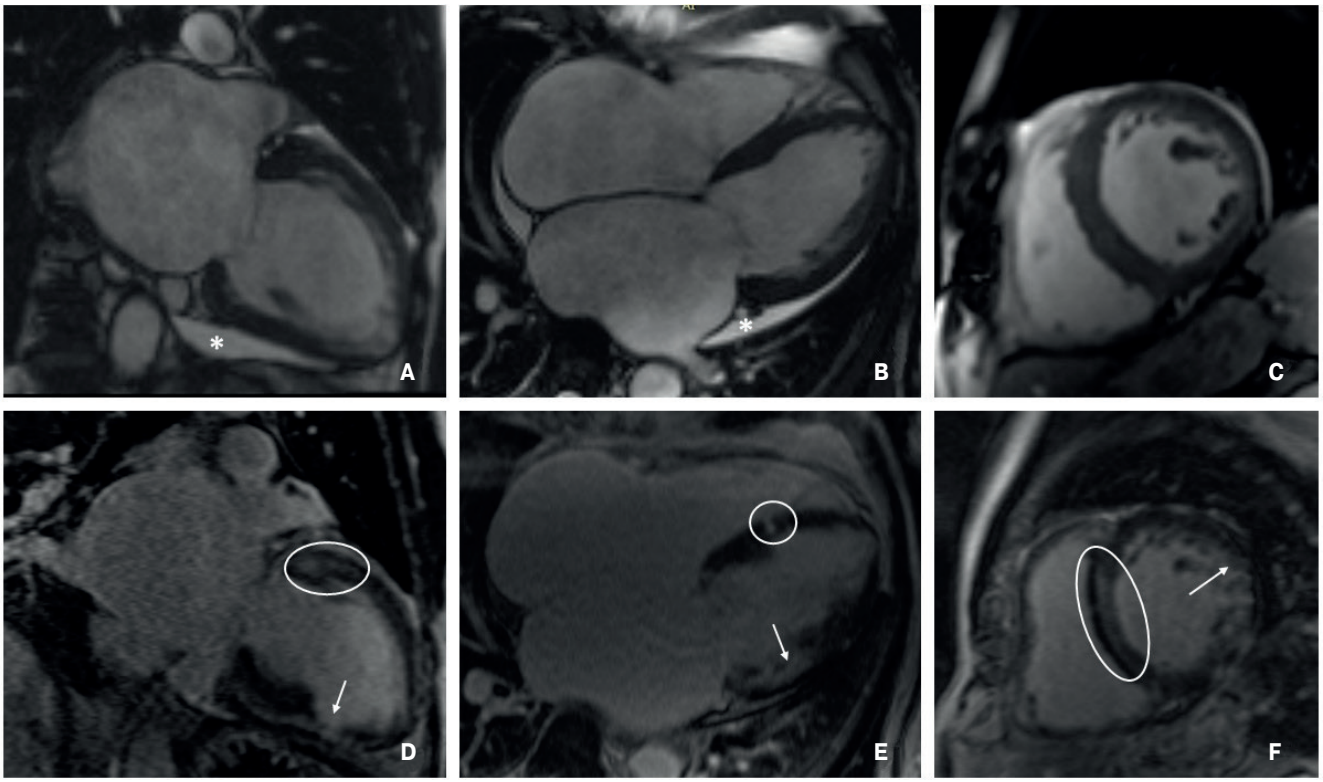


Figure 3. MRI of the **father**'s heart, **proband** in cine mode, SSFP sequence:
A — long axis, 2-chamber projection, **B** — long axis, 4-chamber projection, **C** — short axis at the level of apical segments.
Notes: significant atrial dilation (LA 7,7×7,2 cm, RA — 11,5×8,0 cm), moderate dilation of LV cavity (LV EDD — 6,3 cm, LV EDV — 263 ml), LV EF — <20%; * — moderate hydropericardium; (**D**-**F**) — delayed contrast, IR sequencing with myocardial signal suppression.
The basal and middle septal segments show areas of intramyocardial contrast of non-coronary nature (circled); the basal anterolateral and middle inferior segments show scar changes (indicated by arrows).
Abbreviations: EDV — end-diastolic volume, EDD — end-diastolic dimension, LV — left ventricle, LA — left atrium, RA — right atrium, EF — ejection fraction.

hypertrophy, LLBBB. According to XM-ECG data: AF of 45-144 per minute, transient LLBBB, 215 runs of ventricular tachycardia from 3-4-5-6 complexes. According to EchoCG data: LA — 6,2 cm, EDD — 6,2 cm, IVST — 1,1 cm, LV EF — 42%, zones of non-compact myocardium in the apex, anterior and lateral walls, Stolberger criterion. According to MRI of the heart (Figure 3): EF — 12%, IVST — 1,4 cm, EDV 263 ml; in the area of the apex, apical segments of the anterior and lateral walls, increased trabecularity with a ratio of compact and non-compact layer >2. After contrast agent injection, the basal and middle septal segments show areas of intramyocardial contrast of non-coronogenic nature; basal anterolateral and middle inferior segments show cicatricial changes; marked mitral regurgitation, tricuspid regurgitation, significant dilation of both atria (restrictive cardiomyopathy pattern, NLVM syndrome). According to multispiral computed tomography of coronary arteries: stenosing atherosclerosis with a three-vessel disease. Blood clot in the LA atrial appendage. During coronary angiography: stenosis of the proximal segment of anterior interventricular artery — 80%, stenosis of the middle segment of right coronary artery — 80%, angioplasty with stenting of the anterior interventricular artery and the right coronary artery was performed. After discharge, he felt satisfactory and regularly took the recommended therapy. In the period from 2016 to 2020, he was repeatedly hospitalized for HF decompensation. According to EchoCG data dated March 2021: LA — 7,5 cm, EDD — 6,5 cm, IVST — 1,4 cm, EF — 37%, systolic pulmonary artery pressure — 55 mm Hg, hypokinesis of apical, middle, basal, anterior septal, anterior segments. Currently on therapy: sacubitril/valsartan, dapagliflozin, rosuvastatin, bisoprolol, spironolactone, furosemide, apixaban.

The proband's mother is 65 years old, underwent a comprehensive cardiological and neurological examination. According to the results of EchoCG, there were no signs of non-compact myocardium. IVST — 1,0 cm, EDD — 5,2 cm. No data were obtained for the presence of myopathy.

Son proband 3 years was performed echocardiography, the results of which revealed signs of noncompact myocardium in the area of the apex of the LV.

Proband's 3-year-old daughter also underwent an echocardiogram, according to the results of which no pathology was detected.

Genetic analysis. Genetic analysis by next-generation sequencing in three family members (II-2, III-2, IV-2) revealed an rs1554398369 variant in the gene *FLNC* (hg19:chr7:128481344, NM_001458.5:c.1934A>C, NP_001449.3:p.Asp645Ala). Based on the ACMG/AMP pathogenicity criteria 2015 (Richards et al., 2015), this nucleotide sequence variant was classified as a probable-pathogenic variant (class IV of pathogenicity). In Sanger sequencing, the nucleotide sequence variant rs1554398369 in the gene *FLNC* was confirmed in all three family members (II-2, III-2, IV-2).

Discussion

In the last few years, due to the progressive development of genetic research and the accumulation of knowledge in the field of cardiomyopathy research, more and more attention has been paid to various phenotypic manifestations of cardiomyopathies with variants in the same gene. Within 5 years, a number of papers have been published describing variants in the gene *FLNC* in various cardiopathies. In 2017, Gomez et at. published a paper showing the importance of the gene *FLNS* in HCMP development [10]. 448 patients with HCMP who underwent NGS for the genes *MYH7*, *MYBPC3*, *TNNT2*, *TNNI3*, *ACTC1*, *TNNC1*, *MYL2*, *MYL3*, *TPM1* and *FLNC* were examined. After that, 20 variants of *FLNC* candidates were identified in 22 patients. Based on familial segregation and performed functional studies, 6 of the possible variants (in 7 patients) were definitively classified as probable pathogenic, 10 as variants of uncertain significance, and 4 as probable benign. This was the basis for assuming the influence of *FLNC* as a cause of HCMP development. Later, in the study by Cirino AI, et al., 41 patients with HCMP underwent genetic testing, and a variant in the gene *FLNC* was identified in 1 patient [18].

In addition to HCMP development, a number of studies have demonstrated the relationship between variants in the gene *FLNC* with DCM development, for example, in the study by Ader F, et al. 2019 surveyed 1150 patients with various cardiomyopathy (700 HCMP, 300 DCM, 50 RCMP and 100 NLVM), and 28 patients were identified pathogenic variants in the gene *FLNC* (13 patients with HCMP, 10 with DCM, 4 with RCMP and 1 with NLVM). At the same time, missense variants in the gene *FLNC* led to HCMP development, and nonsense variants led to DCM development [5]. There are significantly fewer studies devoted to the combination of NLVM with variants of the gene *FLNC* [4]: in a 2018 study, a likely pathogenic variant in the gene *FLNC* was identified in one patient with NLVM.

Our work presents a family with proven NLVM in the absence of neurological manifestations and myopathy clinic. Attention is drawn to the burdened inheritance on the paternal side for cardiovascular diseases, with a sufficiently favorable course of the disease in the proband's father. The proband's father did not exhibit clinical symptoms of the disease until he was over the age of 60, and there was no regular medication therapy. It is also worth noting the presence of pronounced restriction and marked fibrosis in the proband's father, which is characteristic of pathogenic variants in the gene *FLNC* [19]. On the contrary, no data for restriction were obtained in the proband, i.e., one can speak about the presence of different NLVM phenotypes within one family, which was demonstrated in our previously published work [12]. The disease in the proband debuted with clinics of CH and progressed after surgical intervention on the mitral valve, which is probably caused by altered myocardial structure, which, in turn, could be the cause of poor response to resynchronization therapy.

The presence of thromboembolic complications (acute cerebrovascular accident, LV cavity thrombosis) in both patients is noteworthy, which may be due to several reasons: the presence of direct noncompaction layer, decreased PV, as well as FP. In this case, there is a set of reasons that led to development of thromboembolic complications.

Given that *FLNC* variants lead to such a variety of clinical manifestations, it is worth mentioning that it is poorly studied, so a more detailed study of mutations in the gene, as well as the identification of links between the genotype and phenotype, will help to identify the connection and predict the development of the disease and its consequences, as well as to develop targeted therapies.

Conclusion

This paper presents a case of familial form of cardiomyopathy in patients with a nucleotide sequence variant in the gene *FLNC*. Proband and his relatives had various phenotypic manifestations of NLVM. The obtained clinical and molecular genetic data confirm the genetic and phenotypic heterogeneity of NLVM, with features characteristic of pathogenic variants in the gene *FLNC*. The study of familial forms of cardiomyopathies expands the information on the disease genesis and confirms the necessity of family screening in order to carry out timely measures aimed at the prevention of cardiovascular complications.

Relationships and Activities: none.

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Clinical features of post-COVID-19 period. Results of the international register "Dynamic analysis of comorbidities in SARS-CoV-2 survivors (AKTIV SARS-CoV-2)". Data from 6-month follow-up

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Aim. To study the clinical course specifics of coronavirus disease 2019 (COVID-19) and comorbid conditions in COVID-19 survivors 3, 6, 12 months after recovery in the Eurasian region according to the AKTIV register.

Material and methods. The AKTIV register was created at the initiative of the Eurasian Association of Therapists. The AKTIV register is divided into 2 parts: AKTIV 1 and AKTIV 2. The AKTIV 1 register currently includes 6300 patients, while in AKTIV 2 — 2770. Patients diagnosed with COVID-19 receiving in- and outpatient treatment have been anonymously included on the registry. The following 7 countries participated in the register: Russian Federation, Republic of Armenia, Republic of Belarus, Republic of Kazakhstan, Kyrgyz Republic, Republic of Moldova, Republic of Uzbekistan. This closed multicenter register with two non-overlapping branches (in- and outpatient branch) provides 6 visits: 3 in-person visits during the acute period and 3 telephone calls after 3, 6, 12 months. Subject recruitment lasted from June 29, 2020 to October 29, 2020. Register will end on October 29, 2022. A total of 9 fragmentary analyzes of the registry data are planned. This fragment of the study presents the results of the post-hospitalization period in COVID-19 survivors after 3 and 6 months.

Results. According to the AKTIV register, patients after COVID-19 are characterized by long-term persistent symptoms and frequent seeking for unscheduled medical care, including rehospitalizations. The most common causes of unplanned medical care are uncontrolled hypertension (HTN) and chronic coronary artery disease (CAD) and/or decompensated type 2 diabetes (T2D). During 3- and 6-month follow-up after hospitalization, 5,6% and 6,4% of patients were diagnosed with other diseases, which were more often presented by HTN, T2D, and CAD. The mortality rate of patients in the post-hospitalization period was 1,9% in the first 3 months and 0,2% for 4-6 months. The highest mortality rate was observed in the first 3 months in the group of patients with class II-IV heart failure, as well as in patients with cardiovascular diseases and cancer. In the pattern of death causes in the post-hospitalization period, following cardiovascular causes prevailed (31,8%): acute coronary syndrome, stroke, acute heart failure.

Conclusion. According to the AKTIV register, the health status of patients after COVID-19 in a serious challenge for healthcare system, which requires planning adequate health system capacity to provide care to patients with COVID-19 in both acute and post-hospitalization period.

Keywords: COVID-19, AKTIV register, cardiovascular diseases, diabetes, post-COVID-19 period.

Relationships and Activities: none.

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To study the features of coronavirus disease 2019 (COVID-19) course and changes in comorbid conditions in patients 3, 6, 12 months after recovery from COVID-19 in the Eurasian region, the international register "Dynamic analysis of comorbidities in SARS-CoV-2 survivors (AKTIV SARS-CoV-2)" was created [1], which included specialists from 7 following countries: the Russian Federation, the Republic of Armenia, the Republic of Belarus, the Republic of Kazakhstan, the Kyrgyz Republic, the Republic of Moldova, the Republic of Uzbekistan. The AKTIV register was divided into 2 parts: AKTIV 1 and AKTIV 2 [2]. Currently, the AKTIV 1 register includes 6300 patients, while the AKTIV 2 (analysis of the COVID-19 2nd wave) — 2770. In total, 9 fragment analyzes of the register data are planned. This fragment of the study presents the data from 3 and 6-month follow-up of patients after hospitalization due to COVID-19.

Material and methods

The study design and statistical processing methods were published earlier [2]. The register provides 6 visits: 3 in-person visits during the acute period and 3 telephone calls after 3, 6, 12 months. Patient recruitment started on June 29, 2020 and completed on October 29, 2020. Completion of the register on October 29, 2022. ClinicalTrials.

Table 1

Clinical characteristics of patients in the AKTIV register within 6-month follow-up after hospitalization, n=2256

Comorbid disease or risk factor	6-month follow-up
HTN, %	53,0
Obesity, %	27,7
CAD, %	16,4
T2D, %	15,4
HF, %	9,8
CKD, %	6,0
AF, %	4,7
Cancer, %	4,4
Asthma, %	4,4
COPD, %	4,3
Prior myocardial infarction, %	4,2
Prior stroke	2,7
T1D, %	0,5
Hepatitis, %	0,5

Abbreviations: HTN — hypertension, CAD — coronary artery disease, MI — myocardial infarction, T1D — type 1 diabetes, T2D — type 2 diabetes, HF — heart failure, CKD — chronic kidney disease, AF — atrial fibrillation, COPD — chronic obstructive pulmonary disease.

Table 2

Persistent symptoms in the post-hospitalization period

Symptom	3 months, n=2185	6 months, n=1208
Weakness, %	30,9	21,1
Shortness of breath, %	28,3	19,0
BP increase, %	18,6	19,1
Heartbeat, %	11,2	5,8
Cough, %	7,9	4,9
Chest pain, %	4,8	3,9
Loss of taste and smell, %	2,9	1,4

Abbreviation: BP — blood pressure.

Table 3

Reasons for seeking unscheduled health care in the post-hospitalization period

Reasons for seeking care	3 months, n1*=638	6 months, n2**=361
Uncontrolled HTN, %	40,2	37,1
T2D decompensation, %	13,2	10,6
CAD destabilization, %	10,3	9,7
Digestive disease, %	7,5	8,4
Cancer, %	4,8	3,8
Asthma exacerbation, %	3,1	1,3
HF decompensation, %	3,1	1,3
Atrial fibrillation, %	2,9	1,9
URTI symptoms, %	2,3	3,9
CKD destabilization, %	1,5	1,9
COPD exacerbation, %	1,5	1,0
Hypothyroidism, %	1,5	1,6
Stroke, %	1,2	0,3
Arthritis, %	1,2	3,9
DVT, %	0,8	0,3
T1D decompensation, %	0,6	0,3
Viral hepatitis, %	0,4	0,0
HIV infection, %	0,4	0,0
PE, %	0,2	0,0
MI, %	0,0	1,0

Note: * — n1, number of patients seeking medical care after 3 months; ** — n2, number of patients seeking medical help after 6 months.
Abbreviations: HTN — hypertension, HIV — human immunodeficiency virus, CAD — coronary artery disease, MI — myocardial infarction, URTI — upper respiratory tract infection, T1D — type 1 diabetes, T2D — type 2 diabetes, DVT — deep vein thrombosis, PE — pulmonary embolism, HF — heart failure, CKD — chronic kidney disease, AF — atrial fibrillation, COPD — chronic obstructive pulmonary disease.

gov identifier: NCT04492384. Control telephone interviews were planned for 3500 patients included in AKTIV 1. Control telephone calls were made to 3007 patients after 3 months and 2011 patients after 6 months. Telephone interviews with patients go on. Out of 3000 patients after 3 months, 432 did not answer the control call (14,4%), while 383 (12,8%) answers were regarded as incorrect (during the telephone survey, <50% of the answers were received). Out of 2000 telephone calls made after 6 months, 398 (19,9%) received no response and 394 (19,7%) received incorrect answers. Thus, the analysis is carried out using data from 2185 telephone interviews after 3 months and 1208 — after 6 months. A total of 2256 patients were interviewed, of which 2185 patients were interviewed after 3 months and 1137 of them were re-interviewed after 6 months, while 71 patients were interviewed only after 6 months. A standard form for phone survey is presented at https://activ.euat.ru/documents.

Table 4A

Newly diagnosed diseases in the post-COVID-19 period in relation to the total cohort of patients

Disease	3 months, n=2185	6 months, n=1208
HTN, %	2,3	3,0
T2D, %	1,4	0,7
CAD, %	0,5	1,4
AF, %	0,3	0,3
Arthritis, %	0,3	0,3
Stroke, %	0,2	0,2
Asthma, %	0,2	0,1
Cancer, %	0,1	0,1
HF, %	0,04	0,1
MI, %	0,04	0,2
CKD, %	0,04	0,0
T1D, %	0,04	0,0

Abbreviations: HTN — hypertension, CAD — coronary artery disease, MI — myocardial infarction, T1D — type 1 diabetes, T2D — type 2 diabetes, CKD — chronic kidney disease, HF — heart failure, AF — atrial fibrillation.

Table 4C

Pattern of newly diagnosed diseases in the post-COVID-19 period

Disease	3 months, n=123	6 months, n=77
HTN, %	41,5	46,7
T2D, %	25,2	10,4
CAD, %	9,7	22,1
AF, %	5,7	5,2
Arthritis, %	4,9	5,2
Stroke, %	4,0	2,6
Asthma, %	3,2	1,3
Cancer, %	2,4	1,3
HF, %	0,8	1,3
MI, %	0,8	3,9
CKD, %	0,8	0,0
T1D, %	0,8	0,0

Abbreviations: HTN — hypertension, CAD — coronary artery disease, MI — myocardial infarction, T1D — type 1 diabetes, T2D — type 2 diabetes, CKD — chronic kidney disease, HF — heart failure, AF — atrial fibrillation.

Table 4B

The incidence of newly diagnosed diseases per 100 thousand population in comparison with the incidence in the Russian population in 2018 [3]

Disease	Incidence 3 months/12 months (recalculation per 100000 population)	Incidence 4-6 months/12 months (recalculation per 100000 population)	Incidence of the Russian population for 12 months in 2018 (newly diagnosed diseases per 100000 population)
HTN (n/100000)	2334,0/9336	2980,0/11920,0	1047,9
CAD (n/100000)	549,2/2196,8	1407,3/5629,2	710,2
MI (n/100000)	45,8/183,2	248,3/993,2	138,2
Diabetes (n/100000)	1464,5/5858,0	662,2/2649,2	251,7
Cancer (n/100000)	137,3/549,2	82,8/331,2	425,5

Abbreviations: HTN — hypertension, CAD — coronary artery disease, MI — myocardial infarction.

The diagnosis was made based on the ICD-10 criteria.

Results

The mean age of patients (n=2256) was 55,27±13,00 years (men, 43,7%). More than half of the patients had hypertension (HTN), almost 1/3 of patients — obesity, almost every sixth patient — coronary artery disease (CAD) and or type 2 diabetes (T2D), every tenth patient — heart failure (HF) (Table 1). There were somewhat less common diseases such as chronic kidney disease (CKD), atrial fibrillation (AF), cancer, asthma, chronic obstructive pulmonary disease (COPD), prior stroke, type 1 diabetes mellitus (T1D) and hepatitis (Table 1).

In the post-hospitalization period, many patients continued to have various complaints (Table 2). After 3-month follow-up, at least 1 symptom persisted in 38,2% of patients, and after 6 months — in 27,7%. The most common symptoms that persisted in patients up to the 3rd and 6th months were weakness and shortness of breath. These symptoms were observed in every third patient after 3 months and every fifth after 6 months. Attention was drawn to the fact that in the first 3 months, many patients (18,6%) with previously effective antihypertensive therapy complained of blood pressure increase, as well as palpitations (11,2%) (Table 2). Less commonly, patients experienced prolonged chest pain and loss of taste and smell.

Table 5

Comparative analysis of patients with and without newly diagnosed diseases, 6-nonth follow-up (n=2256)

Parameter	Patients without newly diagnosed diseases, n=1959	Patients with newly diagnosed diseases, n=297	U-test t-test p-value
Men, %	44,97	42,42	0,410
Age, years M±σ	54,4±14,75	56,14±11,26	0,050
Age <40 years, %	18,13	6,78	0,000
Age of 40-59 years, %	42,39	54,24	0,000
BMI ⩾30 kg/m^2, %	26,39	36,7	0,000
Obesity with age <60 years, %	14,8	25,34	0,000
T <37 in the acute period, 0° C, %	7,61	4,05	0,043
RR of 22-29 bpm in the acute period, %	27,7	34,2	0,042
hsCRP ⩽10 mg/L in the acute period, %	27,59	17,45	0,001
hsCRP >40 mg/L in the acute period, %	38,13	53,62	0,000
Myocarditis in the acute period, %	0,1	1,05	0,002
SpO_2 in the acute period, %, M±σ	95,53±3,41	94,64±3,43	0,000
Lymphocytes in the acute period, %, M±σ	23,28±13,52	17,9±15,85	0,000
hsCRP in the acute period, mg/L, M±σ	48,01±78,69	56,93±51,65	0,000
Glucose in the acute period, mmol/l, M±σ	6,32±2,57	6,64±2,68	0,010
Fibrinogen in the acute period, g/l, M±σ	4,78±1,64	5,33±1,59	0,000

Note: with a standard deviation >30% of the mean, the significance was determined by the nonparametric U-test (Mann-Whitney test).
Abbreviations: hsCRP — high-sensitivity C-reactive protein, BMI — body mass index, T — temperature, RR — respiratory rate, SpO_2 — blood oxygen saturation.

Table 6

Mortality of patients in the 3- and 6- month post-hospitalization period depending on comorbidity

Diseases	3 months, n=2185			6 months, n=1208		
Whole group, n/%	41/1,9			3/0,2		
	+ CVDs	- CVDs	p	+ CVDs	- CVDs	p
CVDs, n/%	34/3,2	7/0,6	0,0001	1/0,2	2/0,17	0,724
Class II-IV HF, n/%	13/8,7	28/1,4	0,0001	0/0,0	3/0,26	0,624
COPD and/or asthma, n/%	4/2,6	2/1,8	0,521	1/1,75	2/0,18	0,020
Cancer, n/%	5/5,7	36/1,7	0,008	0/0,0	3/0,25	0,741

Abbreviations: CVDs — cardiovascular diseases, HF — heart failure, COPD — chronic obstructive pulmonary disease.

Almost a third of patients (29,2%) after hospitalization receive unscheduled health care, and within the first 3 months, at least 2 times. Outpatient health care was sought after 3 and 6 months by 29,2% and 29,9% of patients, respectively. In addition, 4,2% and 4,4% of patients were hospitalized after 3 and 6 months. Also, 2,5% and 2,3% of patients contacted with an ambulance service within 3 and 6 months. In patients who receive unscheduled health care within 3 (n=638) and 6 months (n=361) the most frequent reason for treatment was uncontrolled HTN (40,2% and 37,1%) (Table 3). Almost every tenth patient who applied for medical care complained about chronic CAD and/or type 2 diabetes decompensation. Somewhat less often, the

Table 7

Death causes in patients in the 3- and 6-month post-hospitalization

Death causes	3 months, n=41	6 months, n=3
ACS, %	20,5	
Stroke, %	4,5	
ACF, %	6,8	
Cancer, %	6,8	
Pneumonia, %	9,1	33,4
PE, %	2,3	
Other reasons, %	27,3	66,6
Unknown, %	22,7	

Abbreviations: ACS — acute coronary syndrome, AHF — acute heart failure, PE — pulmonary embolism.

Table 8

Comparative analysis of deceased and surviving patients within 3 months depending on sex and comorbidity

Parameter	Deceased patients, N=41	Surviving patients, N=2144	P	OR (95% CI)
Men, %	36,59	45,2	0,272	0,700 (0,368-1,328)
Age of 40-59 years, %	7,32	44,84	0,000	0,097 (0,030-0,316)
Age of 60-80 years, %	53,66	35,78	0,018	2,078 (1,118-3,864)
Age >80 years, %	39,02	2,62	0,000	23,829 (12,056-47,097)
Male age ⩾60, %	34,15	15,69	0,001	2,785 (1,446-5,367)
Female age ⩾60, %	58,54	22,7	0,000	4,808 (2,562-9,022)
HTN, %	82,93	47,16	0,000	5,442 (2,402-12,330)
HTN, ⩾60 years, %	78,05	28,65	0,000	8,857 (4,202-18,665)
HTN, <60 years, %	4,88	18,47	0,026	0,226 (0,054-0,942)
Smoking, %	7,32	5,3	0,569	1,411 (0,429-4,642)
Obesity, BMI ⩾30 kg/m^2, %	24,39	27,8	0,629	0,838 (0,408-1,720)
Obesity, ⩾60 years, %	21,95	11,2	0,032	2,230 (1,052-4,729)
Obesity, <60 years, %	2,44	16,57	0,015	0,126 (0,017-0,919)
BMI <18,5 kg/m^2, %	8,33	0,53	0,000	17,030 (3,477-83,416)
BMI ⩾40 kg/m^2, %	4,17	3,13	0,772	1,347 (0,179-10,161)
AF, %	19,51	3,93	0,000	5,932 (2,658-13,241)
AF, ⩾60 years, %	19,51	3,13	0,000	7,511 (3,340-16,891)
Coronary artery disease, %	24,39	10,41	0,004	2,777 (1,343-5,742)
History of myocardial infarction, %	12,2	3,36	0,002	3,996 (1,523-10,489)
Class II-IV HF, %	31,71	7,99	0,000	5,343 (2,717-10,508)
Prior stroke, %	12,2	2,18	0,000	6,244 (2,343-16,636)
T2D, %	21,95	13,62	0,125	1,783 (0,842-3,775)
T2D, ⩾60 years, %	19,51	8,67	0,016	2,554 (1,162-5,611)
T2D, <60 years, %	2,44	4,88	0,471	0,487 (0,066-3,580)
CKD, %	21,95	4,54	0,000	5,912 (2,745-12,735)
CKD, ⩾60 years, %	19,51	3,08	0,000	7,631 (3,392-17,169)
CKD, <60 years, %				
GFR ⩾90 ml/min/1,73 m^2, %	5,56	28,49	0,002	0,148 (0,035-0,617)
GFR of 89,9-60 ml/min/1,73 m^2, %	27,78	51,45	0,005	0,363 (0,174-0,757)
GFR 59,9-45 ml/min/1,73 m^2, %	30,56	13,71	0,004	2,770 (1,345-5,705)
GFR 44,9-30 ml/min/1,73 m^2, %	22,22	4,45	0,000	6,133 (2,704-13,914)
GFR 29,9-15 ml/min/1,73 m^2, %	8,33	0,77	0,000	11,692 (3,181-42,980)
COPD, %	4,88	3,36	0,594	1,476 (0,349-6,232)
COPD, ⩾60 years, %	4,88	2,79	0,426	1,784 (0,421-7,561)
Active cancer, %	12,2	3,93	0,008	3,399 (1,300-8,883)
Cancer ⩾60 years, %	12,2	2,46	0,000	5,499 (2,074-14,581)
Anemia, %	50,0	18,69	0,000	4,350 (2,279-8,302)

Abbreviations: HTN — hypertension, CI — confidence interval, CAD — coronary artery disease, MI — myocardial infarction, BMI — body mass index, OR — odds ratio, T2D — type 2 diabetes mellitus, GFR — glomerular filtration rate, AF — atrial fibrillation, HF — heart failure, CKD — chronic kidney disease, COPD — chronic obstructive pulmonary disease.

reasons for treatment were gastrointestinal disease, cancer, asthma exacerbation, decompensated HF, AF, and upper respiratory tract infection. Even less common were treatment for CKD, COPD exacerbation, hypothyroidism and arthritis. The rarest reasons for seeking medical help were deep

Table 9

Comparative analysis of deceased and surviving patients within 3 months depending on multimorbidity characteristics

	Deceased patients, N=41	Surviving patients, N=2144	P	OR (95% CI)
No comorbidities, %	10,0	39,04	0,001	0,174 (0,052-0,574)
1 comorbidity, %	6,67	28,39	0,009	0,180 (0,043-0,759)
2-3 comorbidities, %	40,0	24,69	0,055	2,033 (0,971-4,258)
⩾4 comorbidities, %	43,33	7,88	0,000	8,938 (4,246-18,814)
No comorbidities, ⩾60 years, %	6,67	5,3	0,742	1,276 (0,299-5,444)
No comorbidities, <60 years, %	3,23	37,05	0,000	0,057 (0,008-0,417)
2-3 comorbidities, ⩾60 years, %	33,33	13,63	0,002	3,170 (1,464-6,861)
2-3 comorbidities, <60 years, %	6,67	11,1	0,442	0,572 (0,135-2,422)
⩾4 comorbidities, ⩾60 years, %	43,33	6,54	0,000	10,937 (5,174-23,118)
HTN+CAD, %	9,76	2,41	0,003	4,373 (1,502-12,728)
HTN+HF, %	31,71	7,28	0,000	5,909 (3,000-11,640)
HTN+CAD+HF, %	24,39	5,72	0,000	5,313 (2,545-11,092)

Abbreviations: HTN — hypertension, CI — confidence interval, CAD — ischemic heart disease, OR — odds ratio, HF — heart failure.

Table 10

Comparative analysis of deceased and surviving patients within 3 months depending on the acute COVID-19 course characteristics

Parameter	Deceased patients, N=41	Surviving patients, N=2144	P	OR (95% CI)
CT 0, %	17,65	10,61	0,190	1,806 (0,737-4,425)
CT 1-2, %	61,76	78,6	0,019	0,440 (0,218-0,888)
CT 3-4, %	20,59	10,8	0,071	2,142 (0,919-4,994)
RR ⩾30 bpm, %	5,88	1,69	0,068	3,630 (0,828-15,918)
SpO_2 ⩾95%, %	78,05	89,26	0,023	0,428 (0,202-0,908)
SpO_2 of 75-94%, %	21,95	10,74	0,023	2,338 (1,102-4,959)
hsCRP ⩽10 mg/L, %	8,11	26,65	0,011	0,243 (0,074-0,795)
hsCRP >40 mg/L, %	62,16	39,64	0,006	2,502 (1,278-4,897)
DVT in the acute period, %	2,44	0,33	0,029	7,471 (0,898-62,153)
AKI in the acute period, %	2,44	0,29	0,017	8,721 (1,026-74,122)

Abbreviations: hsCRP — high-sensitivity C-reactive protein, CI — confidence interval, CT — computed tomography, AKI — acute kidney injury, OR — odds ratio, DVT — deep vein thrombosis, RR — respiratory rate, SpO_2 — blood oxygen saturation.

vein thrombosis (DVT), T1D decompensation, viral hepatitis, HIV infection, pulmonary embolism (PE), and myocardial infarction (MI) (Table 3).

During 3- and 6-month follow-up, 5,6% and 6,4% of patients had newly diagnosed diseases (Tables 4A, 4B, 4C). Incidence rate of newly diagnosed HTN, CAD, myocardial infarction and diabetes in patients after COVID-19 was significantly higher compared to Russian population level in 2018; however, approximately the same level of newly diagnosed cancer incidence rate was revealed (Table 4B) [3].

Among patients with newly reported diseases after 3- and 4-6-month follow-up, patients with HTN predominated, which accounted for 41,5% and 46,7% in the structure of newly diagnosed diseases. It is noteworthy that the proportion of hypertensive patients increased during 4-6-month follow-up compared with the first 3 months. In addition, the proportion of patients with newly diagnosed CAD during 4-6-month follow-up (22,1%) compared to 3 months (9,7%). For 4-6 months there were more myocardial infarction cases than in the first 3 months (3,9% vs 0,8%). A similar pattern was observed for

Table 11

Comparative analysis of deceased and surviving patients within 3 months depending on the data from acute COVID-19 course

Parameter	Deceased patients, N=41	Surviving patients, N=2144	P
Age, years	73,2±14,38	53,28±13,5	0,000
RR, breaths per minute	21,1±4,77	19,51±3,01	0,020
HR, beats per minute	88,83±14,55	85,31±12,61	0,040
SpO_2, %	92,34±5,26	95,45±3,38	0,000
Hb, g/l	120,68±23,27	135,83±18,18	0,000
Lymphocytes, %	15,64±9,51	22,57±14,03	0,000
hsCRP, mg/L	76,82±65,85	47,3±68,77	0,350
D-dimer, FEU/ml	107,86±287,2	16,94±134,41	0,010
GFR, ml/min/1,73 m^2	53,59±21,2	77,02±20,83	0,000
Troponin I, ng/ml	81,04±141,32	0,32±2,17	0,000
Potassium, mmol/l	3,88±0,51	4,27±0,59	0,000

Abbreviations: hsCRP — high-sensitivity C-reactive protein, GFR — glomerular filtration rate, RR — respiratory rate, HR — heart rate, Hb — hemoglobin, SpO_2 — blood oxygen saturation.

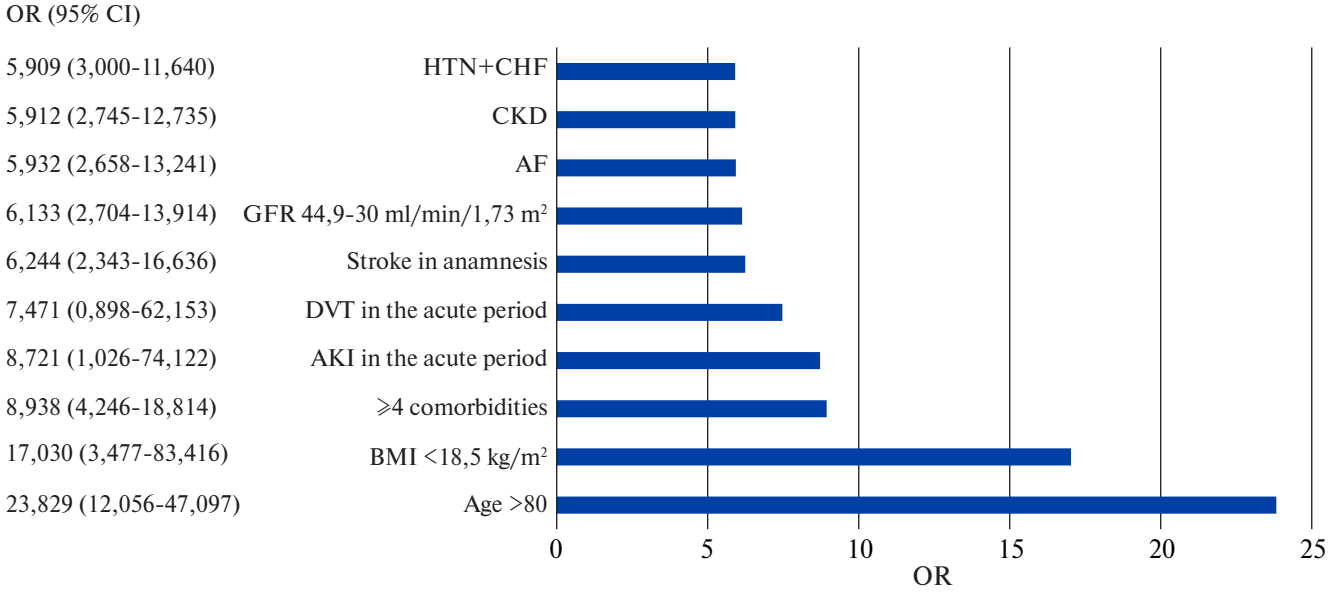

Figure 1. Ten main death RFs in the early 3-month post-hospitalization period.
Abbreviations: HTN — hypertension, CI — confidence interval, BMI — body mass index, AKI — acute kidney injury, OR — odds ratio, GFR — glomerular filtration rate, DVT — deep vein thrombosis, AF — atrial fibrillation, CKD — chronic kidney disease, CHF — chronic heart failure.

arthritis, the proportion of which was higher for 4-6-month follow-up (5,2%) in comparison with the first 3 months (4,9%), as well as for newly diagnosed HF, which was registered in 0,8% in the first 3 months and 1,3% during 4-6-month follow-up. The ratio of other newly diagnosed diseases has changed in the opposite direction. So, the incidence rate of T2D, AF, stroke, asthma, cancer, CKD and type 1 diabetes was lower in the period of 4-6 months compared with the first 3 months (Table 4B).

Despite the fact that patients with newly diagnosed diseases were older than those without them, (Table 5) there were more people aged 40-59 in their group in percentage terms. Obesity was more common in patients with newly diagnosed diseases. It was noteworthy that patients with newly diagnosed diseases suffered from more severe COVID-19 with higher fever, respiratory rate (RR), high-sensitivity C-reactive protein (hsCRP), lymphocyte percentage, glucose and fibrinogen levels. In addition, patients with newly diagnosed diseases were more often diagnosed with myocarditis in the acute COVID-19 period (Table 5).

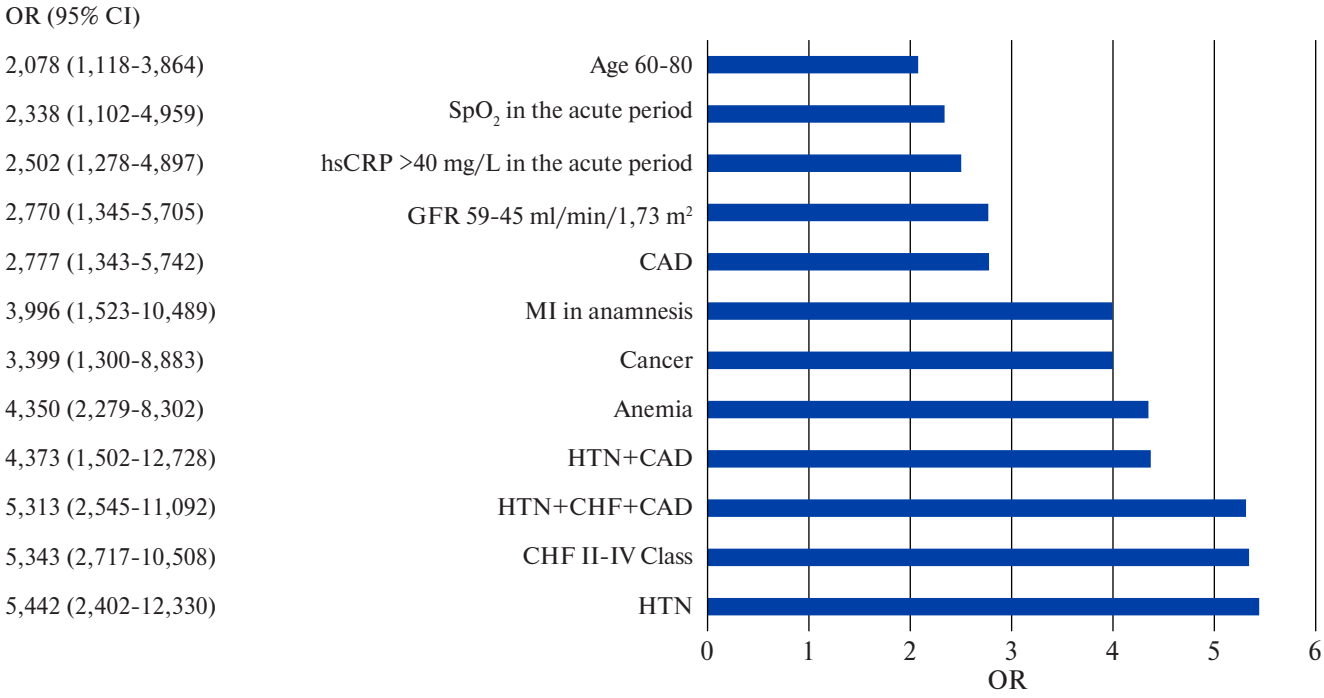

Figure 2. Death RFs in the early 3-month post-hospitalization period.
Abbreviations: HTN — hypertension, hsCRP — high-sensitivity C-reactive protein, CI — confidence interval, CAD — coronary artery disease, MI — myocardial infarction, BMI — body mass index, OR — odds ratio, GFR — glomerular filtration rate, CHF — chronic heart failure, SpO_2 — oxygen saturation.

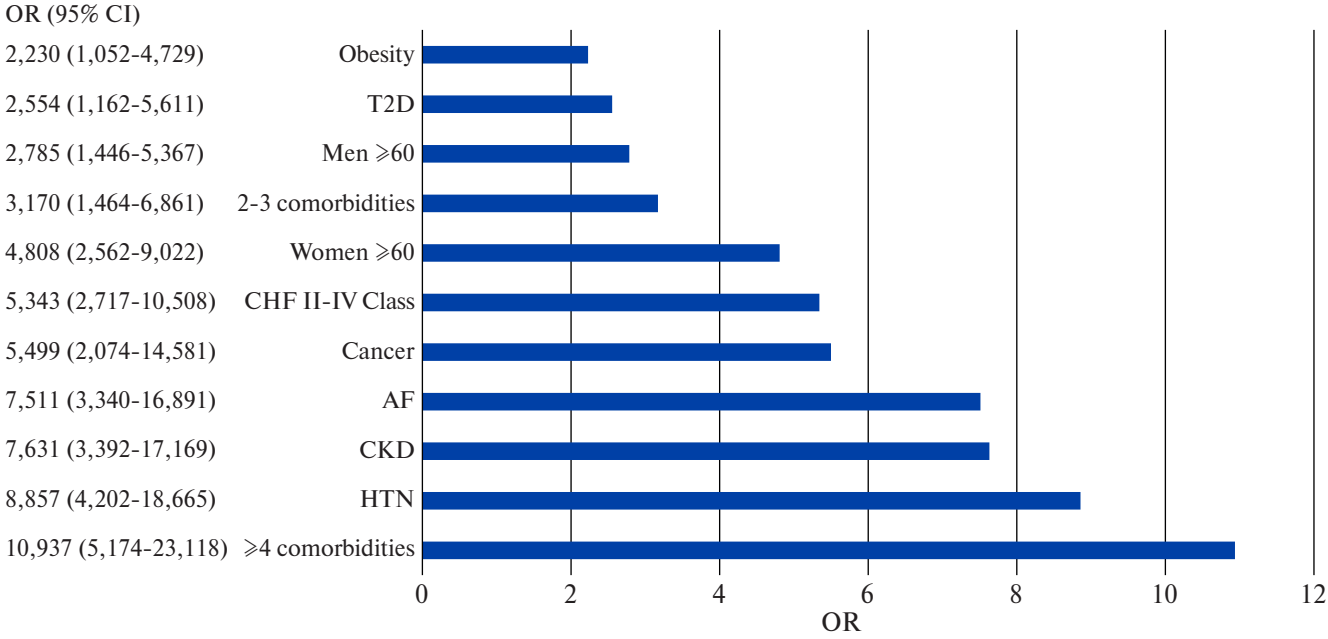

Figure 3. Death RFs in the early 3-month post-hospitalization period in patients aged ⩾60.
Abbreviations: HTN — hypertension, CI — confidence interval, OR — odds ratio, T2D — type 2 diabetes, GFR — glomerular filtration rate, AF — atrial fibrillation, CKD — chronic kidney disease, CHF — chronic heart failure.

The mortality rate of patients in the post-hospitalization period was 1,9% in the first 3 months and 0,2% during 4-6-month follow-up (Table 6). According to a differentiation analysis of mortality, depending on the type of comorbidity, the highest mortality rate was observed in the first 3 months in the group of patients with class II-IV HF, as well as in patients with cardiovascular diseases (CVDs) and

cancer. The presence of COPD and asthma did not affect the mortality of patients.

In the pattern of death causes in the post-hospitalization period, the following CVDs predominated (31,8%): acute coronary syndrome, stroke, acute heart failure (Table 7). In addition, pneumonia, cancer and pulmonary embolism were among the known causes of death.

Patients who died in the post-hospitalization period were significantly different from those who survived. The deceased patients were older. Age >60 years was associated with an increased risk of death by 3,324 and 4,765 times for men and women, respectively (Table 8). Patients who died in the post-hospitalization period differed from the survivors in comorbidity rates. HTN was associated with an increased risk of death, which was most pronounced in the group of patients >60 years old (Table 8). The following factors was associated with the death risk: body mass index (BMI) <18,5 kg/m^2, AF, especially in patients >60 years old, and CAD, especially with prior myocardial infarction. For patients ⩾60 years old, obesity was a risk factor (RF) for death. One of the strongest risk factors for lethal outcome was class II-IV HF, the presence of which was associated with an almost 5-fold increase in risk. A strong risk factor for lethal outcome was a history of stroke and CKD. The risk of death increased as the glomerular filtration rate (GFR) decreased. So, GFR of 59,9-45 ml/min/1,73 m^2 was associated with an increased risk of death by 2,770 times, GFR of 44,9-30 ml/min/1,73 m^2 — 6,133 times, and GFR of 29,9-15 ml/min/1,73 m^2 — 11,692 times. The presence of cancer, especially for patients ⩾60 years of age, and anemia were associated with an increased risk of death.

Patients who survived and died in the post-hospitalization period differed in multimorbidity severity (Table 9). Among the survivors, there were significantly more patients with no concomitant diseases or with only 1 comorbidity. The presence of 2-3 and especially 4 comorbid diseases was associated with an increased death risk. This was especially important for patients ⩾60 years old. Among the combinations of comorbid diseases, the most common were combinations of CVD and RF, such as HTN, CAD, HF, and obesity. The combination of HF with hypertension and/or CAD was a strong risk factor for lethal outcome in the post-hospitalization.

Surviving and deceased patients differed depending on the severity of infection during hospitalization or outpatient treatment in the acute period. The deceased patients suffered from more severe COVID-19 than the survivors (Tables 10, 11). They more often had severe shortness of breath with a respiratory rate of ⩾30 bpm, a decrease in SpO_2 within 75-94%, serum hsCRP >40 mg/L, which was associated with a significant increase in the death risk. Strong RFs were complications of the acute period of infection, such as acute renal injury and deep vein thrombosis (DVT) (Table 10). In addition, deceased patients in the acute infection period had higher heart rate, D-dimer levels, and troponin I levels, as well as lower hemoglobin, lymphocyte proportion, GFR and potassium levels (Table 11).

Thus, there were following 10 strongest RFs of lethal outcome in the early post-hospitalization period (3 months) in decreasing order of factor value (Figure 1): age >80 years, BMI <18,5 kg/m^2, ⩾4 comorbid conditions, acute renal injury and DVT in the acute period, prior stroke, GFR of 44,9-30 ml/min/1,73 m^2, presence of AF, CKD, and a combination of HTN+HF.

In addition, significant death RFs were as the factor value decreased (Figure 2): HTN, class II-IV HF, HTN+CAD+HF combination, HTN+CAD combination, anemia, cancer, prior myocardial infarction, CAD, GFR of 59,9-45 ml/min/1,73 m^2, hsCRP >40 mg/l in the acute period, SpO_2 of 75-94% in the acute period, and the age of 60-80 years.

For patients ⩾60 years old, the main risk factors for lethal outcome were in decreasing order of factor value (Figure 3): ⩾4 comorbidities, HTN, CKD, AF, cancer, class II-IV HF, 2-3 comorbidities, T2D and obesity. It was noteworthy that the combination of age ⩾60 years and female sex was associated with a 4,808-fold increased risk of death.

Discussion

The incidence of comorbidities in patients after COVID-19 generally corresponds to the population levels in a similar age [3, 4], as well as data from other observational studies of patients after hospitalization. So, according to Günster C, et al. [5] the most common comorbidities in patients discharged from the hospital were HTN (56,7%), diabetes (uncomplicated — 22%; complicated — 8,5%), cardiac arrhythmias (27,3%), CKD (23,0%) and HF (19,0%).

According to the AKTIV register, 38,2% of patients after COVID-19 had long-term persistence of symptoms. Most often, patients complained of weakness, shortness of breath, chest pain, increased blood pressure and palpitations. Other researchers demonstrate similar data. Thus, according to Huang C, et al. from Wuhan [6] with 6-month follow-up of 1733 patients after discharge from hospital, the most common persisting symptoms were fatigue or muscle weakness (63% of patients), as well as sleep problems (26%) and anxiety and/or depression (23%). According to the National

Institute for Health and Care Excellence (NICE) guidelines for long COVID [7], about one in five people had symptoms that lasted ⩾5 weeks, and 1 in 10 people had symptoms that lasted ⩾12 weeks. Most often, patients complained of chronic cough, shortness of breath, chest tightness, cognitive dysfunction, and extreme fatigue. Many publications on tachycardia in patients after COVID-19 have appeared recently [8-10]. Ståhlberg M, et al. [8] in their review emphasize the presence of tachycardia in long COVID and introduce a new term "post-COVID-19 tachycardia syndrome", considering it as a special phenotype of long COVID, which is defined as symptoms after COVID-19 infection persisting for 4-12 or >12 weeks [7].

One of the most significant results of data from the AKTIV register is information on an increase in the incidence of newly diagnosed diseases in patients after COVID-19. The incidence rate of HTN, CAD, MI and diabetes significantly exceeds that in the general Russian population (Table 4B). Other authors cite similar data. According to a retrospective study by Ayoubkhani D, et al. [11], patients after hospitalization due to COVID-19 were diagnosed with following diseases more often than in the corresponding control group: 3,0 (2,7-3,2) times for CVDs, 2,8 (2,0-4,0) times for chronic liver disease, 1,9 (1,7-2,1) times for CKD and 1,5 (1,4-1,6) times for diabetes.

According to the AKTIV register, more often newly diagnosed diseases developed in patients aged 49-50 years. According to the study by Ayoubkhani D, et al. [11], more often newly diagnosed diseases in the post-hospitalization period developed in patients under 70 years of age in comparison with patients in older age groups. Newly diagnosed diseases in patients with a low death risk from COVID-19 was studied in a prospective cohort observational study by Dennis A, et al. [12]. The study found that 70% of middle-aged patients without pronounced comorbidity after COVID-19 develop *de novo* damage to one or more organs 4 months after COVID-19 onset, which, according to the authors, should have serious consequences for society and the health system as a whole.

Rehospitalizations and increased mortality in the first 3-6 months are a serious problem in the post-hospitalization period. After hospitalization, according to our data, almost 1/3 of patients sought unscheduled health care (outpatient, inpatient), and during the first 3 months, at least 2 times. In addition, mortality of patients within 6 months amounted to 2,1%. An analysis of >100 thousand hospitalized patients with COVID-19 in the United States showed that 6 months after discharge, the readmission rate to the same hospital was 9%, while 1,6% was hospitalized >1 time. The presence of prior lung diseases, HF, T2D, CKD and age ⩾65 years increased the risk of readmission [13].

The UK retrospective cohort study by Ayoubkhani D, et al. [11] with 47780 patients showed that during an average follow-up period of 140 days, almost a third (29,4%) of those discharged from the hospital after an acute COVID-19 were rehospitalized (n=14060), and 12,3% (n=5875) died early after discharge (first 90 days). A study of 1775 US veterans hospitalized for COVID-19 found that 20% were readmitted and 9% died within 60 days of discharge [14]. According to the study by Leijte WT, et al. [15] with 769 patients after COVID-19 found that the all-cause mortality rate after discharge was 6,4%, and the re-hospitalization rate was 11,7%. The main reasons for readmission were respiratory failure (31%), arterial and venous thrombotic events (16%), or events associated with decompensated comorbidity (14%). Mortality was significantly higher in the cohort of elderly patients and patients with acute delirium. According to the study by Chopra V, et al. (n=1250) [16], 6,7% of patients died 60 days after discharge from the hospital. The observational study by Günster C, et al. with 8679 patients from Germany [5] showed that 26,8% of patients were re-hospitalized within 180 days, while the 90- and 180-day mortality rate was 27,9% (n=2425) and 29,6% (n=2566), respectively. For patients aged ⩾80 years, the 180-day mortality rate was 52,3% (n=1472). The RFs for 180-day all-cause mortality were HF, CKD, diabetes, cancer, liver disease, coagulopathy, BMI ⩾40 kg/m^2, and age. For patients with symptomatic HF, the 180-day all-cause mortality was 49,8%, for patients with CKD — 47,2%, for patients with complicated diabetes — 45,4%.

Ninety-day follow-up of severe COVID-19 patients after hospitalization has been reported in various observational studies, a large percentage of which had single-center design. The 90-day mortality rate ranged from 11% in Spain [17] to 29% in Denmark [18] (single-center studies), 27% in Sweden [19], 31% in Belgium, France and Switzerland [20]. In the multicenter study from 3 countries above, which included 4643 patients with severe COVID-19, the early independent predictors of 90-day mortality were old age, immunosuppression, severe obesity, HTN, diabetes, CKD, CVD, and severe acute respiratory distress syndrome.

Brieghel C, et al. [18] found that the risk of 90-day mortality increased in proportion to the patient age and the Charlson comorbidity index. In the study by Zettersten E, et al. [19], 3-month mortality depended on the patient's sex (older in men), age,

presence of COPD, asthma, immunodeficiency and active treatment of cancer.

Thus, the patients included in the AKTIV register were found to have comorbidities comparable to those cited above, which led to an increase in readmission rates and mortality in the post-hospitalization period. Apparently, the predominance of CVDs in patients after COVID-19 is universal in all countries. The AKTIV registry working group suggests that the post-hospitalization problems of COVID-19 can be explained by the destabilization of comorbid diseases due to COVID-19 [21], direct damaging effects of the virus on tissues and organs [22], expressed by the health system overload [23].

Study limitations. An insufficiently accurate assessment of the mortality rate in the post-hospitalization period is possible. This is due to the fact that no answer to the phone call cannot rule out the death of a patient. The accuracy of data presented is limited by the fact that they were obtained by contact with patients or their relatives, and not by the analysis of medical documents.

Conclusion

According to the AKTIV register and other studies, the health status of people after COVID-19 is a serious problem for the health care system in all countries. These patients are characterized by common seeking for health care, including rehospitalizations, worsening of existing disease course, high mortality rate, and diagnosis of "new" diseases in the post-hospitalization period.

The accumulated information on the frequency and RFs of rehospitalization and the development of *de novo* diseases allows the working group of the AKTIV register to suggest the formation of a new phenotype of patients. We believe that in a routine clinical practice, a new patient phenotype has appeared — a patient after a severe COVID-19, which required hospitalization. This patient is characterized by a high risk of progression of HTN, T2D, atherosclerosis and associated complications, as well as the development of *de novo* HF and/or progression of prior HF.

Discussion on this issue will allow, in our opinion, to optimize the solution of such priority tasks as health care capacity planning to provide care to patients with COVID-19 in both acute and post-hospitalization periods and can influence decision-making both at the local and national levels.

Relationships and Activities: none.

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