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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.

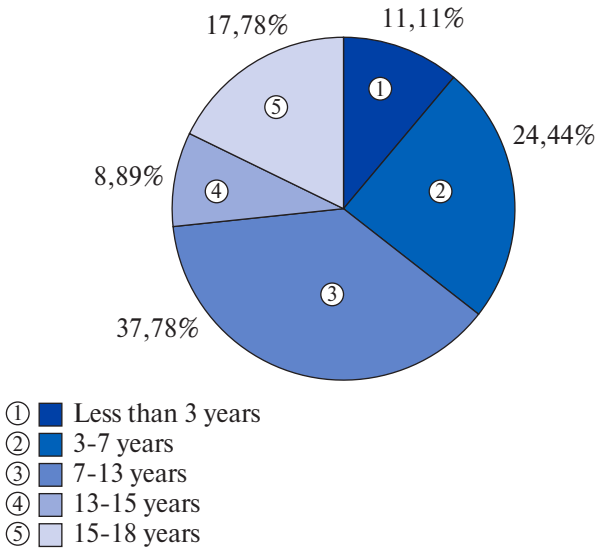

Figure 1. Distribution of patients by age at the time of the last discharge from the Federal State Autonomous Institution "National Medical Research Center for Children's Health".

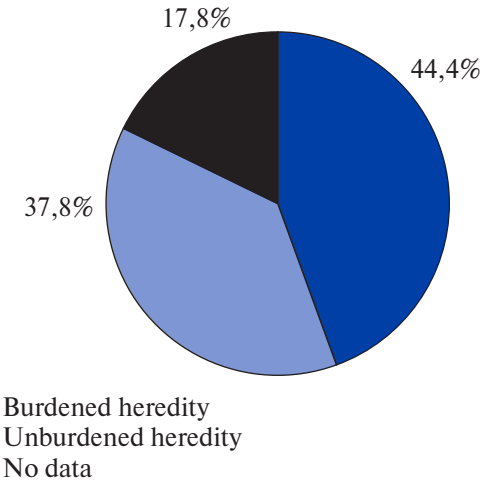

Figure 2. Distribution of patients by presence of hereditary pathology in biological parents.

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.

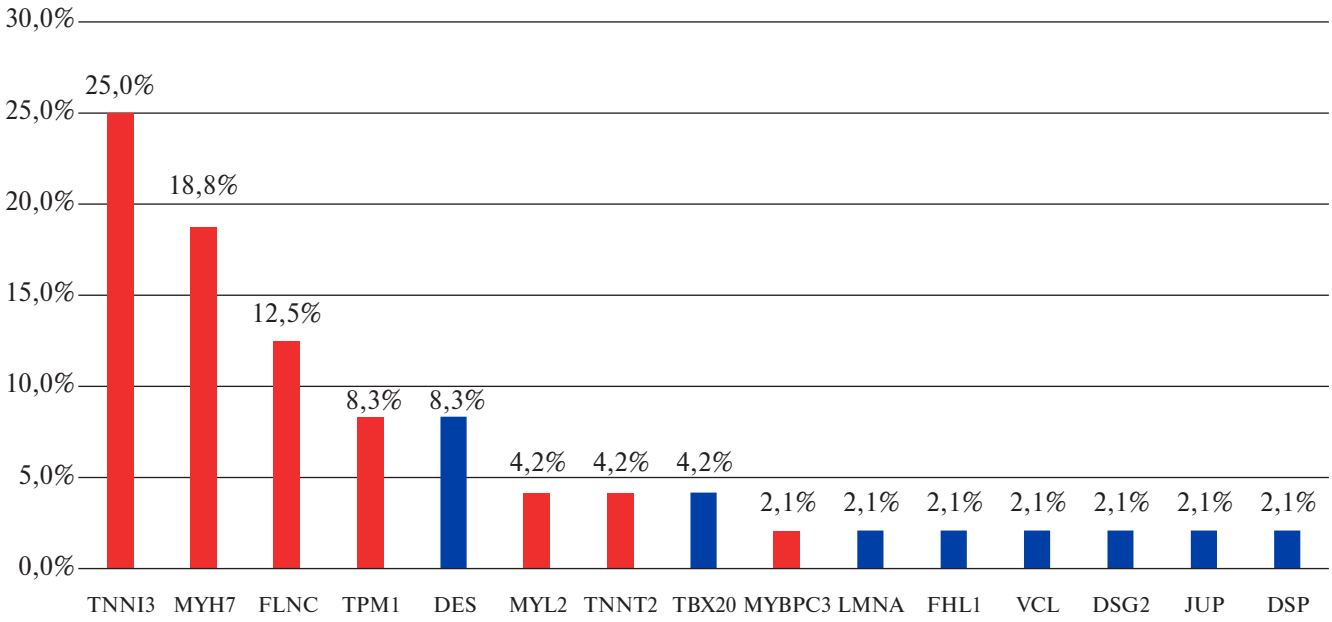

Figure 3. Percentage of genes containing mutations that caused RCMP and CMP with a restrictive type of hemodynamics in the examined Russian children.
Note: red — sarcomeric genes, blue — non-sarcomeric genes.

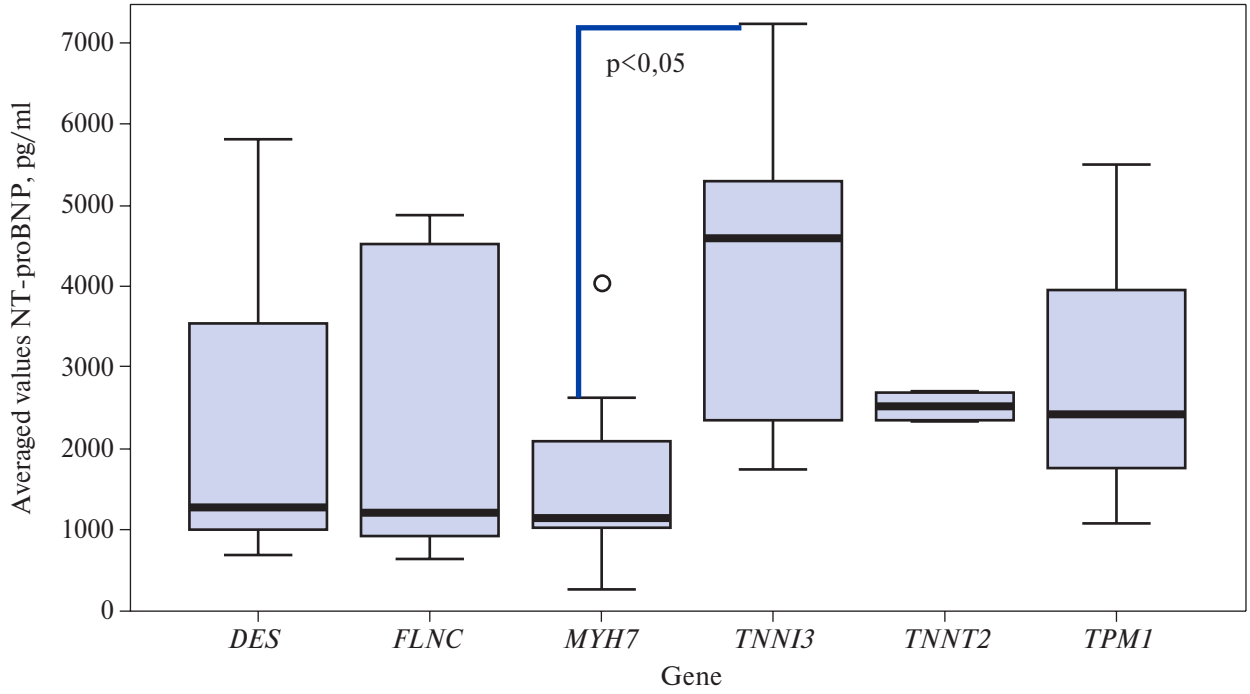

Figure 4. Distribution of the averaged values of NT-proBNP according to affected genes.

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).

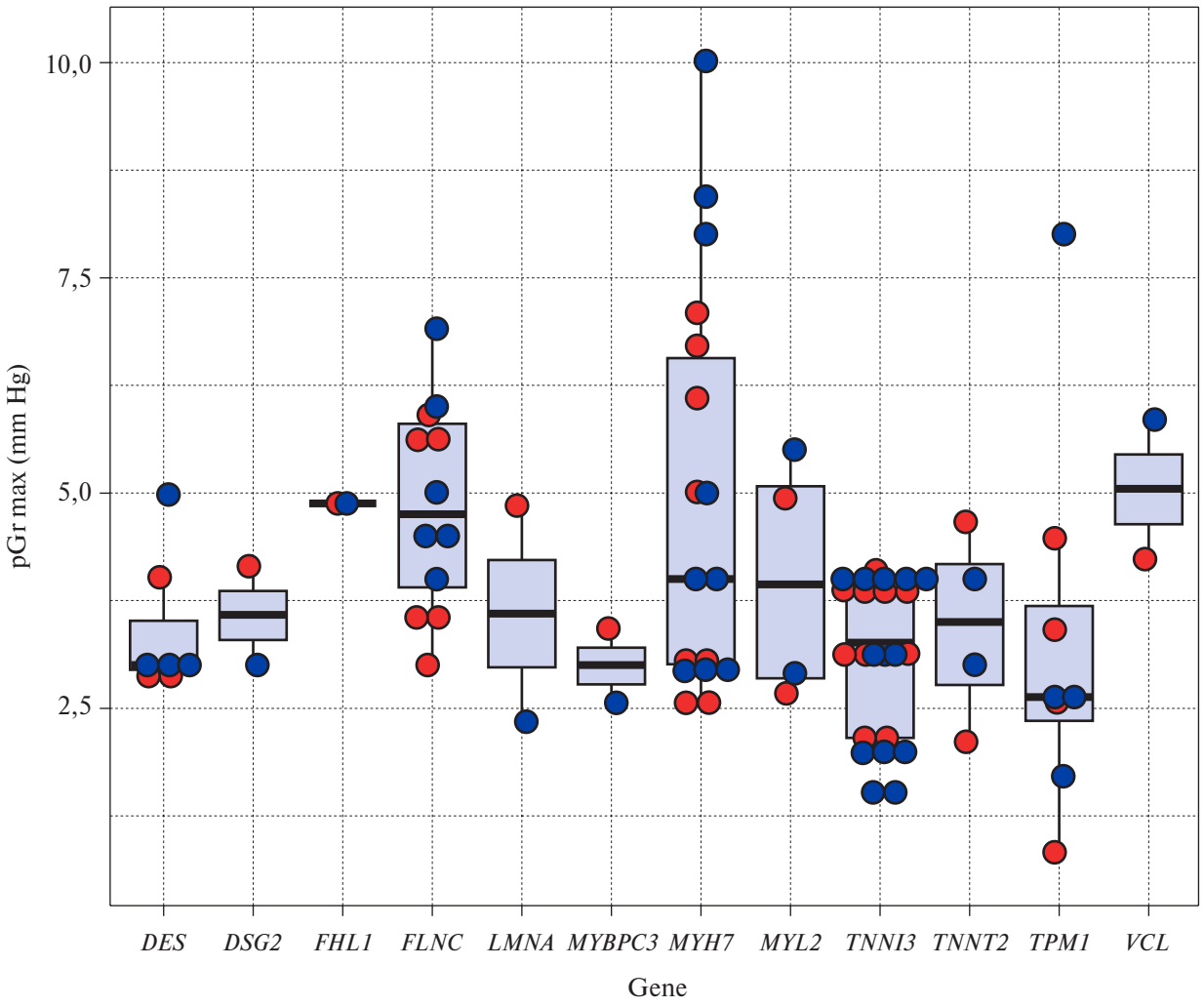

Figure 5. Distribution of values of the parameter PGr max (mm Hg) of blood flow on pulmonary artery valve in the general group of children with RCMP and children with CMP with restrictive hemodynamics.
Note: individual indicators of PGr max (mm Hg) are presented in the form of dots: red indicates the 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

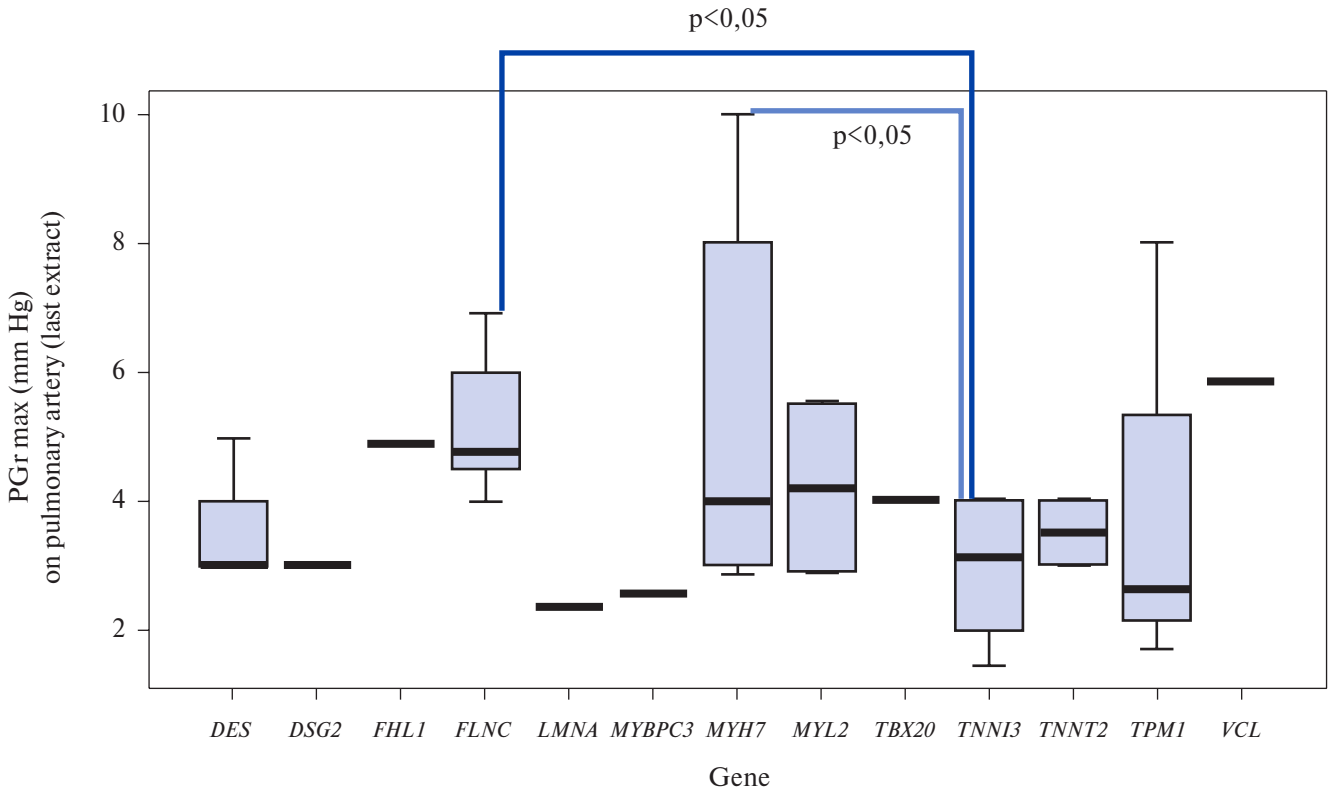

Figure 6. Distribution of registered values of pGr max (mm Hg) during the last discharge from the hospital in children with mutations in various genes.

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.

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