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IMPACT OF GENOTYPE ON CLINICAL COURSE IN BIVENTRICULAR ARRHYTHMOGENIC CARDIOMYOPATHY

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Aim. To analyze the correlation between genotype and phenotype in patients with biventricular arrhythmogenic right ventricular cardiomyopathy (ARVC).

Methods. The clinical phenotype of 9 unrelated probands (89 % men, median age 35 [34; 37]) with biventricular ARVC were observed. The clinical and instrumental examination included a 12-lead ECG, 24-hour Holter ECG monitoring, transthoracic echocardiography and cardiac magnetic resonance imaging with late gadolinium enhancement. Biventricular variant of ARVC was diagnosed according to the 2020 Padua criteria for both right and left ventricles involvement. High-throughput sequencing was utilized to search for mutations in genes linked to the onset of cardiomyopathies and other inherited rhythm disorders. Statistical analysis procedures were performed using the STATISTICA-12 program.

Results. In all patients with biventricular ARVC, according to late gadolinium enchansment magnetic resonance imaging, left ventricular involvement of varying degrees was detected, characterized by fibrous or fibrofatty infiltration of the myocardium, as well as regional or global systolic dysfunction. Genotyping in 9 patients with biventricular ARVC revealed 10 variants of the nucleotide sequence of III-V classes of pathogenicity according to the criteria of ACMG (2015) in 4 genes associated with ARVC (*PKP2*, *DSP*, *DSC2*, *DSG2*). Of these, 7 variants belonged to classes IV and V (*PKP2* - 4 mutations, *DSP* - 2 mutations, *DSG2* - 1 mutation); 3 nucleotide substitutions were variants with uncertain significance (VUS, class III) - 2 in *DSC2* gene and 1 in *DSP* gene. A combination of nucleotide variants in two genes (*DSP* and *DSC2*) was detected in 1 patient. The findings highlight that mutations in *DSP* gene were associated with more severe systolic dysfunction and left ventricle dilation compared to carriers of mutations in *PKP2* gene. In patients with variants of class III pathogenicity in *DSC2* gene the most adverse clinical course of the disease was observed with the early onset of the first sustained ventricular tachycardia and the development of severe dysfunction and dilation of both ventricles requiring heart transplantation in comparison with carriers of mutations in other genes.

Conclusion. The results obtained in a cohort of patients with biventricular ARVC demonstrate a specific correlation between genotype and clinical course and disease severity as well.

Key words: arrhythmogenic right ventricular cardiomyopathy; biventricular variant; CMR imaging; mutations in *PKP2*; mutations in *DSP*; mutations in *DSC2*; mutations in *DSG2*

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The prevailing guidelines established by the European Society of Cardiology (ESC) in 2023 for the management of patients afflicted with cardiomyopathies have effectuated a noteworthy revision in the classification of a subset of conditions previously subsumed under the overarching nomenclature of arrhythmogenic cardiomyopathy (ACMP). This categorization is defined by inherent structural and functional aberrations within the myocardium, concomitant with the manifestation of ventricular arrhythmias [1]. Originally, in clinical practice, the term used was «arrhythmogenic right ventricular cardiomyopathy" (ARVC), and the criteria for confirmation were based on modifications outlined by the International Task Force (ITF) led by F. Marcus and others in 2010 [2]. Over the preceding decade, the nosological framework pertaining to this condition has undergone significant expansion, notably incorporating biventricular and left-dominant variants. This evolving comprehension has precipitated a terminological refinement, transitioning from the erstwhile «arrhythmogenic right ventricular cardiomyopathy» to the more encompassing designation of «arrhythmogenic cardiomyopathy» [3]. Revised criteria, grounded in the morphofunctional

and structural attributes derived from magnetic resonance imaging (MRI) data for the left (LV) and right ventricles (RV) of the heart, have been formulated for the diagnostic assessment of arrhythmogenic cardiomyopathy (ACMP). This initiative was undertaken collaboratively by an interdisciplinary cadre of international researchers and clinical cardiologists affiliated with the Faculty of Medicine at the University of Padua [4].

Nevertheless, in the most recent European Society of Cardiology (ESC) guidelines from 2023, there is a strong endorsement from experts advocating for adherence to the terminology «arrhythmogenic right ventricular cardiomyopathy». According to this guideline, the term ARVC can be used to describe a variant in which cavity dilatation and/or local contractility abnormalities are predominantly confined to the RV, with or without LV involvement, and modified International Task Force 2010 (ITF 2010) criteria can be applied [2].

Over the past two decades, pathogenic mutations have been identified in the genes for desmosomal proteins associated with ARVC: plakoglobin (*JUP*) [5], plakophilin-2 (*PKP2*) [6], desmoplakin (*DSP*) [7], desmoglein-2 (*DSG2*) [8], and desmocollin-2 (*DSC2*) [9]. Earlier investigations have posited that genotype could exert an impact on the susceptibility to both life-threatening tachyarrhythmias and the onset of heart failure [10]. However, given

Table 1.

Genotyping results in patients with biventricular arrhythmogenic cardiomyopathy

No.	PC	Gene	Exon	Nucleotide substitution / Rs	Amino acid substitution	MAF mutation class (gnomAD)
1	793c	*PKP2*	11	c.2014-1G>C rs193922674	-	V 0.00003184
2	707c	*PKP2*	4	c.1057_1058del	p.Leu353GlyfsTer33	V* 0.00
3	18m	*PKP2*	9	c.1912C>T / rs397517012	p.Gln638Ter	V 0.000007074
4	17m	*PKP2*	5	c.1237C>T / rs372827156	p.Arg413Ter	V 0.00001415
5	778c	*DSP*	23	c.5212C>T/ rs794728124	p.Arg1738Ter	V 0.00003187
6	6m	*DSP*	23	c.3494delA	p.Lys1165Argfs*10	IV-V 0.00
7	4m	*DSP*	3	c.364 G>T	p.Asp122Tyr	III* 0.00
		DSC2	4	c.394 C>T rs727504578	p.Arg132Cys homozygote	III-IV 0.00003539
8	801c	*DSC2*	5	c.488C>A rs758759298	p.Thr163Lys	III 0.00
9	798c	*DSG2*	3	c.137G>A rs121913008	p.Arg46Gln	IV-V 0.000003560

Notes: hereinafter PC - patient code; *, new - previously undescribed mutations; MAF (gnomAD)- minor allele frequency in the gnomAD database.

Table 2.

Comparative characterization of patients with different genetic variants of biventricular arrhythmogenic cardiomyopathy

	Patients with mutations in the PKP2 gene (n=4)	Patients with mutations in other genes (n=5)	P
Age of disease manifestation, years	27 [23; 31]	33 [32; 36]	0.063
Male sex, n (%)	4 (100)	3 (60)	0.193
Familial form, n (%)	2 (50)	3 (60)	0.797
Cardiac arrest (VT/VF)	2 (50)	2 (40)	0.798
Syncope, n (%)	4 (100)	4 (80)	0.407
Spontaneous sustained VT, n (%)	4 (100)	4 (80)	0.407
T inversion in V1-V6, n (%)	4 (100)	5 (100)	-
Low-voltage ECG, n (%)	0	2 (40)	0.193
Presence of LGE, n (%)	4 (100)	4 (80)	0.407
LV dilatation, n (%)	0	4 (80)	0.017
RV dilatation, n (%)	0	1(20)	0.407
LV dysfunction (LVEF <50%), n (%)	0	4 (80)	0.017
RV dysfunction (RVEF <45%), n (%)	3 (75)	5 (100)	0.292
Implantable cardioverter-defibrillator, n (%)	3 (75)	5 (100)	0.292
Orthotopic heart transplantation, n (%)	0	1 (20)	0.407
Fatal outcome, n (%)	0	1 (20)	0.407

Notes: hereinafter, VT/VF - ventricular tachycardia/ventricular fibrillation; LV and RV - left and right ventricles; EF - ejection fraction.

Таблица 3.

Клинико-инструментальная характеристика пациентов с бивентрикулярной аритмогенной кардиомиопатией

PC	Sex/YB	ASM, years	FF	SS	VT	VES for 24 hours, n	Tc V1-3	Cardiac MRI							Endocardial EPS	Events and outcomes
								LVEF, %	LV iEDV, ml/m2	LGE LV	LV fibrosis, %	RV iEDV, ml/m2	LGE RV	RVEF, %		
793c	m/1996	26	-	+	+	4000	+	64	88	+	4.4	86	+	50	Induced by the PVT	ICD, ES, RFA VES/VT
707c	m/ 1987	31	-	+	+	613	+	63	62	+	4.9	64	-	44	Induced by the MVT	RFA VES/VT
18m	m/1977	32	+	+	+	2500-4000	+	57	69	+	3.6	107	+	42	-	ICD
17m	m/1988	22	+	+	+	2280	+	53	73	+	12.7	109	+	40	-	ICD
778c	f/1977	40	+	+	+	14905	+	48	107	+	12	83	+	37	-	ICD
6m	m/1998	23	-	+	+	17526	+	49	109	+	36.5	95	+	41	Induced by the MVT	ICD, RFA VES/VT
4m	m/1988	32	+	-	-	3576	+#	26	110	+	23.2	227	+	10	-	ICD, WL OHT, LO
801c	f/1989	33	+	+	+	1953	+#	21	104	-	-	95	-	44	-	ICD, OHT
798c	m/1986	36	-	+	+	1079	+	56	85	+	8.1	87	-	40	Induced by the PVT	ICD

Notes: MRI - magnetic resonance imaging; YB - year of birth; ASM - age of symptom manifestation; FF - family form; SS - syncopal states; VES - ventricular extrasystole; Tc - electrocardiogram T-chart changes; EPS - electrophysiologic study; iEDV - end-diastolic volume index; LGE - late gadolinium enhancement; PVT and MVT - polymorphic and monomorphic VT; ICD - implantable cardioverter-defibrillator; ES - electrical storm; RFA - radiofrequency ablation; WL - waiting list; OTS - orthotopic heart transplantation; LO - lethal outcome; #, low-voltage.

the evolving conceptualization of the disease, which underscores its phenotypic diversity, there arises a necessity for elucidating genotype-phenotype relationships for a more comprehensive understanding.

Aim. To study the influence of genotype on the phenotype of patients with biventricular variant of ARVC.

METHODS

A cohort of nine unrelated probands, predominantly male (89%), with a median age of 35 (interquartile range: 34 to 37), underwent thorough clinical and instrumental assessments. The evaluations encompassed a 12-lead electrocardiogram (ECG), daily ECG monitoring, transthoracic echocardiography, and cardiac magnetic resonance imaging (MRI) incorporating delayed contrast enhancement.

The diagnosis of ARVC was established according to modified criteria developed by the ITF 2010 multidisciplinary working group [2]. The criteria defining a «definite» diagnosis of ARVC necessitated the fulfillment of either two major criteria, one major and two minor criteria, or four minor criteria drawn from six distinct categories. These categories encompassed ventricular morphofunctional abnormalities, myocardial structural abnormalities, depolarization abnormalities, repolarization abnormalities, ventricular arrhythmias, and family history/genetics. «Biventricular» variant of ARVC was diagnosed when ≥1 morphofunctional and/or structural abnormality of both the RV and LV was detected at the same time according to the 2020 Padua criteria[4].

Cardiac MRI was performed on a 1.5 T tomograph (Siemens Healthineers, Magnetom Aera, Germany) using ECG synchronization. All studies were performed according to the Society for Cardiovascular Magnetic Resonance (SCMR) 2020 recommended protocol for ARVC [11]. Additionally, native and postcontrast T1-mapping sequences of myocardium were used for tissue characterization purposes.

LV systolic dysfunction was defined as LV ejection fraction (EF) <50%, and severe dysfunction was defined as LV EF <35%. LV dilatation was defined when end-systolic volume on MRI >214 mL or LV diastolic diameter >58 mm in men and end-diastolic volume >176 mL or diastolic diameter >52 mm in women [12]. Severe RV systolic dysfunction was defined as RV EF <45%

Evaluation of myocardial fibrosis involved the utilization of delayed contrast enhancement through two-dimensional segmented inversion-recovery sequences administered 10 minutes subsequent to the intravenous injection of a contrast agent (gadobutrol, 0.15 mmol/kg body weight). This assessment was conducted across multiple projections, including 4-chamber, 2-chamber, 3-chamber, short-axis, 3-chamber left ventricular (LV), and LV outflow tract projections.

A left ventricular lesion was characterized by the manifestation of one or more of the subsequent features: (1) diminished global and/or compromised local myocardial contractility, and (2) subepicardial or intramural contrast enhancement within one or more LV segments situated in the region of the interventricular septum and/or LV free wall, excluding the junctures connecting the interventricular septum and ventricles.

The evaluation of fatty infiltration encompassed the analysis of T1-weighted images obtained through Turbo Spin Echo sequence, cine images, post-contrast delayed enhancement series, and native T1 mapping of the myocardium.

The exploration for mutations within the coding sequences of genes linked to the progression of cardiovascular pathology was executed through high-throughput sequencing utilizing the MiSeq and NextSeq genetic analyzers manufactured by Illumina, USA. Sample preparation was performed using the TruSight Cardio Sequencing Kit gene panel (Illumina, USA). The pathogenicity of new and previously described genetic variants was interpreted according to the 2015 recommendations of the American Society of Medical Genetics. [13]. Genotyping and anonymous publication of results were performed with the written consent of the patient.

The conducted study was approved by the local ethical committee (Minutes No. 9 of the Independent Ethical Committee meeting dated 05.07.2022). All patients signed a written informed voluntary consent to participate in the study.

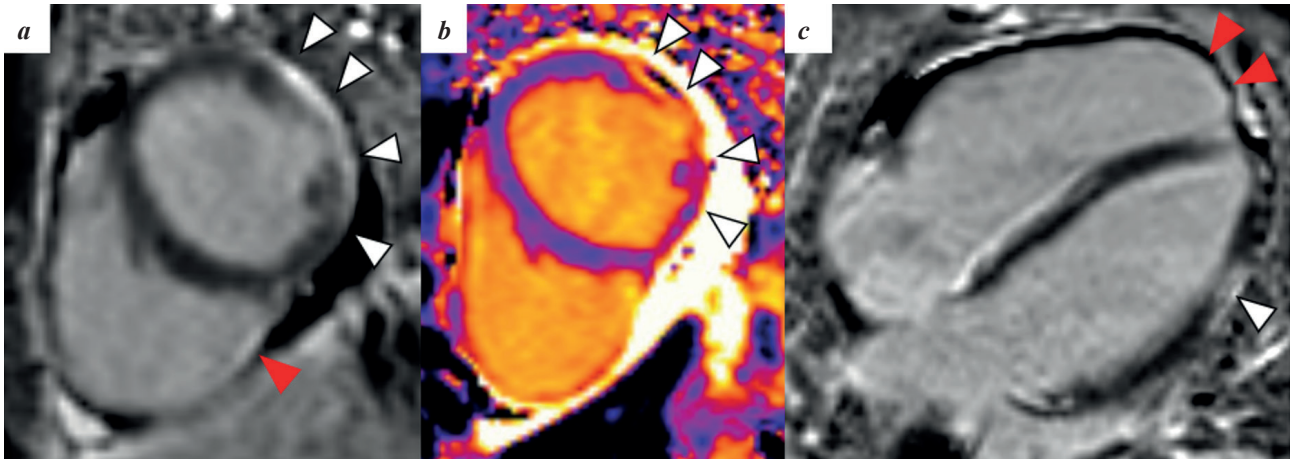

Fig. 1. Magnetic resonance imaging of the heart of a 793c patient. with images of the heart in the delayed contrast enhancement sequence: end-diastolic phase, in short-axis projection (a), native T1-mapping (b), and four-chamber projection (c); areas of subepicardial and transmural fibrofatty replacement of the myocardium of the lateral wall of the left ventricle (white arrow heads), areas of fibrosis of the myocardium of the inferior wall and apex of the right ventricle (red arrow heads).

Statistical analysis

Statistical analysis procedures were performed using the STATISTICA-12 program. The normality of distribution of quantitative signs in separate comparison groups was checked using Kolmogorov-Smirnov and Shapiro-Wilk criteria. Nonparametric quantitative measures are represented by median and quartiles as Me [LQ; UQ]. Qualitative indicators are described by absolute values and percentages (n, %). The two groups were compared using Student's t-criterion, Mann-Whitney criterion, and median criterion. The level of statistical significance in the study was taken as $p < 0.05$.

RESULTS

Genotyping of 9 patients with biventricular ARVC (Table 1) revealed 10 nucleotide sequence variants of pathogenicity classes III-V according to ACMG (2015) criteria in 4 genes associated with ARVC (*PKP2*, *DSP*, *DSC2, DSG2*). Of these, 7 variants belonged to classes IV and V (*PKP2* - 4 mutations, *DSP* - 2 mutations, *DSG2* - 1 mutation); 3 nucleotide substitutions were variants of uncertain significance (class III) - 2 in the *DSC2* gene and 1 in the *DSP gene.* One patient had a combination of nucleotide variants in two genes: *DSP* and *DSC2*. The probands were divided into two groups depending on the genotyping results: 1) patients with mutations in the *PKP2* gene; 2) patients with mutations in other desmosomal genes. The comparative characteristics of these groups are presented in Table 2.

All patients with mutations in *PKP2* gene and 80% of the second group (the exception was patient 4m, Table 3) had syncope and sustained hemodynamically significant VTs. Five patients (group 1: 17m, 18m; group 2: 778c, 4m, 801c) were found to have a familial form of the disease, with two patients (codes 17m, 18m) having sudden cardiac death (SCD) in first-degree relatives.

The median age at disease manifestation in patients with mutations in the *PKP2* gene was 27 [23; 31] years, whereas in patients with mutations in other desmosome genes it was 33 [32; 36] years (p=0.063). Male patients were predominant in both groups.

The disease manifested cardiac arrest with ECG-proven ventricular fibrillation and successful resuscitation followed by implantation of a cardioverter-defibrillator (ICD) for secondary prevention of SCD in two patients (codes 17m and 18m) with nonsense mutations c.1237C>T (p.Arg413*, rs372827156) and c.1912C>T (p.Gln638*, rs397517012) in the *PKP2* gene resulting in premature arrest of plakophilin-2 protein synthesis.

ECG abnormalities (inversion of the T plaque in precordial leads V1-V6) were recorded in all patients regardless of genotype, low-voltage ECGs in standard leads - in 2 carriers of VUS c.394C>T (p.Arg132Cys, rs727504578) and c.488C>A (p.Thr163Lys, rs758759298) in the *DSC2* gene (codes 4m and 801c). Of note, the missense substitution of p.Arg132Cys was observed in the homozygous state. Given the exceedingly low prevalence of this variant (minor allele frequency of 0.00004) and its absence in homozygous form in extensive population studies, coupled with the identification of other rare homozygous substitutions in this patient, there is a reasonable inference that the individual may have originated from a closely related marriage. In addition to the variant in the *DSC2* gene, the patient had a novel missense variant c.364G>T (p.Asp122Tyr, rs756013600) in a heterozygous state in the *DSP* gene.

LV dilatation (LV end-diastolic volume index (iEDV) >107 ml/m^2) and its dysfunction (LVEF <50%) were de-

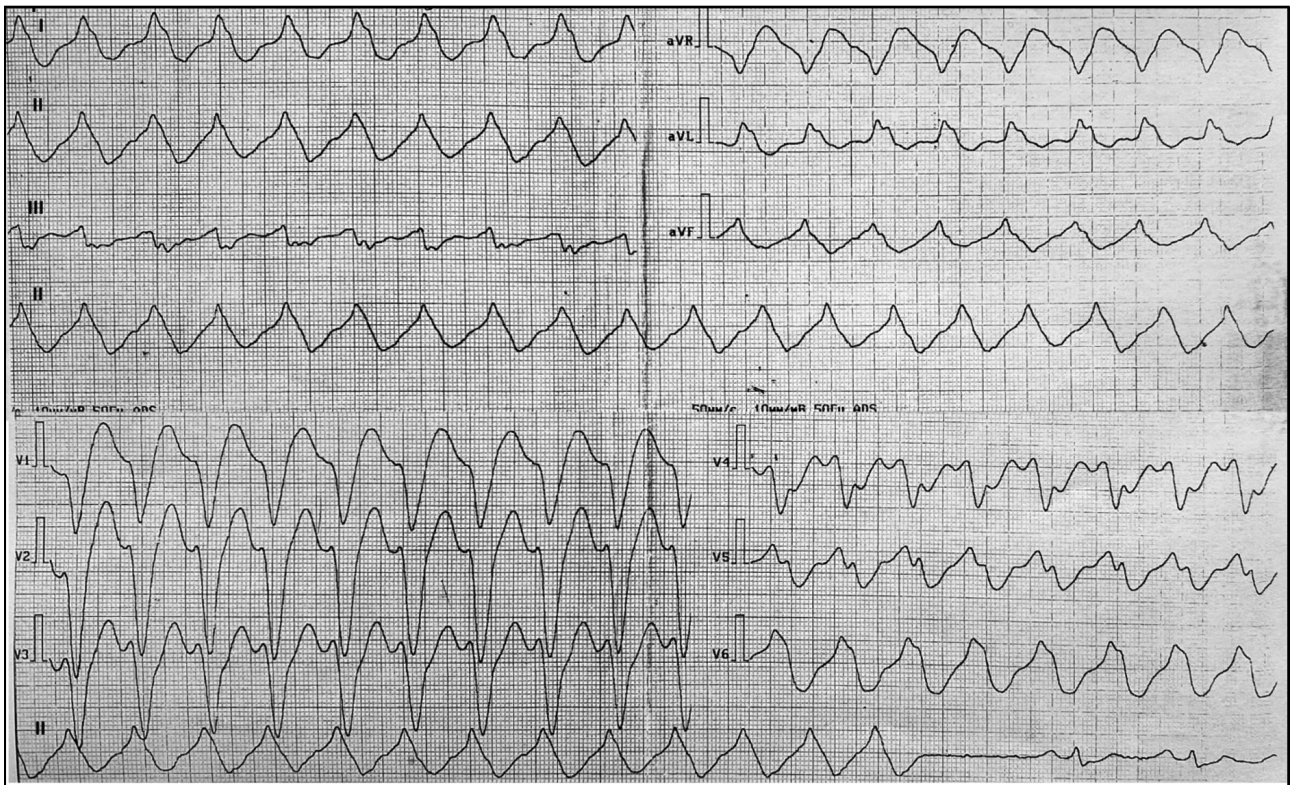

Fig. 2. ECG-12 of patient 793c: sustained ventricular tachycardia with HR 200 beats/min, with morphology of complete blockade of the left bundle branch of Hiss with restoration of sinus rhythm.

tected only in group 2 patients: in all carriers of mutations in *DSP* and DSC2 genes. At the same time, the patient with homozygous mutation in *DSC2* gene also had dilatation of RV (iEDV of RV >123 ml/m^2). RV dysfunction (RV EF <45%) was recorded in 89% of patients regardless of genotype: 3 (75%) individuals with mutations in the *PKP*2 gene and all patients in group 2. The presence of myocardial fibrosis was detected in all patients with nucleotide sequence variants in the *PKP2* and *DSP* genes.

ICDs were implanted in 3 (75%) patients with mutations in the *PKP2* gene and 4 (80%) patients with variants in other genes, i.e., the need for ICDs was also independent of the localization of the mutation in the genes.

When comparing the clinical manifestations of the disease among patients with mutations in the *PKP2* gene (Table 3), the most severe form was observed in patients 17m and 18m with nonsense mutations p.Arg413* and p.Gln638* in the *PKP2* gene. Along with the manifestation of the disease by cardiac arrest with successful resuscitation followed by ICD implantation, both patients had a family history of close relatives' SCD. According to cardiac MRI, no LV dilatation (LV iEDV 73 ml/m^2) and no abnormalities of global (LV EF 53%) LV contractility were detected. Regional hypokinesis of the mid lateral segments of LV myocardium was observed. The RV is not dilated (iEDV of the RV 109 ml/m^2). However, a decrease in global contractility of RV (EF 42% and 40%, respectively) and impaired local contractility of RV myocardium in the form of regional dyskinesis of peritricuspidal zone and small aneurysmal bulges of RV free wall and outflow tract were discovered.

Two patients (codes 707c and 793c) who also had pathogenic variants in the *PKP2* gene (deletion resulting in a stop codon, c.1057_1058del (p.Leu353Glyfs*33) and a splicing site mutation, c.2014-1G>C, rs193922674) had a relatively less severe disease course. Cardiac MRI in both patients showed normal LV and RV function, but areas of subepicardial accumulation of contrast agent in basal and middle segments of LV and in lateral and middle segments of RV were detected (Fig. 1). In patient 707c with p.Leu353Glyfs*33 deletion, the disease was manifested by frequent VES from the output tract of the RV and a single syncope. Endocardial electrophysiologic study (EPS) induced monomorphic ventricular tachycardia (VT) and performed radiofrequency ablation (RFA) of VT. The second patient (code 793c), with a splice site mutation - c.2014-1G>C, rs193922674), had disease debut at age 26 years. The main clinical manifestations were persistent paroxysms of monomolar VT (Fig. 2) with syncopal states requiring hospitalization. For the purpose of primary prevention of SCD, the patient was implanted with an ICD, against the background of repeated motivated triggering of which an electrical storm occurred. The decision was made to perform EPS with RFA of the VT in order to eliminate the symptoms and alleviate the patient's condition.

In 2 patients (codes 6m and 778c) with pathogenic mutations leading to premature arrest of protein synthesis, LV lesions were dominant in the *DSP* gene compared with carriers of variants in other genes. Thus, in a patient (code 6m) with a new deletion of c.3494delA (p.Lys1165Argfs*10) in the *DSP* gene, cardiac MRI revealed LV dilatation (iEDV LV 109 ml/m^2), LV systolic dysfunction (LV EF 49%), whereas RV dilatation (iEDV RV95 ml) and marked RV systolic dysfunction (RV EF 41%) were absent. Extensive subepicardial accumulation of contrast agent in lateral and middle segments of LV and transmural accumulation in basal and middle segments of RV was revealed. In a 40-year-old patient (code 778c) with a nonsense mutation c.5212C>T (p.Arg1738*, rs794728124) in the *DSP* gene, no significant LV and RV dilatation was found, but RV dysfunction (RV EF 37%) and subepicardial accumulation of contrast agent along the posterior wall of the RV were determined. In the absence of pronounced LV systolic dysfunction, subepicardial accumulation of contrast agent along the inferior LV wall and transmural accumulation in the basal segment of the anterolateral LV wall were ob-

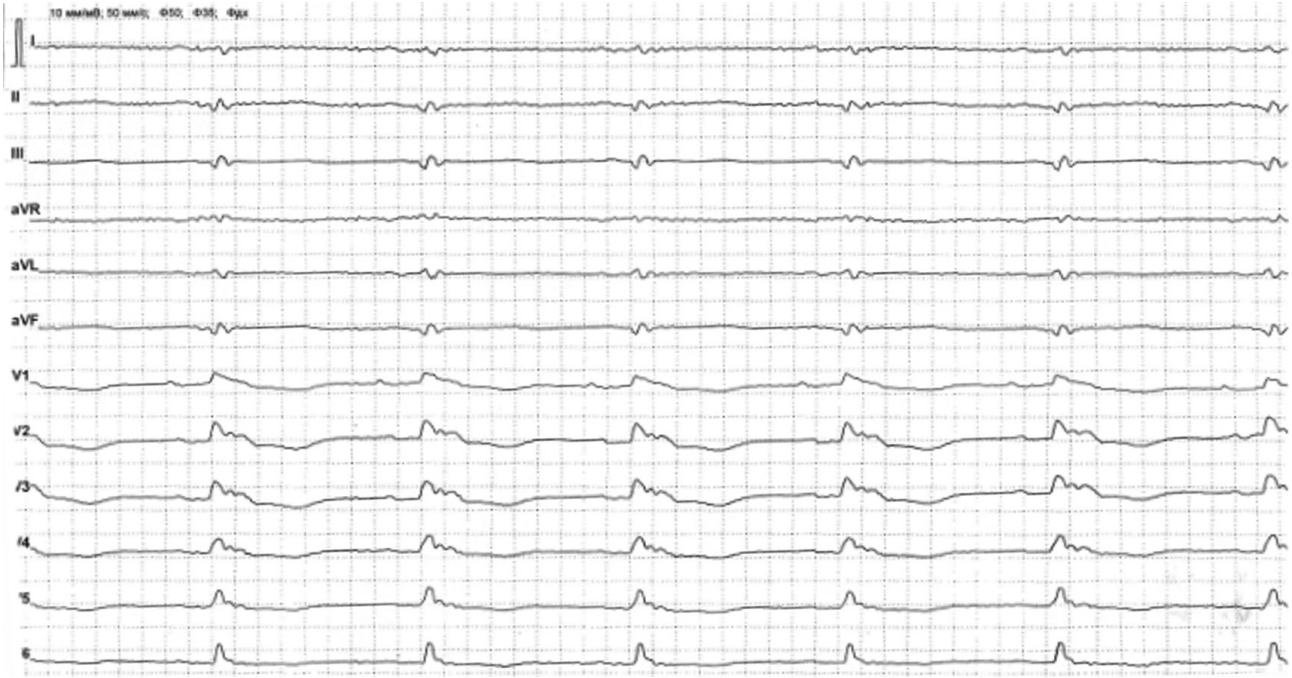

Fig. 3. Low-amplitude ECG-12 of patient 4m: inversion of the T-chart in leads V1-V6.

served. The presence of intramyocardial fatty infiltration adjacent to myocardial fibrosis was detected in the same segments. In both patients, the disease manifested with arrhythmic events: in patient 778c with the nonsense mutation, cardiac arrest due to the development of VT/VF with successful resuscitation and subsequent ICD implantation for secondary prevention of SCD; in patient 6m with the deletion, development of sustained VT paroxysms with syncope requiring ICD implantation for primary prevention of SCD. On the background of multiple motivated ICD triggers, 6m had an electrical storm and underwent EPS with RFA of the VT to relieve symptoms.

The 2 patients (codes 4m and 801c) with VUS variants in the *DSC2* gene and T inversion in precordial leads V1-V6 (Fig. 3) had the most severe course of the disease compared with other patients diagnosed with ARVC, characterized by the development of life-threatening arrhythmias requiring ICD implantation and subsequent progression of heart failure requiring orthotopic heart transplantation (OHT). For example, patient 801c with a p.Thr163Lys substitution in the *DSC2* gene was resuscitated after cardiac arrest caused by an episode of sustained VT/VF followed by ICD implantation at the age of 33 years. Cardiac MRI revealed marked LV systolic dysfunction (LVEF 21%), LV dilatation (iEDV RV 104 ml/m^2) and minor systolic dysfunction of the RV (RVEF 44%). Extensive subepicardial accumulation of contrast agent along the inferior LV wall and in the basal segment of the anterolateral LV wall was revealed (Fig. 4). Due to progression of CHF requiring continuous inotropic support, the patient underwent OHT at the age of 34 years. In a 32-year-old patient 4m with a homozygous variant in the *DSC2* gene and an additional substitution in the *DSP* gene at the time of hospitalization, the clinical picture was represented by symptoms of HF of functional class III according to NYHA. From the history, he was known to have had an ICD implanted at the age of 27 years for primary prevention of SCD. According to cardiac MRI data, marked dilatation of LV and RV (LV EDV 217 ml, RV EDV 448 ml) and systolic dysfunction of both ventricles (LV EF 26%, RV EF 10%); transmural accumulation of contrast agent in LV and RV was determined. Due to frequent hospitalizations due to progression of HF requiring inotropic support, the decision was made to perform OHT. The patient was placed on the waiting list, however, 1 month later, death occurred.

In a 36-year-old patient 798 with a pathogenic c.137G>A mutation (p.Arg46Gln, rs121913008) in the *DSG2* gene, the disease manifested with frequent VES and repeated episodes of unstable VT. The ECG showed inversion of the T in leads V1-V3. Cardiac MRI revealed systolic dysfunction of the RV (RV EF 40%), no ventricular dilatation of the heart was noted. It was decided to perform an EPS, during which polymorphic VT was induced. The patient was implanted with an ICD for secondary prevention of SCD.

DISCUSSION

This study undertook a comprehensive characterization of the clinical trajectory, the emergence of adverse events, and ultimate outcomes. Additionally, it conducted an analysis of phenotypic manifestations contingent upon the genotype in patients presenting with biventricular ARVC. These patients have been shown to be at high risk for cardiac arrest, sustained VT, and the development of heart failure. In our observation, patients with *DSP* and *DSC2* genotype had a more severe clinical course of the disease with unfavorable outcome than patients with *PKP2* genotype, which is consistent with observations in other cohort studies [9, 14].

We show that carriers of pathogenic nonsense mutations p.Arg413*, p.Gln638* and deletion of p.Leu353Glyfs*33 leading to premature stop codon in the *PKP2* gene were associated with earlier development of life-threatening ventricular arrhythmias with the need for ICD implantation. Carriers of p.Lys1165Argfs*10 and p.Arg1738* deletions in the *DSP* gene, leading to premature arrest of protein syn-

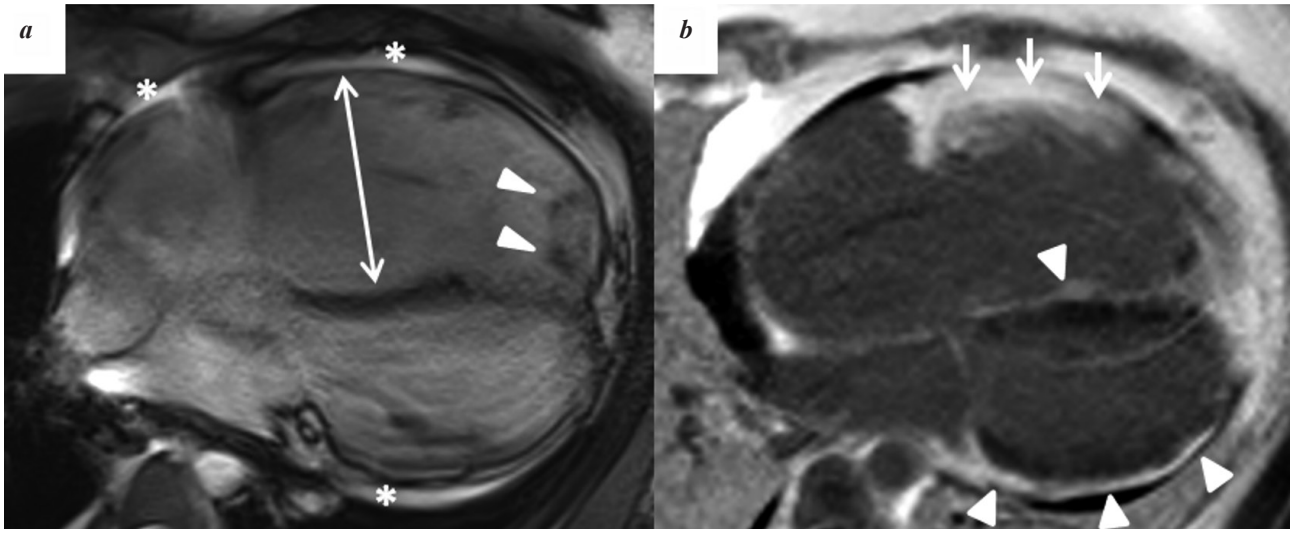

Fig. 4. Cardiac magnetic resonance imaging of patient 4m: images of the heart in four-chamber projection in the end-diastolic phase. Cine-image (a): marked dilation of the right ventricular cavity (thin white arrow), thrombus in the right ventricular cavity (white arrow heads), effusion in the pericardial cavity (white asterisks). Delayed contrast enhancement (b): transmural myocardial fibrosis of the lateral wall of the right ventricle (white arrows), subepicardial myocardial fibrosis of the lateral wall of the left ventricle and interventricular septum (white arrow heads).

thesis, had more pronounced LV dysfunction and dilatation compared with carriers of mutations in the *PKP2 gene.* Patients with variants of pathogenicity class III (VUS) in *DSC2* gene had the most unfavorable clinical course of the disease with early onset of the first sustained VT and a higher probability of development of VT/VF and increased risk of severe LV dysfunction and CHF requiring OHT compared to carriers of mutations in other genes. Of note, one patient had a variant in the *DSC2* gene in a homozygous state and, in addition, the patient had a novel substitution, pathogenic by in silico predictors, in the *DSP* gene, which may have exacerbated the phenotypic manifestations of the disease.

In our observed patients with biventricular variant of ARVC, decreased LVEF <35% and right ventricular dysfunction were associated with a high risk of sustained VT. On the other hand, among patients with LVEF >50%, 5 of 9 patients had persistent VT requiring ICD implantation regardless of genotype. Overall, stratification of the risk of SCD among patients with biventricular variant ARVC remains unspecified and needs to be refined.

In biventricular ARVC, both ventricles of the heart are affected with the development of systolic dysfunction and ventricular dilatation, which is accompanied by the development of heart failure of progressive nature, requiring OHT. In our observation, carriers of *DSP*, *DSC2* genotypes were associated with CHF progression at a young age.

Finally, our observation shows that the modified ITF 2010 criteria [2] do not allow us to diagnose the biventricular form of ARVC because there are no developed criteria for LV. In this case, the Padua criteria of 2020 are more diagnostically accurate. [4], which include morphofunctional changes in both ventricles. Consequently, it is not recommended to rely solely on the ITF 2010 criteria for the diagnosis of biventricular ARVC.

Variants in the *LMNA* and $FLNC_{LOF}$ genes, as well as desmosomal genes, have been recognized as a potential cause of biventricular ARVC and associated with LV systolic dysfunction and extensive subepicardial myocardial fibrosis (a peculiar «ring-shaped» pattern), predisposing to life-threatening arrhythmias and SCD, as well as progressive conduction disturbance in *LMNA* gene mutations [15, 16].

The present results emphasize the importance of genetic testing not only for patients with suspected ARVC, but also for cases with biventricular ventricular heart disease, frequent ventricular arrhythmias, and development of HF symptoms at a young age.

Thus, the biventricular form of ARVC is a disease with a wide range of clinical manifestations, ranging from virtually asymptomatic patients to severe cases with fatal outcome due to SCD or the development of heart failure requiring heart transplantation or ICD implantation.

CONCLUSION

The results obtained in a cohort of patients with biventricular ARVC demonstrate a definite association of genotype with the clinical course and severity of the disease. ITF 2010 criteria have been shown to have limited diagnostic value for the biventricular variant of ARVC.

REFERENCES

1. Arbelo E, Protonotarios A, Gimeno JR, et al. 2023 ESC Guidelines for the management of cardiomyopathies. *European Heart Journal*. 2023;44(37): 3503-3626. https://doi.org/10.1093/eurheartj/ehad194.

2. Marcus FI, McKenna WJ, Sherrill D, et al. Diagnosis of arrhythmogenic right ventricular cardiomyopathy/dysplasia: proposed modification of the Task Force Criteria. *European Heart Journal.* 2010;31(7): 806-814. https://doi.org/10.1093/eurheartj/ehq025.

3. Corrado D, van Tintelen PJ, McKenna WJ, et al. Arrhythmogenic right ventricular cardiomyopathy: evaluation of the current diagnostic criteria and differential diagnosis. *European Heart Journal*. 2020;41(14): 1414-1429. https://doi.org/10.1093/eurheartj/ehz669.

4. Corrado D, Perazzolo Marra M, et al. Diagnosis of arrhythmogenic cardiomyopathy: The Padua criteria. *International Journal of Cardiology*. 2020;319: 106-114. https://doi.org/10.1016/j.ijcard.2020.06.005.

5. McKoy G, Protonotarios N, Crosby A, et al. Identification of a deletion in plakoglobin in arrhythmogenic right ventricular cardiomyopathy with palmoplantar keratoderma and woolly hair (Naxos disease). *Lancet.* 2000;355(9221): 2119-2124. https://doi.org/10.1016/S0140-6736(00)02379-5.

6. Gerull B, Heuser A, Wichter T, et al. Mutations in the desmosomal protein plakophilin-2 are common in arrhythmogenic right ventricular cardiomyopathy. *Nature Genetics*. 2004;36(11): 1162-1164. https://doi.org/10.1038/ng1461.

7. Rampazzo A, Nava A, Malacrida S, et al. Mutation in Human Desmoplakin Domain Binding to Plakoglobin Causes a Dominant Form of Arrhythmogenic Right Ventricular Cardiomyopathy. *American Journal of Human Genetics*. 2002;71(5): 1200-1206.

8. Awad MM, Dalal D, Cho E, et al. DSG2 mutations contribute to arrhythmogenic right ventricular dysplasia/cardiomyopathy. *American Journal of Human Genetics*. 2006;79(1): 136-142. https://doi.org/10.1086/504393.

9. Syrris P, Ward D, Evans A, et al. Arrhythmogenic right ventricular dysplasia/cardiomyopathy associated with mutations in the desmosomal gene desmocollin-2. *American Journal of Human Genetics*. 2006;79(5): 978-984. https://doi.org/10.1086/509122.

10. Bhonsale A, Groeneweg JA, James CA, et al. Impact of genotype on clinical course in arrhythmogenic right ventricular dysplasia/cardiomyopathy-associated mutation carriers. *European Heart Journal*. 2015;36(14): 847-855. https://doi.org/10.1093/eurheartj/ehu509.

11. Kramer CM, Barkhausen J, Bucciarelli-Ducci C, et al. Standardized cardiovascular magnetic resonance imaging (CMR) protocols: 2020 update. *Journal of Cardiovascular Magnetic Resonance*. 2020;22(1): 17. https://doi.org/10.1186/s12968-020-00607-1.

12. Kawel-Boehm N, Hetzel SJ, Ambale-Venkatesh B, et al. Reference ranges (‘normal values’) for cardiovascular magnetic resonance (CMR) in adults and children: 2020 update. *Journal of Cardiovascular Magnetic Resonance*. 2020;22(1): 87. https://doi.org/10.1186/s12968-020-00683-3.

13. Richards S, Aziz N, Bale S, et al. Standards and guide-

lines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in Medicine*. 2015;17(5): 405-424. https://doi.org/10.1038/gim.2015.30.
14. Reza N, de Feria A, Chowns JL, et al. Cardiovascular Characteristics of Patients with Genetic Variation in Desmoplakin (DSP). *Cardiogenetics*. 2022;12(1): 24-36. https://doi.org/10.3390/cardiogenetics12010003.
15. Miller DT, Lee K, Chung WK, et al. ACMG SF v3.0 list for reporting of secondary findings in clinical exome and genome sequencing: a policy statement of the American College of Medical Genetics and Genomics (ACMG). *Genetics in Medicine: Official Journal of the American College of Medical Genetics*. 2021;23(8): 1381-1390. https://doi.org/10.1038/s41436-021-01172-3.
16. Carruth ED, Qureshi M, Alsaid A, et al. Loss-of-Function FLNC Variants Are Associated With Arrhythmogenic Cardiomyopathy Phenotypes When Identified Through Exome Sequencing of a General Clinical Population. *Circulation. Genomic and Precision Medicine*. 2022;15(4): e003645. https://doi.org/10.1161/CIRCGEN.121.003645.