

## PKP2 and DSG2 genetic variations in Latvian arrhythmogenic right ventricular dysplasia/cardiomyopathy registry patients

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### ABSTRACT

**Objective:** The Latvian arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD-C) registry was established to determine the genetic background of ARVD-C for analyzing discovered genetic variation frequencies in the European and Latvian populations.

**Methods:** In total, 38 patients with suspected ARVD-C were selected. The clinical parameters were defined according to the ARVD-C guidelines, PKP2 and DSG2 gene analysis was performed using the Sanger sequencing. Additionally, large deletions/duplications were analyzed using the multiplex ligation-dependent probe amplification (MLPA) analysis.

**Results:** Twenty symptomatic patients were enrolled in the study. Typical ARVD abnormalities were found in electrocardiography for 10 (50%) patients, in Holter monitoring for 19 (95%), in transthoracic echocardiography for 20 (100%), and in cardiac magnetic resonance for 6 (30%). Different benign genetic variations were found. Three novel, unregistered, possibly benign variations were found in the PKP2 gene: c.2489+131G>A, c.2489+72delA, and c.1035-5T>C and three in the DSG2 gene: c.404G>A, c.1107G>A, and c.379-15A>G. Two genetic variations in the PKP2 gene: c.1592T>G, c.2489+1G>A are possibly pathogenic. For the first time, variation c.1592T>G, has been discovered in the homozygote form. Using the MLPA analysis, large deletions or duplications were not found.

**Conclusion:** The prevalence of the majority of non-pathological genetic variations is similar in the Latvian ARVD-C patients and the European population. Possibly, pathogenic variations were found only in 10% of our registry patients, which could mean that PKP2 and DSG2 are not the most commonly affected genes in the Latvian population. (*Anatol J Cardiol* 2018; 20: 296-302)

**Keywords:** arrhythmogenic right ventricular dysplasia-cardiomyopathy, ARVD-C, cardiomyopathy, genetics, PKP2, arrhythmia

### Introduction

The prevalence of ARVD-C in the general population ranges from 1 in 2,000 to 1 in 5,000 (1, 2). The diagnosis is confirmed by the criteria of the Task Force of the Working Group (3).

ARVD-C is a rare form of cardiomyopathy in which the heart muscle of the right ventricle (RV) is replaced by fat and/or fibrous tissue, but the more detailed pathogenesis is largely unknown (4). The development of ARVD-C is due to the genetic variants of desmosomal protein encoding genes. ARVD-C is usually inherited as an autosomal dominant trait with incomplete penetrance and variable expression (4). Several studies have confirmed that the PKP2 gene variants in patients with ARVD-C are the most common ones,

with the prevalence ranging from 11% to 51%, mainly truncating genetic variants (up to 73%) (5-7). In 10-40% of the ARVD-C patients, causative variations are found in the DSG2 gene (8).

Up to 57% of patients with ARVD-C have compound heterozygosity (a different pathogenic allelic variant in both alleles of the same gene) or digenic heterozygosity (a heterozygous pathogenic allelic variant in two different genes). It is reasonable to expect a more severe phenotype in patients who have compound and digenic heterozygosity, especially when at least one pathogenic variant is affecting the PKP2 gene (9).

There are numerous national registries around the world and one international registry (1). This is the initial stage to establish a Latvian ARVD-C registry in collaboration with cardiologists, family physicians, and geneticists. The registry for ARVD-C patients

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and their first-degree relatives is necessary to evaluate their clinical condition, genetic background, and assess the natural course of the disease as well as help patients with education, consultation about their lifestyle, family planning, first-degree screening of relatives, and treatment opportunities.

### Aims

The specific aims of the multidisciplinary study of ARVD-C were to establish a Latvian ARVD-C registry, enrolling ARVD-C patients and their family members, based on the standardized diagnostic test criteria, according to the 2010 Task Force Criteria (3), in a prospective longitudinal follow-up study; determine the genetic background of ARVD-C by identifying genetic variations in the *PKP2* and *DSG2* genes; and determine the sudden cardiac death risk of patients with ARVD-C and to improve therapy.

### Methods

The Multidisciplinary Prospective Longitude Follow-up Study of Latvian ARVD-C patients, which was started in 2014, represents an effort of the research group from the University of Latvia, Pauls Stradins University Hospital, in cooperation with the Genetic Laboratory of Riga Stradins University Scientific Laboratory of Molecular Genetics.

In total, 38 patients with suspected ARVD-C due to complaints of uncertain syncope, pre-syncope, chest pain, confounding ventricular tachycardia, and a positive family history or typical findings in electrocardiography (ECG) and transthoracic echocardiogram (TTE) were selected. Previous test results were analyzed and a detailed medical history regarding ARVD-C and related cardiovascular or systemic conditions was assessed. Twenty patients with possible ARVD-C were directed for further examinations of ECG, TTE, cardiac magnetic resonance (CMR), and 24-hour Holter monitoring, and the risk assessment was done. After confirming definite or borderline diagnosis according to the revised Task Force Criteria of the European Society of Cardiology/International Society and Federation of Cardiology (3), genetic analysis of the *PKP2* and *DSG2* genes was performed. Sudden cardiac death (SCD) risk assessment was done based on the article by the ESC Council for Cardiology Practice (10). The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Central Medical Ethics Committee of Latvia. All study participants provided informed consent. During the study annual follow-up has been organized. This study is planned to last for 5 years with annual visits. A follow-up visit includes medical history, clinical evaluation, standard ECG, TTE, and Holter monitoring for 24 hours.

For estimating the frequency of novel variants in the population of Latvia, 50 self-reported healthy individuals from the general population of Latvia were selected.

DNA from patients and control individuals were isolated using the standard phenol chloroform method (11). For all patients,

the *PKP2* gene (Gene Bank Accession no: NC 000012) and *DSG2* gene (Gene Bank Accession no: NC\_000018) coding sequence and exon/intron boundaries was directly sequenced. *PKP2* gene primer sequences for exons 2-14 were adapted from the publication (12), and the first exon was designed in the Primer 3 program; primer sequences are available upon request. *DSG2* gene primer sequences were adapted from publication (13). All sequences were verified in the Basic Local Alignment Search Tool (BLAST) database and compared to the *PKP2* gene reference sequences (NM\_004572.3 and NG\_009000.1) and *DSG2* gene (NM\_001943 and NG\_007072). The nomenclature and position were checked using the Mutalyser software (<https://mutalyzer.nl>) and further the HGVS nomenclature was used (14). All discovered genetic variations were consulted in the ARVD-C database (<http://www.arvcdatabase.info>) to study their possible connection with ARVD-C. The possible pathogenicity of genetic variations was analyzed using the ClinVar database. For novel variants, the pathogenicities were analyzed according to the American College of Medical Genetics guidelines (15). Multiple computational tools, such as PolyPhen, Sift, SNAP, PhD-SNP et al, were used. The minor allele frequency (MAF) of novel genetic variations was compared with the European population sample using data from the 1000 Genome project browser (<https://1000genomebrowser.org>) using the Fisher exact test or  $\chi^2$  test. Three exons (4, 7, and 12) of the *PKP2* gene and three exons (5, 8, and 9) of the *DSG2* were analyzed in 50 unaffected Latvian individuals to determine the frequency of undescribed genetic variants in the Latvian population. Multiplex ligation-dependent probe amplification (MLPA) was used to detect possible large deletions and duplications in the *PKP2* and *DSG2* genes. The MLPA analysis was done using P168-C2 MRC-Holland probemix according to the manufacturer's guidelines.

### Results

#### Clinical data

Out of 38 patients with suspicions of ARVD-C, 20 symptomatic patients were enrolled with 12 females (60%) and eight males (40%); the median age was  $43 \pm 14.3$  years. The high risk of SCD was identified in four (20%) patients—all the patients had experienced sustained ventricular tachycardia. The risk of SCD for 13 (65%) patients was moderate and for three (15%) patients was low. Abnormalities in the ECG were found for 10 (50%) patients, in Holter monitoring for 19 (95%) patients, and in echocardiography for 20 (100%) patients; structural changes in cardiac magnetic resonance were seen in six (30%) patients. Twelve (60%) patients had a history of medium to high intensity physical activities, from whom five patients had high risk of SCD and seven had moderate risk of SCD. For treatment, beta-blockers were used in nine patients (45%), radiofrequency catheter ablation in seven (35%) patients, and implantable cardioverter-defibrillator in three (15%) patients. No gender-based or racial/ethnic-based differences were present.

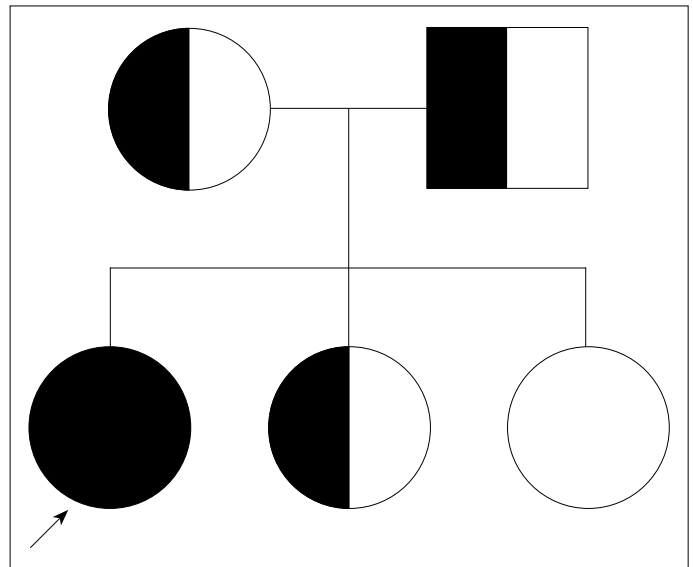
**Table 1. Clinical characteristics of the symptomatic ARVD-C patients**

Parameters	Value
<b>Study population</b>	20 patients
<b>Age (years)</b>	43±15.4 (min 21, max 72)
<b>Male (gender)</b>	8 (40%)
<b>Definite diagnosis</b>	16 (80%)
<b>Borderline diagnosis</b>	4 (20%)
<b>Electrocardiography findings</b>	
Epsilon waves in V1 - V3	2 (10%)
TAD of QRS ≥55 ms	2 (10%)
Inverted T-waves in right precordial leads (V1, V2, and V3)	5 (25%)
Non-sustained VT	13 (65%)
Sustained VT	4 (20%)
<b>Echocardiography</b>	
RV aneurysm, akinesia, dyskinesia	15 (75%)
PALX RVOT ≥32 mm	9 (45%)
PSAX RVOT ≥36 mm	14 (70%)
Fractional area change ≤33%	0
<b>24-hour Holter monitoring</b>	
Ventricular extrasystoles in 24h≥500	13 (65%)
<b>Cardiac magnetic resonance</b>	
Structural changes	6 (30%)
RVEDV/BSA >100 mL/m <sup>2</sup>	1 (5%)
RV EF <45%	3 (15%)
<b>Therapy</b>	
Beta blockers	9 (45%)
RFA	7 (35%)
ICD	3 (15%)
<b>Sudden cardiac death risk</b>	
High risk	4 (20%)
Moderate risk	13 (65%)
Low risk	3 (15%)

During the second visit (after 1 year), no patient had noteworthy changes in the standard ECG recording. Right ventricular aneurysm developed in two patients during 1 year, and non-sustained ventricular tachycardia was detected during 24-hour Holter monitoring in one patient. Detailed characteristics of the study group are described in Table 1.

#### Genetic data

Different genetic variations previously described as benign were found in the *PKP2* and *DSG2* genes (Tables 2 and 3). All the patients had at least two different genetic variations. The maxi-

**Figure 1.** Family tree of the patient with the c.1592T>G genetic variation

imum genetic variation for one patient is 11. Three novel, unregistered, likely benign genetic variations were found in the *PKP2* gene: c.2489+131G>A, c.2489+72delA, and c.1035-5T>C and three were found in the *DSG2* gene: c.404G>A, c.1107G>A, and c.379-15A>G (Table 4). These identified genetic variations were likely benign because there were no changed amino acid sequences in the protein (missense variant) or changes in the splice site, and there were no reports regarding their clinical importance. Using multiple computational tools, such as PolyPhen, Sift, SNAP, PhD-SNP, and others, to predict the effect of an individual, it was also visible that these genetic variations are likely benign. For variant, c.404G>A, where amino acid is changed, due to the high population frequency is suggesting that variant is benign according to the ACMG classification (BS1 criteria). Clinical significance of genetic variation c.430G>A recently is described as uncertain, but after analyzing the effect of amino acid substitution by using multiple computational tools, this variation likely appears to be benign (16). One genetic variation (located in splice site) c.2489+1G>A is reported in ClinVar database as pathogenic/likely pathogenic. No specific functional studies have been conducted for this splice site genetic variation, but it is predicted that the added amino acids interfere with the structure of the repeat arm units, which could result in the weak binding of desmosomal proteins (17).

One of the identified missense genetic variants c.1592T>G (MAF in European populations 0.006), which was previously described only in the heterozygous state and reported in the ClinVar database as likely benign in one of patients, was identified using Sanger sequencing as homozygous. Using multiple computational tools, c.1592T>G was found more likely to be deleterious (Table 5). By comparing the MAF of the mutation with the Latvian and European populations, it appeared that c.1592T>G is not found in the Latvian control population and is statistically significant and commonly presented in ARVD-C patients com-

**Table 2. Identified previously described PKP2 gene genetic variations**

Variation	Protein change	SNP db#	Frequency in ARVD patients	Frequency in LV individuals	Frequency in EUR individuals
<b>PKP2 gene benign genetic variations</b>					
c.1097T>C	p.(Leu366Pro)	rs1046116	0.10	0.17 (P=0.43)	0.21 (P=0.24)
c.2145+45G>A	p.(=)	rs10772008	0.22	NA	0.19 (P=0.5)
c.2145+72A>G	p.(=)	rs7967264	0.04	NA	0.067 (P=1)
c.2578-69G>A	p.(=)	rs7956824	0.45	NA	0.32 (P=0.08)
c.2489+14insC	p.(=)	NA	0.10	0.13 (P=0.58)	NA
c.2300-21delG	p.(=)	rs57690055	0.12	0.13 (P=1)	NA
<b>PKP2 gene pathogenic genetic variation</b>					
c.2489+1G>A	Splice site affected	rs111517471	0.02	0	0
c.1592T>G	p.(Ile531Ser)	rs147240502	0.07	0	0.006 (P=0.0005)

**Table 3. Identified previously described, benign DSG2 genetic variations**

Variation	Protein change	SNP db#	Frequency in ARVD patients	Frequency in EUR individuals
c.828+16C>A	p.(=)	rs3737378	0.12	0.27 (P=0.049)
c.877A>G	p.(Ile293Val)	rs2230234	0.18	0.10 (P=0.13)
c.861C>T	p.(=)	rs2230233	0.33	0.38 (P=0.48)
c.1014+27G>A	p.(=)	rs62095194	0.12	0.28 (P=0.03)
c.2505A>G	p.(=)	rs1042769	0.12	0.10 (P=0.59)
c.3321T>C	p.(=)	rs1791235	0.40	0.37 (P=0.69)
c.1174G>A	Val392Ile	rs193922639	0.04	0 (P=0.038)
c.2318G>A	p.Arg773Lys	rs2278792	0.15	0.28 (P=0.07)
c.2137G>A	p.Glu713Lys	rs79241126	0.07	0.07 (P=1)
c.1651+70T>C	p.(=)	rs2276150	0.15	0.5 (P=0.0001)
c.828+13C>A	p.(=)	rs180926981	0.04	0 (P=0.038)
c.430G>A	Glu144Lys	rs199842209	0.04	0 (P=0.038)

**Table 4. PKP2 and DSG2 genes novel, likely benign genetic variations**

Variation	Protein change	Frequency in ARVD patients	Frequency in LV individuals
<b>PKP2 gene</b>			
c.2489+131G>A	p.(=)	0.14	0.19 (P=0.8)
c.2489+72delA	p.(=)	0.25	0.12 (P=0.13)
c.1035-5T>C	p.(=)	0.5	0.5 (P=1)
<b>DSG2 gene</b>			
c.1107G>A	p.(=)	0.04	0 (P=0.96)
c.379-15A>G	p.(=)	0.04	0.3 (P<0.001)
c.404G>A	Arg135Lys	0.04	0.05 (P=0.52)

pared to the unaffected individuals in the European population. Parents of the index patient are from unrelated marriages, and genetic variation c.1592T>G is found in a heterozygote form for

both. Two siblings of the index patient were also tested. One of the siblings is heterozygote, and the other sibling was not affected. The family tree is shown in Figure 1. Homozygote overall

**Table 5. Possibility of mutation being pathogenic**

Variation	PredictSNP	MAPP	PhD-SNP	Poly Phen-1	Poly Phen-2	SIFT	SNAP
c.1592T>G	72%	56%	88%	33%	59%	79%	65%

frequency and agreement with phenotype is suggesting that this variant in homozygote state could be pathogenic, conforming the ARVD-C diagnose molecularly.

## Discussion

The registry of ARVD-C patients in Latvia is the first one in the Baltic States. It is important to identify ARVD C patients and their first-degree relatives for the evaluation of the clinical diagnosis, long-term outcome, and therapy.

The standard ECG results showed that the Epsilon wave, which is a typical finding of ARVD-C, was rarely seen in our registry patients. The bipolar Fontaine precordial ECG leads I–III may also be used to enhance the recording of Epsilon waves (18). In our opinion, standard ECG sensitivity and specificity is not significant, and the standard ECG part in Task Force Criteria 2010 should be reviewed. Other investigation methods, such as surface multi-lead ECG, which is a relatively new; non-invasive method; or right-sided precordial lead electrocardiography (R-ECG), is used to detect the Epsilon waves (19). Conceivably, some of these methods might be considered in the ARVD-C diagnostic criteria.

Although *PKP2* is the most common gene that is affected in ARVD-C patients, of the 20 patients, only one case showed a likely pathogenic genetic variant. Moreover, the present study is also one of several studies that has shown multiple allelic variation identification in ARVD-C patients, although those are not pathogenic. Negative genetic testing may be due to genetic variants within other ARVD-C associated genes, undiscovered genes, or due to the presence of large duplications or deletions involving known genes (20). Some authors have stated that a conventional mutation screening fails to detect pathogenic genetic variants in up to 50% of index cases (7). Large duplications or deletions can be detected using microarray analysis and/or MLPA (7, 20). If there is no pathogenic mutation in others with ARVD-C associated genes, microarray analysis and MLPA should be done. Recent research has also shown a potentially new diagnostic method for ARVD – presence of anti-*DSG2* antibodies in patients with definite or borderline diagnosis – appears to be highly sensitive and specific for the detection of ARVD-C (21).

It was a challenge to evaluate the pathogenicity of newly discovered genetic variations; this problem is more actual in all types of genetic studies that involve new variant identification (22, 23) in all fields (24). Firstly, after analyzing genetic variations' pathogenicity in the official ARVD-C genetic database, we realized that the last update in this website was in February 2015 (25).

It is important to submit and verify all variations in such projects as the ClinVar database, where all the data are regularly updated and interpreted by common guidelines to prevent missing new, potentially pathogenic variations. Special software programs (PolyPhen, SIFT, SNAP, and others) were used to predict the effect of amino acid substitutions and to analyze single nucleotide variant possible pathogenicity. Secondly, the MAF comparison of genetic variation between patients and healthy individuals is important and can be used as evidence of benignity, but also can lead astray, as there remains a possibility of incomplete penetrance, variable expression, or age dependent effects (26). The clinical guidelines by the American College of Medical Genetics can be used for interpretation of sequence variants' pathogenicity (15). Functional studies are an important tool in support of pathogenicity (15), and the effects of genetic variations, both exonic and intronic, could be shown by experimental mRNA analysis (27). Animal models also play an important role in understanding the pathogenicity of ARVD-C. Not only knockout mouse models, where targeted deletion of single desmosomal genes is done, but also cardiac-specific transgenic mouse models with specific genetic variations have shown new insights in the etiology of ARVD-C (28, 29). After using the aforementioned methods to predict genetic variation pathogenicity, the results are sometimes ambiguous. In the case of ARVD-C, functional studies are also difficult to accomplish, and animal models are mainly for understanding the general pathogenesis of ARVD-C (30) and definitely cannot be used in daily practice in a clinical laboratory for the identification of pathogenicity; therefore, risk stratification remains challenging.

All newly discovered, unregistered genetic variations are synonymous, which are mostly interpreted as benign; however, they are not always so innocent (31). Synonymous variations can affect processes, such as transcription, splicing, translation, and protein secondary structure formation (32).

## Study limitations

As the awareness of ARVD-C as a diagnosis has been raised quite recently, our registry still has very small proportion of what is thought should be in the Latvian population. Consequently, conclusions of most common genetic variants and thus time and cost effective genetic screening in suspected individuals is restricted. Functional studies to confirm unknown genetic variations' pathogenicity were not performed.

Also, due to technical limitations, we were not able to estimate criteria, such as fQRS, terminal QRS, and terminal activation duration of QRS.



## Conclusion

The Latvian ARVD-C registry has been established. The prevalence of the majority of non-pathological genetic variations is similar in Latvian ARVD-C patients and European population. Possibly, pathogenic variations were found only in 10% of our registry patients, which could mean that *PKP2* and *DSG2* are not the most commonly affected genes in the Latvian population. For the first time, c.1592T>G was found in the homozygote form and inherited in an autosomal recessive trait, which is not common for ARVD C. Further studies are warranted, and other ARVD-C associated genes will be sequenced to genetically confirm the ARVD diagnosis and help determine the risk for first-degree relatives.

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