Clinical Classification of Arrhythmogenic Right Ventricular Cardiomyopathy

Yulia Lutokhina\(^a\) Olga Blagova\(^a\) Alexander Nedostup\(^a\)
Svetlana Alexandrova\(^b\) Anna Shestak\(^c\) Elena Zaklyazminskaya\(^c\)

\(^a\)Department of Cardiology of the V.N. Vinogradov Faculty Therapeutic Clinic, I.M. Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russia; \(^b\)Department of Computer and Magnetic Resonance Tomography, A.N. Bakoulev Center for Cardiovascular Surgery RAMS, Moscow, Russia; \(^c\)Laboratory of Medical Genetics, B.V. Petrovsky Russian Research Center of Surgery, Moscow, Russia

Keywords
Arrhythmogenic right ventricular dysplasia/cardiomyopathy · Premature ventricular beats · Ventricular tachycardia · Chronic heart failure · Left ventricular noncompaction

Abstract

**Introduction:** Commonly accepted clinical classification of arrhythmogenic right ventricular cardiomyopathy (ARVC) is still not developed. **Objective:** To study the clinical forms of ARVC. **Methods:** Fifty-four patients (38.7 ± 14.1 years, 42.6% men) with ARVC. Follow-up period: 21 (6–60) months. All patients underwent electrocardiography, 24 h-Holter monitoring, echocardiography, and DNA diagnostic. Magnetic resonance imaging was performed in 49 patients. **Results:** According to the features of clinical course of ARVC, 4 clinical forms were identified. (I) Latent arrhythmic form (\(n = 27\)) – frequent premature ventricular contractions and/or nonsustained ventricular tachycardia (VT) in the absence of sustained VT and syncpe; characterized by absence of fatal arrhythmic events. (II) Manifested arrhythmic form (\(n = 11\)) – sustained VT/ventricular fibrillation; the high incidence of appropriate implantation of cardioverter-defibrillator (ICD) interventions (75%) registered. (III) ARVC with progressive chronic heart failure (CHF, \(n = 8\)) as the main manifestation of the disease; incidence of appropriate ICD interventions was 50%, mortality rate due to CHF was 25%. (IV) Combination of ARVC with left ventricular noncompaction (\(n = 8\)); characterized by mutations in desmosomal or sarcomere genes, aggressive ventricular arrhythmias, appropriate ICD interventions in 100% patients. Described 4 clinical forms are stable in time, do not transform into each other, and they are genetically determined. **Conclusions:** The described clinical forms of ARVC are determined by a combination of genetic and environmental factors and do not transform into each other. The proposed classification could be used in clinical practice to determine the range of diagnostic and therapeutic measures and to assess the prognosis of the disease in a particular patient.

© 2020 S. Karger AG, Basel
Introduction

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a hereditary myocardial disease with a predominant RV involvement characterized by ventricular arrhythmias and a high risk of sudden cardiac death (SCD) [1]. Its prevalence is 1:2,000–1:5,000 [2], which makes it impossible to classify ARVC as a rare cardiomyopathy. The early diagnosis and treatment of ARVC and prevention of SCD are extremely important.

ARVC was first described in 1977 [3] and over the past 4 decades, a great deal of information has been accumulated. A real breakthrough was made in the study of molecular genetic mechanisms of the disease development and progression [4]. But there is still no unified clinical classification of ARVC, which would define approaches to the treatment of patients.

The first classification was developed by Fontaine et al. [5], based on a 9-year observation of 4 patients with different clinical course of ARVC: 3 clinical forms were identified. Three years later another variant of the ARVC classification was proposed, in which the RV and left ventricular forms were distinguished and a total of eleven different clinical forms were identified [2]. None of these classifications was widely used in clinical practice, as they did not define prognosis and approaches to treatment. Thus, the development of clinical classification seems to us an important task and an ultimate challenge.

Methods

The research was conducted at the V.N. Vinogradov Faculty Therapy Clinic of I.M. Sechenov First Moscow State Medical University (Sechenov University) in 1997–2019. The investigation conforms with the principles outlined in the Declaration of Helsinki and was approved by the University’s Local Ethics Committee. All patients signed an informed consent to participate in the study. The study included 54 patients with ARVC diagnose in accordance with the Task Force Criteria 2010 (definite diagnosis was established if the patient fulfilled 2 major or 1 major and 2 minor criteria from different categories; borderline if the patient fulfilled 1 major and 1 minor or 3 minor criteria from different categories; possible if the patient fulfilled: 1 major or 2 minor criteria from different categories) [6]. The average age was 38.7 ± 14.1 years (18–79), 31 women and 23 men (57.4 and 42.6%, respectively). A definite diagnosis of ARVC was established in 34 patients (63%), borderline – in 18 (33.3%), and possible – in 2 (3.7%). Patients were observed 21 (6–60) months (from 1 month to 19 years). The period from the onset of the disease to the last contact with the patient was 6.5 (2.9–10.3) years.

Clinical Examination of Patients

All patients underwent initial electrocardiography (ECG) in 12 leads, transthoracic echocardiography (EchoCG), and 24-h ECG monitoring. Cardiac magnetic resonance imaging (MRI) was performed in 49 (90.7%) patients, signal-averaged ECG – in 18 (33.3%), computed tomography of the heart – in 17 (31.5%). EchoCG (n = 28 [52%], the period between the initial and the last study of 30 [12–59] months) and 24-h ECG monitoring (n = 31 [57.4%], the period between the initial and the last study of 28 [12–60] months) were estimated in dynamics. Conventional visual criteria were used to diagnose left ventricular noncompaction syndrome (LVNC, [7–10]) in the combination with ARVC. For a definite diagnosis of LVNC, it was confirmed by at least 2 from imaging methods (EchoCG, MRI, computed tomography). According to the main clinical manifestation of ARVC, the patients were divided into 4 phenotypes: (1) patients with frequent premature ventricular contractions (PVCs) with/without nonsustained ventricular tachycardia (VT); (2) patients with sustained VT; (3) patients who have prominent symptoms of chronic heart failure (CHF), RV dilatation with an RV ejection fraction (EF) decrease and the presence of a severe tricuspid regurgitation; (4) all patients with combination of ARVC with LVNC.

Genetic Examination of Patients

All patients underwent DNA-diagnostics. Analysis of coding and adjacent intron sequences of genes from the ARVC diagnostic list was carried out: plakoglobin 2 (PKP2), desmoglein 2 (DSG2), desmoplakin (DSP), desmocolline, placoglobin, transmembrane protein 43, transforming growth factor beta-3, phospho-
lamban, lamin, desmin, αT-catenin, as well as genes of emerin, α-subunit of sodium channels, LIM-binding domain, crystalline and filamin C (FLNC). The analysis was performed by direct bi-directional Sanger’s sequencing or high-performance semiconductor sequencing on the PGM IonTorrent platform. The presence of the revealed mutations was confirmed during sequencing by Sanger. Further the bioinformatic analysis of the effect of the detected mutations was performed.

**Treatment of Patients**
Medication treatment included selection of optimal antiarrhythmic and cardiotropic therapy. Interventional treatment included radio-frequency ablation (RFA) in frequent monomorphic PVCs or sustained VT and implantation of cardioverter-defibrillator (ICD). Antiarrhythmic or interventional treatment was considered effective in case of complete suppression of both sustained and nonsustained VT and reduction of PVCs number by at least 75%.

**Statistical Analysis**
IBM SPSS Statistics version 22 was used. Discrete data are presented as absolute values and percentages. Continuous data are presented in the form of arithmetic mean ± mean square deviation in case of normal distribution or in the form of quartiles 50 (25–75) if the distribution differs from normal. The Wilcoxon criterion was used to estimate the differences in repeated measurements. Statistically significant differences were considered to be those at \( p \leq 0.05 \). Graphically, the survival rate, in dependence on the clinical form, is presented in the form of Kaplan-Meyer curves.

**Results**
In accordance with the peculiarities of clinical manifestations and course of the disease, we have identified 4 clinical forms of ARVC:
(I) Latent arrhythmic form – frequent PVCs and/or nonsustained VT in the absence of sustained VT and syncope;
(II) Manifested arrhythmic form – sustained VT/ventricular fibrillation (VF);
(III) ARVC with progressive CHF as the main manifestation of the disease;
(IV) Combination of ARVC with LVNC.

Summary clinical parameters of patients with different clinical forms of ARVC are presented in Table 1.

1. Latent arrhythmic form is manifested by frequent PVCs and/or nonsustained VT, stable VT is not registered. This variant was diagnosed in half of the patients \((n = 27)\). Among the patients, \(2/3\) were women. Average age was 36.7 ± 11.9 years (from 18 to 69 years). Follow-up was 26 (12–60) months. A definite diagnosis was made in 51.9% of patients, a borderline in 44.4%, and a possible in 3.7%.

In most patients, ARVC diagnosis was suspected due to frequent PVCs: 20 (9.5–36) 1,000/day, family history of SCD (11.1%), and syncope (11.1%). On the ECG of 4 patients, ɛ-wave was revealed, late potentials (LP) were registered in 7 patients, and in 2 patients there was an increase of terminal activation duration of QRS > 55 ms. Repolarization abnormalities were noted in 8 patients: in 5 negative T wave in leads V1–3, and in 3 – in leads V1–2. In 18.5% of patients, low QRS voltage was registered. Normal ECG (except PVCs) was registered in 15 (55%) patients.

In cardiac MRI, the increase in the end-diastolic diameter (EDD) of the RV > 4.0 cm was observed in 11 patients, but the ratio of the end-diastolic volume to the body surface area was beyond the reference Task Force Criteria 2010 values only in 5 (18.5%). The main MRI sign of suspected ARVC was a decrease in the RV EF. A helpful MRI sign was also the fat in the myocardium of RV which was noted in 55.5% of patients.

Pathogenic mutations were found in 3 patients in the DSG2, FLNC, and α-subunit of sodium channel genes. In addition, in 3 patients in the genes associated with ARVC (PKP2,
Placoglobin, and LIM-binding domain), variants of unknown clinical significance were found.

Patients with this clinical form were distinguished by a relatively benign clinical course of the disease and a good response to the treatment. RFA was performed in 7 patients (25.9%) with frequent PVCs, in 2 of them RFA was performed repeatedly. The intervention was effective in 5 out of 7 patients (71.4%). In the rest of the patients, frequent PVCs were suppressed by antiarrhythmic therapy. In 50% of patients, IC class antiarrhythmic drugs (aethacizin [Diethylaminopropionyletoxycarbonylaminophenothiazine], lappaconitin hydrobromide) or their combination with sotalol were effective, 50% required amiodarone. The ICD was implanted in only 1 patient due to frequent nonsustained VT, syncope, and sinus node dysfunction. There were no appropriate ICD shocks. No fatal arrhythmic events or outcomes have been recorded during follow-up.

2. The manifested arrhythmic form is expressed by sustained VT (usually from RV) and/or VF. This form is diagnosed in 11 (20.4%) patients. Women prevailed (n = 7, 63.6%), average age was 36.1 ± 17.4 years (from 18 to 71 years). The follow-up was 51 (6–107) months. The diagnosis of ARVC is definite in 7 patients and borderline in 4 patients. Family history was burdened by SCD in 3 (27.3%) patients, 7 (63.6%) had syncope.

In the ECG typical changes were noticed. Thus, a typical ɛ-wave was registered in 3 patients, in 1 patient LP were detected, and in 1 patient – an increase of terminal activation duration of QRS up to 65 ms. Negative T waves in the leads V1–2 were detected in 1 patient, and in V1–3 and further – in 4 patients. ECG-criteria of ARVC were registered in 8 patients, in 3 ECG was normal.

At cardiac MRI the increased indexed volume of RV was registered only in 2 patients, the increase of its EDD >4.0 cm was detected in 5, and the decrease of RV EF <45% – in 2 patients. The detection of fat in the RV was found in 6 out of 11 patients.
In 5 patients (45.5%), the diagnosis is genetically verified: mutations in the gene PKP2 ($n = 3$) prevailed, in 1 patient, a mutation in the gene transmembrane protein 43 was detected, in another patient, a mutation in the gene DSG2 in combination with a mutation in the desmin gene. Clinical course and outcome of the disease were less favorable than in the latent arrhythmic form. RFA by endocardial access was performed in 5 patients. The good clinical effect was achieved only in 3 (60%) patients. As antiarrhythmic drugs, 45.5% of patients received amiodarone, and approximately one-third of patients received aethacizin (IC class drug, 36.4%). The ICD was implanted in 4 (36.4%) patients with appropriate shocks in 3 (75%), including 1 patient with multiple shocks due to VF. There was a high mortality rate: 18.2% arrhythmic deaths.

3. ARVC with a progressive CHF. Signs of CHF in our cohort were noted in 21 (38.9%) patients; however, in this form, we have included only patients who have prominent symptoms of CHF, RV dilatation with an EF decrease, and the presence of a severe tricuspid regurgitation. The group included 8 (14.8%) patients, men prevailed (62.5%), the average age was 45.8 ± 19.6 years, and the observation period was 10.5 (4.25–19.25) months.

The diagnosis was definite in all patients. They had a significantly lower number of PVCs than other clinical forms of ARVC: 1,580 (492–3,540)/day. Family history of SCD was noted in 1 patient, syncope – in 3 patients. Along with RV failure, almost everybody had some degree of LV involvement: LV EF < 50% was noted in 87.5%, and the negative T waves in the left, and/or lower chest leads in 75%.

At least one large ARVC ECG-criterion was registered for each patient. Half of the patients had $\varepsilon$-wave, 75% – negative T wave in right precordial leads, and 50% had a low voltage of QRS complex. MRI was performed in 6 out of 8 patients. In 5 patients, the increase of RV EDD (54.3 ± 12.6 mm) was marked. RV EF was estimated in 3 patients: in all patients, it was reduced (25.7 ± 15.0%).

The mutations in the genes DSG2, DSP, desmocolline, and FLNC were detected in half of the patients. Clinical course and outcome were the least favorable. RFA was successfully performed in 1 patient with atrial flutter. Antiarrhythmic therapy included amiodarone (87.5%) and sotalol (12.5%). The ICD was implanted in 4 (50%) patients (secondary prevention in 3) with appropriate shocks in half of the patients. The frequency of fatal outcomes was the highest and reached 50%: 2 patients dyed from terminal CHF, one from stroke, one from cancer.

4. ARVC in combination with LVNC. This clinical form included 8 (14.8%) patients with 2 genetically determined cardiomyopathies simultaneously. The males prevailed ($n = 5$, 62.5%), the average age was 41.6 ± 7.8 years, the follow-up was 12 (2.25–40) months. All 8 patients had no doubts about the diagnosis of LVNC; diagnosis of ARVC was definite in 5 patients, in 2 – borderline, and in 1 – possible. The patients were characterized by severe arrhythmias. The number of PVCs was 19.5 (7.1–31.2) thousand. Sustained VT was registered in 4 patients, syncope were registered in 3 patients. One patient had a case of SCD in the family.

On ECG of 3 (37.5%) patients $\varepsilon$-wave was registered, in one more patient, $\varepsilon$-wave was present unsteadily. Two patients had LP, and 2 more had an increase of terminal activation duration of QRS up to 80 ms. Typical negative T waves in the right precordial leads were registered only in 2 cases, in 2 cases negative T waves were registered in the left precordial leads, and in 3 others – in the inferior ones, apparently due to the presence of LVNC. Low QRS voltage was found in half of the patients. By MRI, 62.5% of patients had RV dilatation, and 37.5% had a decrease in RV EF. This clinical form of ARVC was distinguished from clinical form of ARVC with progressive CHF by bigger size of the LV, but lower class of CHF.

Pathogenic mutations were found in 37.5%: 2 patients had a mutation in the DSP gene, and 1 patient had a combination of 2 mutations in the MYH7 and MyBPC. In other patient, a variants of unknown clinical significance in the PKP2 gene was found.
RFA was performed only in patient with mutations in MYH7 and MYBPC3 genes and was ineffective. Amiodarone was administered in 87.5%, and sotalol was effective in 12.5%. The ICD was implanted in 4 (50%) patients with appropriate shocks in 100% (Fig. 1). Lethality was 12.5% (1 SCD in a patient without an ICD).

Follow-Up of the Patients
Evaluation of 24-h ECG monitoring in dynamics was carried out on the background of the most effective antiarrhythmic therapy. Positive response to antiarrhythmic therapy was observed in most of patients: number of PVCs per day significantly decreased, sustained VT was completely suppressed, and frequency of registration of nonsustained VT decreased threefold (Table 2). No patient with a latent arrhythmic form has developed a sustained VT during follow-up.

We also evaluated the EchoCG parameters in the dynamics. During the analysis of EchoCG parameters of patients from all clinical forms together negative dynamics (progressive dilatation of heart chambers, reduction of LV EF, tricuspidal regurgitation deterioration) was not observed in any of the indicators. As a result of evaluation, the Echo parameters separately in each clinical form, significant increasing of LV EDD (5.4 ± 0.9 vs. 6.0 ± 1.3 cm, \( p = 0.04 \)) and tendency to decrease of LV EF (47.1 ± 3.4 vs. 42.6 ± 7.0%, \( p > 0.05 \)) were noted only in ARVC with progressive CHF.

Thus, in our cohort, there was no transition of patients from one clinical form to another during the follow-up 21 (6–60) months. A careful analysis of the patients histories also did not show any transition from one clinical form to another (a period from the onset of the

Table 2. Structure of ventricular rhythm abnormalities in the dynamics in patients with ARVC

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Initial</th>
<th>During follow-up</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVCs, 1,000/day, median (IQR)</td>
<td>15.3 (3.1–30.9)</td>
<td>0.8 (0.024–3.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sustained VT, %</td>
<td>33.3</td>
<td>0</td>
<td>0.001</td>
</tr>
<tr>
<td>Nonsustained VT, %</td>
<td>60.6</td>
<td>21.2</td>
<td>0.002</td>
</tr>
</tbody>
</table>

ARVC, arrhythmogenic right ventricular cardiomyopathy; PVCs, premature ventricular contractions; VT, ventricular tachycardia.
disease to the last contact with the patient has significantly exceeded the observation period and was 6.5 [2.9–10.3] years). Moreover, the survival in dependence of clinical form was different as well (Fig. 2). The mortality rate was 0, 18.2, 50, and 12.5% in the I, II, III, and IV forms, respectively.

**Discussion**

Although ARVC was described >40 years ago, a single, universally accepted classification that would be used by clinicians in routine practice has not been developed.

In 1995, Fontaine et al. [5] proposed 3 forms of ARVC: (I) LV EF >50% and the arrhythmic risk can be controlled by appropriate antiarrhythmic therapy. (II) Variable degree of LV involvement (30 < EF <50%) either by extension of a disease process as observed in the right ventricle or by the phenomenon of myocarditis. This form may remain stable for many years. (III) Progressive degradation of the myocardium over a period of about 10 years with a clinical presentation comparable to DCM. In our opinion, disadvantage of this classification is the lack of separation of the arrhythmic form into latent and manifested, which is important for the diagnosis and choice of tactics of SCD prevention.

The following classification by Fontaine et al. [2] differed significantly from first. It includes isolated RV forms and ARVC with predominant involvement of LV and also Naxos’ disease, Venetian cardiomyopathy, and many other forms. This classification, in our opinion, is neither pathogenetically nor clinically valid.

What is the difficulty of developing a clinical classification of ARVC? The most obvious reason may seem to be its relatively low prevalence, but the current literature review data on the incidence of ARVC makes it less likely. To date, there are several large registers of ARVC patients and their relatives: Johns Hopkins University ARVD/C Registry, the Interuniversity Cardiology Institute of the Netherlands, and the Italian register of about 300 people (University of Pavia). However, none of the researchers have attempted to propose a classification for ARVC. This is probably due to the fact that among the large amount of statistical information about patients that is observed by different doctors in different cities. In our register, which is created within a single department, we can track and analyze the course of ARVC in details.
Anyway, the relatively small size of our cohort is one of the limitations of this study and its further expansion would be useful to improve our classification. In our opinion, it is essential to bring together all the scientists involved in the analysis of clinical course of ARVC and discuss classification issues.

Our full proposed clinical classification of ARVC is presented as follows:

1. Sudden arrhythmic death as the only clinical manifestation.
2. Latent arrhythmic variant:
   - isolated RV PVCs;
   - RV PVCs with episodes of nonsustained VT;
3. Manifested arrhythmic variant:
   - sustained VT/VF;
4. Variant with progressive CHF:
   - predominantly right heart failure;
   - biventricular heart failure;
5. Combination of ARVC with LVNC:
   - arrhythmic form without CHF;
   - biventricular heart failure;
6. Nonarrhythmic form of ARVC.

The greatest practical interest is represented by 4 clinical variants considered in the section “Results.” It is necessary to note the simplicity of the distinction between these clinical forms. Patient’s belonging to a particular clinical form make it possible to predict the further clinical course of the disease and to choose the optimal tactics of management and prevention of SCD. For example, treatment of patients with a latent arrhythmic form could be limited to prescribing antiarrhythmic therapy and/or conducting RFA in cases of frequent monotopic PVCs. Patients with manifested arrhythmic form are indicated to have ICD implantation due to the high risk of SCD. In the management of patients with ARVC with progressive CHF, optimal cardiotropic and diuretic therapy is necessary, and in combination with ARVC with LVNC, in addition to the treatment of CHF and rhythm disorders, ICD implantation is necessary, due to the high risk of SCD.

Suspicion of a particular clinical variant of ARVC allows to outline the spectrum of diseases for differential diagnosis. Thus, the latent arrhythmic form should be differentiated with idiopathic PVCs; the manifested arrhythmic form – with channelopathies, coronary heart disease, idiopathic VT; ARVC with progressive CHF – with DCM syndrome of other etiology (incl. inflammatory), pulmonary heart, and the combination of ARVC with LVNC- with isolated LVNC and DCM of different etiology.

There is the opinion, that clinical forms are only the phases in the natural course of ARVC [11]: an early concealed phase, overt electrical disorder, isolated right heart failure and biventricular pump failure. It is assumed, that the transition from one phase to another is conditioned by the progressive fibro-fat substitution of RV. The question is, do patients with ARVC always develop clinically significant CHF and when? In Fontaine’s study, it remains unclear whether 10 years should pass from the moment of the disease’s debut or from the previous form [5].

The incidence of CHF in ARVC varies from 4 to 49% [12], with the peak of symptoms occurring between 40 and 50 years old [13]. It is also widely recognized that there are both patients who have a virtually asymptomatic course of disease and those who develop severe biventricular heart failure at a young age [14]. As a result of observing our cohort of patients, we have the impression that the disease does not shift from one form to another. None of the patients with latent arrhythmic form had developed sustained VT during the whole period of follow-up, and none of the patients with arrhythmic forms had CHF symptoms in the first place. It was no worsening of EchoCG parameters in patients without initial CHF.
It is possible to suppose that some patients belonging to other clinical forms were not observed long enough to have significant clinical manifestations of CHF, but 2 patients with manifested arrhythmic form were over 70 years old. The average age of patients in the group with progressive CHF is higher than in other groups, but, first, in this group, there are 3 patients under 35 years of age, and second, the rest of the patients in this group had no period of “arrhythmic forms.” In any case, the relatively short follow-up period is a limitation of our research and further observation is required to confirm the stability of the forms definitively.

According to the available data, clinical forms are stable in the time and determined by a combination of genotype and environmental factors. This is consistent with the extremely variable course of ARVC even in patients with the same mutation within the same family [15].

**Conclusion**

On the basis of the analysis of clinical data and the natural course of the disease 4 stable in time clinical forms of ARVC were identified, which were not prone to transition: latent arrhythmic form, manifested arrhythmic form, ARVC with progressive CHF, and ARVC in combination with LVNC. Latent arrhythmic form is characterized by ventricular PVCs and/or nonsustained VT, absence of fatal arrhythmic events, and lethal outcomes. The manifested arrhythmic form is characterized by the presence of sustained VT, high frequency of appropriate ICD interventions (75%), and lethality (18%). At ARVC with progressive CHF, the right heart failure prevailed with a sharp decrease in its EF and a relatively preserved left heart, the frequency of appropriate ICD interventions was 50%, and the mortality rate was 25%. The combination of ARVC with LVNC may be based on mutations in both desmosomal and sarcomeric genes, this form is characterized by aggressive VT, appropriate ICD shocks were noted in 100%. This classification is simple to use, and it determines the approach to patient management, including the prevention of SCD, which makes it appropriate to use it in daily clinical practice.

**Disclosure Statement**

The authors have no conflict of interest to declare.

**Funding Sources**

Genetic investigation was supported by Russian Science Foundation Grant No. 16-15-10421.

**References**