ASSOCIATION OF POLYMORPHIC VARIANTS OF *ACE* AND *BDKRB2* WITH HEART RATE VARIABILITY IN ATHLETES OF THE REPUBLIC OF KARELIA

Kolomeichuk SN[1] ✉, Alekseev RV[2], Putilov AA[3], Meigal AYu[2]

[1] Laboratory of Genetics,
Institute of Biology, KarRC RAS, Petrozavodsk, Russia

[2] Department of Human and Animal Physiology, Pathophysiology and Histology, Medical Institute,
Petrozavodsk State University, Petrozavodsk, Russia

[3] Research Group for Math-Modeling of Biomedical Systems,
Research Institute for Molecular Biology and Biophysics, Novosibirsk, Russia

This work aims to study distribution of allele frequencies of the *ACE* and *BDKRB2* genes coding for the angiotensin-converting enzyme and the bradykinin receptor β2, respectively, in athletes specializing in different sports and to establish the associations between the studied genotypes and heart rate variability. The study included 75 male athletes. Polymorphisms of *ACE* and *BDKRB2* (I/D and +9/−9, respectively) were studied by PCR. A significant difference was revealed in the −9/−9 genotype frequency between the studied groups of athletes. Parasympathetic nerve activity prevailed in the athletes with the I allele of the *ACE* gene. Time-domain parameters of heart rate variability had low values in the carriers of the D/D genotype. In the athletes with the *ACE* I/I genotype the time-domain parameters differed from those typical for the I/D and D/D genotype carriers. Participants homozygous for −9 *BDKRB2* had the lowest heart rate in the studied sample, implying an increased contribution of parasympathetic activity to heart rate regulation. The −9 allele of *BDKRB2* was found to be associated with the minimal *R* — *R* interval between consecutive hear beats. We conclude that polymorphisms I/D of *ACE* and +9/−9 of *BDKRB2* can indicate individual patterns of heart rate regulation in athletes from the Republic of Karelia.

Keywords: training, sport specialization, heart rate variability, genetic polymorphism, *ACE*, *BDKRB2*

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✉ **Correspondence should be addressed:** Sergey Kolomeichuk
ul. Nevskogo, d. 50, Petrozavodsk, Russia, 185910; sergey_kolomeychuk@rambler.ru

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АССОЦИАЦИЯ ПОЛИМОРФНЫХ ВАРИАНТОВ ГЕНОВ *ACE* И *BDKRB2* С ПАРАМЕТРАМИ ВАРИАБЕЛЬНОСТИ СЕРДЕЧНОГО РИТМА У СПОРТСМЕНОВ РЕСПУБЛИКИ КАРЕЛИИ

С. Н. Коломейчук[1] ✉, Р. В. Алексеев[2], А. А. Путилов[3], А. Ю. Мейгал[2]

[1] Лаборатория генетики, Институт биологии,
Карельский научный центр РАН, Петрозаводск

[2] Кафедра физиологии человека и животных, патофизиологии, гистологии, медицинский институт,
Петрозаводский государственный университет, Петрозаводск

[3] Группа математического моделирования биомедицинских систем,
Научно-исследовательский институт молекулярной биологии и биофизики, Новосибирск

Целью настоящего исследования было изучение распределения частоты аллелей гена ангиотензинпревращающего фермента *ACE* и рецептора β2 брадикинина у спортсменов различной спортивной специализации, а также выявление взаимосвязи генотипа с параметрами вариабельности сердечного ритма. Методом ПЦР в группе атлетов (n = 75, мужчины) исследован полиморфизм генов *ACE* I/D и *BDKRB2* +9/−9. Показано достоверное отличие между группами спортсменов по частоте генотипа −9/−9 гена *BDKRB2*. Уровень парасимпатической активности преобладает у носителей аллеля I гена *ACE*. В группе спортсменов с генотипом D/D регистрируются низкие значения временных параметров вариабельности сердечного ритма. Согласно полученным данным, временные параметры ритма сердца спортсменов с генотипом *ACE* I/I отличаются от значений групп *ACE* I/D и *ACE* D/D. У гомозигот по аллелю −9 гена *BDKRB2* отмечены самые низкие значения ЧСС, что указывает на усиление парасимпатических влияний в системе регуляции сердечного ритма. Аллель −9 гена *BDKRB2* ассоциирован с минимальной продолжительностью последовательных сокращений сердца. Полиморфные локусы *ACE* I/D и *BDKRB2* +9/−9 можно рассматривать как контрольные показатели процесса регуляции параметров сердечной деятельности при проведении первичного отбора спортсменов в Республике Карелии.

Ключевые слова: тренировочный процесс, спортивная специализация, вариабельность сердечного ритма, генетический полиморфизм, *ACE*, *BDKRB2*

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✉ **Для корреспонденции:** Коломейчук Сергей Николаевич
ул. Невского, д. 50, г. Петрозаводск, 185910; sergey_kolomeychuk@rambler.ru

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As more young people are being recruited in professional sports, the latter is becoming more competitive demanding that athletes should be in perfect physical shape throughout the entire training period [1]. Normally, wise training and competition schedules that account for the individual capacity and functional ability of an athlete do not negatively affect performance or trigger pathology. But if physical capacities fail the athlete who is trying to handle extremely high training loads, the vegetative systems may respond with disease.

Many researchers regard the cardiovascular system as an indicator of individual's adaptability [1, 2]. The cardiovascular health of athletes is therefore in the focus of sports science. Based on the continuous monitoring of the cardiovascular system, training loads can be optimized, exercise tolerance assessed, and structural or morphological changes in the circulation predicted. According to the literature, cardiovascular abnormalities are the main cause of sudden death in athletes [2–5]. In this light, discovery of genetic markers that play a role in cardiovascular and muscular health is of great prognostic significance. Of particular importance are their associations with various phenotypes, including anthropometric data, results of load tests and cardiac interval measurements, etc. [6, 7].

Sports genomics is a relatively new discipline that studies the structure and functioning of athletes' genomes [8–11]. The first genetic marker associated with endurance was identified in the late 1990s [12]. The importance of genetic polymorphisms and their associations with athlete's performance and phenotype are widely discussed in the literature [11, 13, 14]. A few methodologies have been proposed to explore associations between genetic polymorphisms and professional achievements of an athlete. Population studies explore the associations between a particular genotype or allele and a phenotypic trait, e.g. oxygen consumption VO_2 max, in groups of athletes [8, 11]. An alternative approach is offered by whole-genome studies of polymorphic DNA markers that may be associated with certain physical characteristics [10, 12]. On the whole, association studies are the most common type of studies in sports genomics. They are based on the assumption that one allele referred to as candidate because of its known function is associated with the studied phenotypic trait, is relatively frequent in elite athletes in comparison with the general population and therefore enhances performance [10, 11, 12].

A review of the literature over the period between 1997 and 2014 revealed that at least 120 genetic markers are associated with the elite athlete status, including 77 genetic markers of endurance and 43 markers of power/strength. But only 11 (9 %) of those markers showed a stable association in 3 or more studies. Among the endurance markers are *ACE* I, *ACTN3 577X*, *PPARA rs4253778* G and *PPARGC1A Gly482*; power/strength markers are *ACE* D, *ACTN3 R577*, *AMPD1 Gln12*, *HIF1A 582Ser*, *MTHFR rs1801131* C, *NOS3 rs2070744* T and *PPARG 12Ala* [8].

The aim of this work was to study frequency distribution of *ACE* and *BDKRB2* allelic variants in athletes specializing in different sports and to establish as association between those allelic variants and parameters of heart rhythm in athletes from the Republic of Karelia.

METHODS

The study was conducted from October 2015 to May 2016 in the city of Petrozavodsk, the Republic of Karelia. The study was approved by the Bioethics Committee of the Institute of Biology KarRC RAS (Protocol No. 21/20/187 dated February 26, 2015). Participants gave their informed consent. The study recruited 75 athletes with different qualifications (from regional champions to Masters of Sports) specializing in different sports, aged 18 to 30 years. Depending on the quality trained, the athletes were distributed into 3 groups: *strength* (n = 25; bodybuilding, power lifting, box, wrestling), *speed* (n = 23; track and field sprinting, middle-distance running) and *endurance* (n = 27; skiing, long-distance running). The study included only male individuals over 18 years of age, with at least 5-year experience in sports and without chronic conditions.

Measurements were taken early in the morning. First, cardiac rhythm parameters and cardiovascular function were evaluated at rest. Anthropometric measurements included height (cm), weight (kg), fat mass (kg), muscle mass (kg), total body water (kg), bone mass (kg), body mass index (BMI, a ratio of body weight to height), and impedance. Height was measured using a stadiometer. Weight and other parameters were measured using the Tanita Body Composition Analyzer SC–330 S (Tanita, Japan).

In the second stage of the study parameters of the resting heart rhythm were analyzed and blood samples were collected for the genetic analysis. DNA was extracted from peripheral blood lymphocytes; the samples were analyzed using the shared facility equipment of the Institute of Biology KarRC RAS. Genomic DNA was extracted from 200 µL of venous blood using the AxyPrep Blood Genomic DNA Miniprep Kit (Axygen, USA) according to the manufacturer's protocol. The *ACE* (I/D) and *BDKRB2* (+9/−9) polymorphisms were studied using polymerase chain reaction and the restriction fragment length polymorphism analysis.

Fragments of the *ACE* and *BDKRB2* genes were amplified with the following forward and reverse primers: 5'-CTGGAGACCACTCCCATCCTTTCT-3 and 5'-ATGTGGCCATCACATTCGTCAGAT-3 for *ACE* and 5'-TCTGGCTTCTGGGCTCCGAG-3' and 5'-AGCGGCATGGGCACTTCAGT-3 for *BDKRB2*. Thermocycling conditions were as follows: initial denaturation at 94 °C for 7 min, followed by 30 amplification cycles at 94 °C (1 min), 62 °C (1 min), and 72 °C (1 min 10 s); final synthesis at 72 °C (5 min). PCR yielded a 477 b. p. product for the *ACE* I allele and a 190 b. p. product for the *ACE* D allele. PCR product sizes for the *BDKRB2* +9 and –9 alleles were 100 b. p. and 90 b. p., respectively. PCR was performed in the programmable thermocycler MaxyGene II (Applied Biosystems, USA) using

Table 1. Results of the bioelectrical impedance analysis of body composition conducted in the study participants

Specialization	n	Height, cm	Weight, kg	Fat mass, kg	Muscle mass, kg	Total body water, kg	Bone mass, kg	BMI, kg/m^2	Impedance
Endurance	27	178.9	72.2*	6.4*	62.6*	46.1*	3.3	22.5*	481.5*
Speed	23	178.6	72.9*	6.5*	63.1*	46.6*	3.4	22.8*	475.4*
Strength	25	177.3	85.1	11.6	69.9	51.4	3.7	26.8	447.9

Note. * — represents statistically significant differences ($p<0.05$) relative to the *Strength* group.

the amplification mixture ScreenMix-HS (Evrogen, Russia) and 25 μL of gene-specific primers.

PCR yield was analyzed by 6 % (for *ACE*) and 8 % (for *BDKRB*) polyacrylamide gel electrophoresis followed by ethidium bromide staining and visualization on the UV transilluminator ECX-F20 at 312 nm wavelength (Vilber Lourmat, France). ECG was recorded using the digital Poly-Spectrum-8/E system (NeuroSoft, Russia) according to the standard technique. Time domain (R — R min, R — R max, RRNN, SDNN, RMSSD, pNN50 and CV) and spectral (TP, VLF, LF norm, HF norm) parameters of heart rate variability (HRV) were computed using the Poly-Spectrum-Rhythm software (Neurosoft, Russia).

Significance of differences in population frequencies was estimated using the standard χ^2 formula (Microsoft Excel). Differences between the groups and factor effects on HRV parameters were estimated by ANOVA and the H-test (STATGRAPHICS Centurion XVI, Statpoint Technologies, USA).

RESULTS

The study was conducted in 75 athletes. The participants were divided into 3 groups depending on their specialization. Comparison of the *Strength* and *Speed* groups revealed that power athletes weigh more, have bigger fat and muscle masses and a higher BMI ($p < 0.05$, Table 1). In the run-up for the competitions body fat percentage is relatively high in power athletes because their diet becomes more diversified.

Significant differences were observed between the *Strength* and *Endurance* groups with regard to almost all studied parameters ($p < 0.05$). The only unreliable difference was registered for height and bone masses. Obviously, differences in weight, fat, muscle and total water masses, as well as BMI, were significant because athletes who train strength and those who train endurance have different phenotypes. Those who train their strength are often hypersthenic (a massive build, a broad frame). Athletes who train their endurance are asthenic (a slender narrow build).

Time domain parameters of heart rate variability in the *Endurance* group differed significantly from those in the *Strength* and *Speed* groups (Table 2).

The highest proportion of significant differences was observed between the *Strength* and *Endurance* groups, namely in mean heart rate, R — R min, R — R max, and RRNN. In this respect patterns of cardiac rhythm modulation in power athletes and stayers are opposing: increased sympathetic vs. vagal modulation.

Based on the spectral analysis, fatigue can be estimated and changes in the physical capacity can be predicted during training and competition periods (Table 3).

The spectral analysis revealed that values of the total power of the spectrum (the total effect of all regulatory mechanisms) were very high in the *Speed* and *Endurance* groups. It is believed that the higher the total power of the spectrum, the lower the strain on the regulatory systems. A considerable contribution to TP can be made by the parasympathetic component (high frequency power spectrum, HF), varying depending on the rate and depth of respiration during measurements.

No significant differences were observed in the spectral parameters of heart rate variability between the groups (Table 3).

That said, the values of spectral parameters were on the whole consistent with patterns of adaptation to different types of physical exercise. The total power of the spectrum tended to be higher in the *Speed* and *Endurance* groups due to the prevalence of sympathetic and parasympathetic effects on the cardiac rhythm. Humoral and metabolic effects on heart function were also significant. Parasympathetic modulation of sinoatrial node activity was a prevalent modulation pattern in the *Strength* group, which is probably due to the lack of exhaustion and strain on the regulatory systems in the beginning of the training cycle.

The obtained allelic frequencies of 4 potential markers indicating an association between the genes and blood pressure are consistent with the data previously obtained for other Russian and European populations [8, 10, 11]. Frequencies of the *ACE* I/D genotype varied in Karelia athletes depending on their specialization (Fig. 2). No significant differences were

Table 2. Time domain parameters of heart rate variability

Specialization	n	Mean heart rate	R — R min, ms	R — R max, ms	RRNN, ms	SDNN, ms	RMSSD, ms	pNN50, %	CV, %
Strength	25	66.0	747.8	1124.9	945.6	60.7	59.7	32.8	6.2
Speed	23	59.7	836.3*	1226.2	1020.8	70.0	64.9	37.8	6.9
Endurance	27	57.3*	835.7*	1258.0*	1068.9*	75.6	66.5	39.5	6.9

Note. R — R min and R — R max are minimal and maximal R-R (beat-to-beat) intervals; RRNN is mean normal-to-normal R-R interval; SDNN is standard deviation of normal-to-normal R-R intervals; RMSSD is root-mean square differences of successive NN intervals; pNN50, % is percentage of successive NN intervals with a >50 ms difference; CV is a variation coefficient. * represents statistically significant differences relative to the *Strength* group (tcrit. = 2.008; $p < 0.05$). Differences in time domain parameters of heart rate variability between the groups were estimated using the nonparametric Mann–Whitney U-test.

Table 3. Spectral parameters of heart rate variability of the participants

Specialization	n	TP, ms^2	VLF, ms^2	LF, ms^2	HF, ms^2	LF norm	HF norm	LF/HF
Strength	25	4167.7	1229.8	1059.4	1878.2	46.3	53.7	1.2
Speed	23	5688.4	1737.8	2076.8	1866.9	46.8	53.2	1.2
Endurance	27	5443.8	2201.2	1460.3	1782.4	44.0	56.1	1.2

Note. TP is total power of the spectrum; VLF is very low frequency oscillations; LF is low frequency oscillations; HF is high frequency oscillations; LF norm and HF norm are normalized low and high frequency oscillations, respectively.

observed between the *Strength* and *Speed* groups (χ^2 = 0.35; d. f. = 2, p = 0.72) and between the *Strength* and *Endurance* groups (χ^2 = 1.71; d. f. = 2, p = 0.43). Frequencies of the *BDKRB2* I/D genotype in the participants also varied depending on their specialization (Fig. 2), but difference between the groups was insignificant.

Average values of time domain parameters of heart rate variability in the athletes divided into groups based on their *ACE* genotype (I/I, I/D, D/D) demonstrate that differences between the groups are genotype-associated (Table 4).

Time domain parameters of heart rate variability were different between the subgroups of athletes with the *ACE* I/D genotype and those with the *ACE* I/D and *ACE* D/D genotypes. Average heart rate values coincided with the bradycardia threshold (a minimal heart rate of 41 beats per min was registered in a Master of sports, professional skier, carrier of the *ACE* I/I genotype).

Parameters of the cardiac rhythm in I/D genotype carriers fell in the middle of the scale, between the I/I and D/D groups, representing an intermediate pattern of heart rate modulation.

The two-way ANOVA (factors involved were sports specialization and distribution of *ACE* genotypes I/I, I/D, and D/D) revealed a statistically significant difference in HF values between I/D and D/D genotype carriers (p < 0.05). The one-way ANOVA applied to the general sample (n = 75) showed that time domain parameters of HRV differed in their degree of variability.

Average values of spectral parameters measured in the carriers of different *ACE* genotypes (I/I, ID, D/D) reflect the share of sympathetic, parasympathetic and humoral-metaboloic contributions to heart rhythm modulation determined by the presence of the I or D allele in the athlete (Table 5).

In contrast, TP, VLF, LF and HF were low in D/D genotype carriers, which is consistent with contemporary views on cardiac rhythm modulation in power and speed athletes.

Differences in the degree of variability of HRV spectral parameters were estimated using the one-way ANOVA.

We discovered that HF values differed significantly between the I/I–I/D and I/D–D/D groups, LF — between the I/I–I/D and I/D–D/D groups, and TP — between the I/I–I/D and I/D–D/D groups. Parasympathetic modulation prevailed in the group of I allele carriers. This is consistent with the well-established association between the I allele and endurance.

Previously we showed that parasympathetic nervous activity is increased in endurance athletes. In the carriers of the D/D genotype vagal effects were less pronounced and the total power of the spectrum was lower. Such heart rate variability is often registered in power and power/speed athletes.

Thereby, we conclude that decreased values of major spectral parameters, including TP, LF, and HF, indicate that the athlete is ready to handle speed or strength training exercise while increased values indicate that he/she is ready for endurance training.

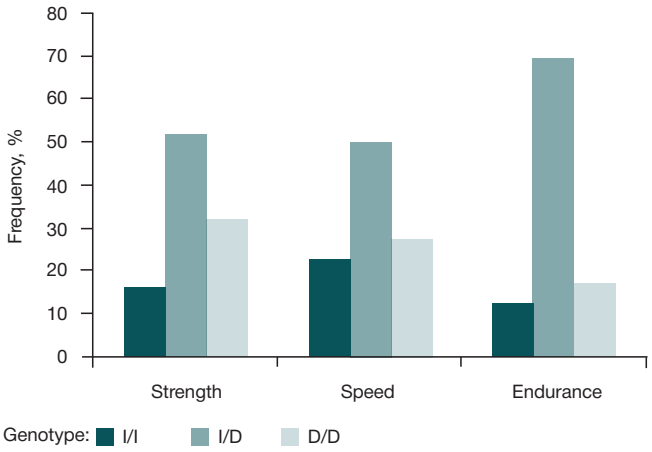

Fig. 1. Frequency distribution of *ACE* allelic variants in the athletes from the Republic of Karelia specializing in different sports

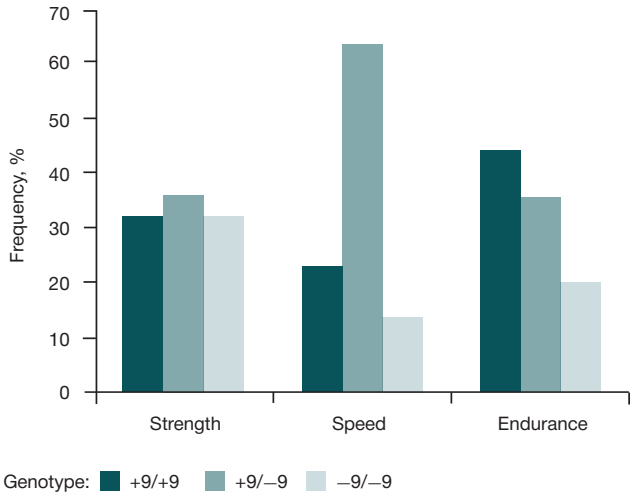

Fig. 2. Frequency distribution of *BDKRB2* allelic variants in the athletes from the Republic of Karelia specializing in different sports

Table 4. Time-domain parameters of heart rate variability in athletes with different *ACE* genotypes

ACE genotype	n	Mean heart rate	R — R min, ms	R — R max, ms	RRNN, ms	SDNN, ms	RMSSD, ms	pNN50, %	CV, %
I/I	14	60.0	843.4	1210.7	1028.4	67.1	60.0	34.4	6.4
I/D	40	60.4	800.8	1220.2	1019.5	71.8	69.4	39.2	6.9
D/D	21	62.3	792.0	1158.3	990.6	62.3	54.6*	33.1	6.1*

Note. * — represents significant difference relative to *ACE* I/I carriers (p < 0.05).

Table 5. Spectral parameters of heart rate variability in athletes with different *ACE* genotypes

ACE genotype	n	TP, ms^2	VLF, ms^2	LF, ms^2	HF, ms^2	LF norm	HF norm	LF/HF
I/I	14	4862.7*	1635.8	1719.0	1507.9*	47.2	52.8	1.3
I/D	40	5723.2	1797.7	1668.1	2257.3	43.0	57.0	1.0
D/D	21	3727.9*	1408.1	1104.5*	1207.6*	48.8	51.2	1.3

Note. * — represents statistically significant differences relative to the *ACE* I/I group (p < 0.05).

Average values of time domain parameters of HRV in the athletes with different *BDKRB2* genotypes (+9/+9, +9/–9, –9/–9) are shown in Table 6, suggesting that heart rate variability depends on the presence of +9 or –9 allele.

Time domain differences in HRV parameters were estimated using the one-way ANOVA.

The one-way ANOVA confirmed the significance of differences in R — Rmin between the *BDKRB2* –9/–9 and *BDKRB2* +9/+9 groups, suggesting the association of the *BDKRB2* –9 allele with the shortest time interval between two consecutive heart beats.

Average values of spectral parameters of heart rate variability in the athletes with different *BDKRB2* genotypes (+9/+9, +9/–9, –9/–9) reflect the share of sympathetic, parasympathetic and humoral-metaboloic contributions to heart rhythm modulation determined by the presence of the +9 or –9 allele (Table 7).

DISCUSSION

Our study recruited athletes specializing in different sports, so we were able to place them into 3 groups based on the trained quality. Members of the *Strength* group differed from other athletes in a number of morphometric parameters, which can be explained by the specifics of their training programs. A particular kind of sport taken up by an athlete shapes their build and body proportions, affects development of the cardiovascular, respiratory and locomotor systems. Long-term engagement in sports stimulates formation of specific morphological traits that can be subsequently used as a criterion for recruiting individuals with the most beneficial phenotype in professional sports [2, 10, 11].

Average values of time domain parameters of heart rate variability give an idea of the range of this variability and reflect cardiac adaptation patterns to different types of physical exercise. Heart rhythm of endurance athletes is low, modulated by increased parasympathetic effects on the sinoatrial node in response to regular physical load of moderate intensity. As a result, R — R min and R — R max intervals become longer. The beat-to-beat interval in these athletes is 1 to 1.5 seconds. This phenomenon is called bradycardia. RRNN and SDNN values in this group are higher than in the *Speed* and *Strength* groups. RMSSD, pNN50 and CV values tend to increase because of the increased vagal tone and the resulting negative chronotropic effect [1, 3, 4].

Power athletes have higher heart rates. It is believed that power and speed training is accompanied by increased sympathetic effects on the cardiac rhythm. The resting heart rate is relatively high, mirrored by low R — R min and R — R max values. We think that increased sympathetic effects determine lower values of RRNN, SDNN, RMSSD, pNN50 and CV.

Comparison of spectral parameters of heart rate variability in athletes specializing in different sports provides sufficient evidence of the contribution of sympathetic, parasympathetic, humoral and metabolic components to cardiac rhythm modulation. As the body adapts to different physical activities, some regulatory mechanisms become more active, while others lose their initial activity.

As demonstrated by the spectral analysis, the total power of the spectrum (the total effect of all regulatory mechanisms) is higher in the *Speed* and *Endurance* groups. The higher the total power, the lower the strain on the regulatory system. Besides, this parameter may be affected by the parasympathetic component (high frequency power spectrum) that depends on the frequency and depth of respiration during measurements. The *Endurance* group demonstrated high values of humoral and metabolic parameters (VLF and VLF%). Normally, the cerebral effect on the cardiac rhythm is manifested as the increased strain on the regulatory mechanisms and indicates lack of adaptation in the athlete. This may be explained by relative fatigue in the endurance athletes during the competition cycle. Perhaps, high values of parasympathetic parameters registered in the *Strength* and *Endurance* groups also originate from fatigue. On the other hand, normalized values of sympathetic and parasympathetic spectrum (VLF excluded) in the endurance athletes are consistent with normal adaptability.

Significant difference in time domain parameters between the *Strength* and *Speed* groups was observed for R — R min values. We believe that the underlying reason for that is running introduced to the raining schedule of sprinters. Running can stimulate parasympathetic effects on the heart function resulting in lower mean heart rates in sprinters in comparison with power athletes. But this difference was insignificant in our study. Perhaps, a larger sample is needed to prove its significance.

No significant differences were observed in time domain parameters of heart rate variability between the *Speed* and *Endurance* groups. Perhaps, the reason here is that rhythm modulation tends to take adaptation shifts in response to physical exercise. During their training cycles, speed and endurance athletes do physical exercise of maximal, submaximal, high and moderate intensity. Among all studied groups, sprinters rank second in the regulation of cardiac rhythm with respect to time domain spectral parameters.

Table 6. Time-domain parameters of heart rate variability in the athletes with different *BDKRB2* genotypes

BDKRB2 genotype	n	Mean heart rate	*R — R* min, ms	*R — R* max, ms	RRNN, ms	SDNN, ms	RMSSD, ms	pNN50, %	CV, %
+9/+9	26	60.6	796.6*	1186.4	1016.8	66.1	61.5	36.43	6.35
+9/–9	32	61.7	794.9*	1213.6	1003.4	70.3	66.25	37.27	6.89
–9/–9	17	59.7	842.9	1199.4	1025.3	67.5	61.6	35.64	6.53

Note. * — represents statistically significant differences ($p < 0.05$) between the *BDKRB2* –9/–9 and *BDKRB2* +9/+9 groups.

Table 7. Spectral parameters of heart rate variability in athletes with different *BDKRB2* genotypes

BDKRB2 genotype	n	TP, ms^2	VLF, ms^2	LF, ms^2	HF, ms^2	LF norm	HF norm	LF/HF
+9/+9	26	4597.1	1664.3	1242.7*	1690.1	45.2	54.8	1.08
+9/–9	32	5437.3	1602.1	1902.6	1927.4	47.3*	52.7*	1.43*
–9/–9	17	4806.8	1762.6	1192.0	1852.2	41.9	58.2	0.77

Note. * — represents statistically significant difference relative to the *BDKRB2* –9/–9 group ($p < 0.05$).

The protein encoded by the *ACE* gene is an important component of the renin-angiotensin system. The mutation in intron 16 of *ACE* yields two allelic variants: D – deletion of a 287 b. p. DNA sequence (Alu sequence) and I — insertion of this fragment. Data on the association of *ACE* I/D variants vary across populations and studies [15]. Athletes with the *ACE* I/I and I/D genotypes have higher BMI, bigger fat and muscle masses in comparison with the carriers of the D/D genotype ($p < 0.05$). The I/I genotype is associated with endurance, the D/D genotype — with speed and power. The I/D genotype of *ACE* is associated with all of these three qualities. Higher frequency of the I allele (the *ACE* I/I genotype) in comparison with the controls was observed in Russian athletes specializing in different sports, such as wrestling, sports games, or middle-distance running [12], Russian rowers [11], elite mountain climbers [13], and marathon swimmers [14]. A number of studies also confirm the association between the *ACE* I/I genotype with predominance of slow-twitch red muscle fibers in thighs [15], high indices of aerobic performance, quick recovery after physical exercise, resilience [13], cardiac output [17], and better ventilatory response to hypoxia [18].

Thus, the *ACE* I allele may be regarded as a genetic marker of endurance validated by a number of Russian and foreign researchers [11, 13, 16, 19].

In our study values of time domain parameters of heart rate variability in athletes with the *ACE* I/I genotype differed from those typical for *ACE* I/D and *ACE* D/D carriers, but the differences were statistically insignificant. Values of time domain parameters were lower in the athletes with the D/D genotype. Lower heart rate brings about an increase in R — R min, R — R max, and RRNN, which indicates the role of the I allele in cardiac rhythm modulation and stimulation of the parasympathetic effects on the sinoatrial node.

Carriers of the *ACE* D/D genotype had higher mean heart rate values and very low values of R–Rmin, R–Rmax, RRNN, RMSSD, pNN50 and CV, which in our opinion may be linked to the activity of the angiotensin converting enzyme associated with the studied genotype. There may be an association between such enzymic activity and the increased sympathetic effect on the cardiac rhythm. Significant difference was observed in RMSSD and CV values between the I/D and D/D genotype carriers. Carriers of the *ACE* D/D genotype had lower RMSSD and CV, which suggests decreased activity of the parasympathetic component of the vegetative system.

Thereby, we conclude that low RMSSD and CV indicate that an athlete is ready to handle speed or power training load while higher values of these parameters are associated with better endurance.

Carriers of the I/I genotype had lower TP values, but higher VLF and LF representing humoral, metabolic and sympathetic contributions to heart rate modulation. We assume that these values result from a small sample size (few I/I carriers in the total sample, in particular, in the *Endurance* group).

Higher values of spectral parameters of heart rate variability in I/D athletes indicate a considerable contribution of parasympathetic and humoral/metabolic components to the total power of the spectrum.

Bradykinin is a member of the kinin family, a polypeptide produced during activation of the kallikrein-kinin system. This polypeptide reduces vascular tone and blood pressure, increases permeability of the vascular wall and modulates signal transmission to the central and peripheral nervous systems. Its activity is mediated by two receptor types: β1 and β2 [8, 20]. The bradykinin receptor β2 encoded by the *BDKRB2* gene is a major mediator of bradykinin activity. It was found to be expressed in different organs and tissues and the vascular endothelium. The *BDKRB2* gene has a functional insertion-deletion polymorphism in its exon 1 (deletion or insertion of 9 nucleotides; +9/−9 or I/D) actively studied in sports genomics. The –9 allelic variant is associated with increased expression of the gene [19, 20]. Williams et al. have shown that the −9 allele of *BDKRB2* is associated with higher efficiency of muscular contractions and positively correlates with improvements in strength [9].

Another study conducted in a group of Russian stayers (long-distance running, swimming, and skiing) revealed a 39.1% frequency of the *BDKRB2* –9/–9 genotype. It was shown that this genotype benefited the elite canoe rowers: they came in 5 seconds earlier than carriers of the +9/+9genotype [11]. The *BDKRB2* –9 allele was also associated with high efficiency of muscular contractions [10] and high peak values of the extensor thigh muscle strength [8]. The *BDKRB2* +9 allele was linked to the risk of right ventricular hypertrophy in response to a 10-week training cycle [20–22].

Thus, according to the literature, the –9 allele of the *BDKRB2* gene can be regarded as a genetic marker of endurance.

We did not observe any significant differences between power and endurance athletes with regard to this parameter, which is probably due to our small sample size.

Homozygous *BDKRB2* −9 carriers had the lowest heart rate indicative of strong parasympathetic effects on the cardiac rhythm. The –9 allele of *BDKRB2* was reliably associated with the maximal duration of a beat-to-beat interval. Our study demonstrated that carriers of the –9 allele (the –9/–9 genotype) had low heart rate and longer R-R intervals (both min and max), which indicates a slightly larger contribution of the sympathetic component to cardiac rhythm modulation. On the whole, the +9/+9 carriers were in the middle of the measurement scale representing a quite balanced pattern of cardiac rhythm modulation shaped by the two components of the vegetative nervous system.

Vagal effects were very pronounced in *BDKRB2* –9/–9 carriers. Both normalized and un-normalized values (HF, HF norm) of the vagal component of the spectrum were higher than in other athletes. This is consistent with the assumption that the -9 allele should be associated with endurance [21–23]. In contrast, the *BDKRB2* +9/+9 group demonstrated relatively low TP and HF and higher LF. Low TP values indicate centralization in cardiac rhythm modulation (VLF included in the analysis). Similar changes in HRV are typical for individuals who train their speed or strength. Differences in spectral parameters with regard to their variability were estimated using the one-way ANOVA. Significant differences were observed only for LF, LF norm, HF norm and the sympathovagal balance. Notably, HF norm was high in *BDKRB2* –9/–9 carriers while LF norm and LF were low. This indicates prevalence of parasympathetic effects on the cardiac rhythm in the *BDKRB2* –9/–9 group. High LF norm and LF and low HF norm are suggestive of better speed and strength qualities.

Extending the range of the studied genes and working with a larger sample will allow us to investigate the mechanisms of heart rate modulation in athletes even more closely.

CONCLUSIONS

Our findings demonstrate that parasympathetic activity dominates other modulation components in the carriers of the *ACE* I allele. We have shown the increased role of the parasympathetic nervous system in *BDKRB2* –9/–9 genotype

carriers. The obtained data indicate that the cardiovascular system is ready to handle dynamic training of different intensity. Our findings are consistent with the assumption about the association between these alleles and sports achievements.

Genetic markers of physical performance can be used to recruit individuals in the professional sport more efficiently and to train qualified professional athletes. Specifically, the study results show that the polymorphisms *ACE* I/D and *BDKRB2* +9/−9 can be used as genetic markers reflective of individual patterns of heart rate modulation.

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