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# Is the -9/+9 polymorphism of the bradykinin receptor beta 2 (*BDKRB2*) gene associated with athlete status?

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**ABSTRACT**

Background: Previous studies concerning the relevance of the BDKRB2 gene polymorphisms revealed that the absence (–9 allele) of a 9 base pair sequence in exon 1 of the BDKRB2 gene is correlated with higher skeletal muscle metabolic efficiency, glucose uptake during exercise, as well as endurance athletic performance. Aim: The aim of the study was to investigate the association between the BDKRB2 -9/+9 polymorphism and elite athletic status in two cohorts of east-European athletes. Therefore, we examined the genotype distribution of the BDKRB2 9/+9 polymorphic site in a group of Polish athletes and confirmed the results obtained in a replication study of Russian athletes. Methods: Three hundred and two Polish athletes and 684 unrelated sedentary controls as well as 822 Russian athletes and 507 unrelated sedentary volunteers were recruited for this study. All samples were genotyped for the -9/+9 polymorphism within exon 1 of the BDKRB2 gene using a polymerase chain reaction (PCR). Significance was assessed by $\chi 2$ analysis with Bonferroni's correction for multiple testing. Results: We have not found any statistical difference in the -9/+9 genotype and allele frequencies in two groups of athletes divided into four subgroups, i.e. endurance, sprint-endurance, sprint-strength and strength athletes, when compared with controls. There weren't any significant differences found in allele frequencies ($P = 0.477$) and genotype distribution ($P = 0.278$) in the initial and replication studies. Conclusion: No association was found between the BDKRB2 -9/+9 polymorphism and elite athletic status in two cohorts of east-European athletes.

**KEY WORDS:** bradykinin, genetics, aeorobic performance, anaerobic performance

## INTRODUCTION

Physical ability is dependent on a combination of environmental as well as genetic factors. Within the group of genetic components that are believed to play a role in athletic performance, there are critical gene variants that have a physiological impact on human body composition and metabolism. One of the most important potential genetic markers for sport performance seems to be the collection of genes involved in blood pressure regulation.

Angiotensin-converting enzyme (ACE) plays a significant role in circulatory homeostasis. It is a key component of the renin-angiotensin system (RAS), being responsible for the production of a vasoconstrictor, angiotensin II. Moreover, it is a very important part of the kallikrein-kinin system (KKS) where ACE degrades kinins into inactive peptide fragments.[1,2] One of these is the vasodilator bradykinin, which acts in opposition to angiotensin II, is an efficacious, short-lived effector of a class of peptides known as kinins, released from kininogenes by proteolytic activity of kallikreins.[3,4] It participates in multiple physiological and pathological processes including vascular dilation, increased vascular permeability, angioedema, smooth muscle contraction, pain, inflammation, neurotransmission as well as cell proliferation.[3,5] Regoli and Barabé[6] suggested that bradykinin acts via two plasma membrane receptors, named the bradykinin $\beta_1$ receptor (BDKRB1) and the bradykinin $\beta_2$ receptor (BDKRB2). The majority of bradykinin physiological effects are mediated by activation of the cell surface BDKRB2, which exhibit high affinity for kallidin (Lys-bradykinin) and bradykinin.[3]

The activation of the BDKRB2 results in increased skeletal muscle glucose uptake during exercise, muscle blood flow and endurance performance.[1] Additionally, the production of the vasodilator nitric oxide (NO) from arginine by the enzyme nitric oxide synthase (NOS)

has been observed.[1,7,8] It is indicated, that NO is one of the key substances that influences blood pressure and basal vascular tone.[9,10]

The bradykinin $\beta_2$ receptor is encoded by a single-copy *BDKRB2* gene and is expressed in most human tissues.[3-5] Using fluorescence in-situ hybridization enabled Ma et al.[11] to localize the *BDKRB2* gene to chromosome 14q32. A three-exon structure for human *BDKRB2* gene has been revealed, with the coding region in exons 2 and 3.[3] Previous studies on the gene sequence have shown that it is characterized by 1 polymorphism in the promoter region and 3 polymorphic sites located in each of the three exons.[3,5] The insertion/deletion polymorphism (-9/+9, rs5810761) in exon 1 has been mainly studied in the context of associations between genotypes and physical performance, as well as hypertension and cardiovascular diseases.[1,2,12,13] The –9 as opposed to the +9 allele, is associated with increased gene transcription and higher receptor mRNA expression.[14,15] As a result, increased activity of the BDKRB2 is observed, which may be involved in determining endurance performance.[2]

These conclusions seem to be supported by Williams et al.[1], who have demonstrated that the absence (-9), rather than the presence (+9), of a 9 base pair (bp) sequence in exon 1 of the *BDKRB2* gene is strongly associated with higher skeletal muscle metabolic efficiency, as well as endurance athletic performance. Additionally, Saunders et al.[2] have confirmed that variants of the *BDKRB2* gene which contribute to increased KKS activity are associated with the endurance performance of South African triathletes. Previous studies have also shown that +9/+9 genotype is strongly associated with left ventricular (LV) growth response in normotensive males undergoing physical training and change in LV mass in response to antihypertensive treatment.[12]

The aim of the study was to analyze the possible importance of the -9/+9 polymorphism within the *BDKRB2* gene in Polish and Russian athletes and sedentary individuals, to examine the possible relationships between genotype and physical

performance. We have examined the genotype distribution of the *BDKRB2* in a group of Polish and Russian athletes who were divided into four groups, covering a spectrum from the more endurance-oriented to the more strength-oriented (power-oriented) disciplines, according to the following values: relative aerobic/anaerobic energy system contribution, time of competitive exercise performance and intensity of exertion in each sport.

## MATERIALS AND METHODS

### Subjects and controls

The initial association study was done in a group of 302 Polish athletes of the highest nationally competitive standard (age 27.8 ± 7.1. yr, male n = 221 and female n = 81). The athletes were prospectively stratified into four groups according to the values of relative anaerobic/aerobic energy system contribution, time of competitive exercise performance and intensity of exertion in each sport. The first group, designated as endurance athletes, consisted of athletes (n = 26) with predominantly aerobic energy production (duration of exertion over 30 minutes, intensity of exertion moderate). This group included triathletes (n = 4), race walkers (n = 6), road cyclists (n = 14) and 15-50 km cross-country skiers (n = 2). The second group, designated as strength-endurance athletes (n = 66), was comprised of athletes whose sports utilise mixed anaerobic/aerobic energy production, with a duration of exertion ranging from 5 to 30 minutes and a moderate to high intensity of exertion. This group included rowers (n = 41), 3-10 km runners (n = 17) and 800-1500 m swimmers (n = 8). The third group (sprint-strength athletes; n = 110) also included athletes with mixed energy production, but when compared to the second group, the time of competitive exercise performance was shorter (1-5 minutes; in the case of combat sports, the duration of a single bout of competition was taken into account), while the intensity of exertion was higher and the balance between

anaerobic/aerobic energy production was shifted towards the anaerobic system. This group was comprised of kayakers (n = 10), 800-1500 m runners (n = 7), 200-400 m swimmers (n = 3), judokas (n = 13), wrestlers (n = 41), boxers (n = 19) and fencers (n = 17). The fourth group (strength athletes) consisted of athletes (n = 100) with predominantly anaerobic energy production (duration of exertion < 1 minute, intensity of exertion submaximal to maximal): 100-400 m runners (n = 29), powerlifters (n = 22), weightlifters (n = 20), throwers (n = 14) and jumpers (n = 15).

All Polish athletes recruited for this study were ranked in the top 10 nationally in their respective discipline. The study population included 63 athletes classified as 'top-elite' (gold medallists in the World and European Championships, World Cups or Olympic Games) and 149 athletes classified as 'elite' (silver or bronze medallist in the World and European Championships, World Cups or Olympic Games). The others (n = 90) were classified as 'sub-elite' (participants in international competitions). Various methods were used to obtain the samples, including: targeting national teams and providing information to national coaching staff and athletes attending training camps.

Control samples were prepared from 684 unrelated, sedentary volunteers (students of the University of Szczecin, aged 19–23; 153 females and 531 males; age 24.3 ± 0.2 yr). All athletes and controls were Caucasian to reduce the possibility of racial gene skew and to overcome any potential problems due to population stratification. The procedures followed in the study were approved by the Pomeranian Medical University Ethics Committee. All participants gave informed consent to genotyping with the understanding that it was anonymous and obtained results would have confidential status.

The replication study was done in 822 Russian athletes of a nationally competitive standard (286 females and 536 males; age 25.3 ± 0.2 yr). The athletes were divided into four

groups according to the parameters established for the initial association study. The group of endurance athletes (n = 100) included biathletes (n = 39), cross-country skiers (n = 44) and long-distance (5-25 km) swimmers (n = 17). The group of strength-endurance athletes (n = 95) consisted of rowers (n = 76), 3-10 km runners (n = 5), 800-1500 m swimmers (n = 9) and 5-10 km skaters (n = 5). The group of sprint-strength athletes (n = 530) was comprised of kayakers (n = 34), 800-1500 m runners (n = 3), 200-400 m swimmers (n = 37), boxers (n = 25), wrestlers (n = 112), alpine skiers (n = 19), short trackers (n=22), 1,5-3 km speed skaters (n = 7), fencers (n = 60), football players (n = 82), ice hockey players (n = 70) and artistic gymnasts (n = 59). The strength athletes group (n = 97) consisted of 100-400 m runners (n = 10), 500-1000 m skaters (n = 13), 50-100 m swimmers (n = 28), weightlifters (n = 34), throwers (n = 5), jumpers (n = 7). There were 364 athletes classified as 'elite' (ranked in the top 10 nationally), of whom 105 were 'top-elite' athletes (award winners of the World and European Championships, World Cups or Olympic Games). There were 272 athletes classified as 'sub-elite' (participants in international competitions). The others (n = 186) were classified as 'non-elite' athletes, being regional competitors with no less than four years experience participating in their sports.

Controls were 507 healthy, unrelated citizens (354 females and 153 males; age 22.1 ± 0.2 yr) of St. Petersburg and Surgut without any competitive sport experience. The geographic ancestry of the athletes and control groups was self-reported. The athletes and control groups were all Caucasian (predominantly Russians). The University of St. Petersburg Ethics Committee approved the study, and written informed consent was obtained from each participant.

**Genetic Analyses**

In the Polish study, genomic DNA was extracted from the buccal cells using a GenElute Mammalian Genomic DNA Miniprep Kit (Sigma, Germany) according to the manufacturer's instructions.

In the Russian study, genotyping was performed on DNA samples obtained from epithelial mouth cells by alkaline extraction[16] or with a DNK-sorb-A sorbent kit according to the manufacturer's instructions (Central Research Institute of Epidemiology, Russia), depending on the method of sample collection (buccal swab or scrape).

All samples were genotyped for the -9/+9 polymorphism within exon 1 of the *BDKRB2* gene using a polymerase chain reaction (PCR). The 100 and/or 91 bp fragments of the gene were amplified by PCR using the forward primer 5'-TCTGGCTTCTGGGCTCCGAG-3' and the reverse primer 5'-AGCGGCATGGGCACTTCAGT- 3' as recommended by Williams et al. (2004). The reaction was carried out in a total volume of 10 μl containing: 1.5 mM $MgCl_2$, 0.75 nM of each dNTP (Novazym, Poland or Sibenzyme, Russia), 4 pM of each primer (Genomed, Poland or Lytech, Russia), 0.5 U of Taq DNA polymerase (Sigma, Germany or Sibenzyme, Russia), and 1 μl (30–50 ng) of genomic DNA. After the first 5 min step at 94 °C, 35 cycles of amplification were performed by using denaturation at 94 °C for 30 s, annealing at 62 °C for 1 min, and elongation at 72 °C for 30 s and a final cycle at 72 °C for 10 min. The amplified PCR fragments were separated by 7.5 % polyacrylamide gel electrophoresis, stained with ethidium bromide, and visualized in UV light.

**Statistical Analysis**

The STATISTICA statistical package, version 7.0, was used to perform all statistical evaluations. A $\chi 2$ test was used to compare the *BDKRB2* -9/+9 alleles and genotype

frequencies between athletes and control subjects. Bonferroni's correction for multiple testing was performed by dividing the p value (0.05) with the number of tests.

## RESULTS

The results of the genotype distribution of -9/+9 *BDKRB2* in Polish and Russian athletes and controls met Hardy-Weinberg expectations ($P > 0.05$ in all groups tested separately). *BDKRB2* genotype distribution results of the Polish control group (+9/+9 – 28.8%; +9/–9 – 50.7%; –9/–9 – 20.5%) and Russian control group (+9/+9 – 29.4%; +9/–9 – 49.5%; –9/–9 – 21.1%) were similar to those reported in previous studies on Caucasian populations. [1, 14,15,17] There were no significant differences in *BDKRB2* genotype and allele frequencies between males and females amongst both athletes and controls of both ethnic groups (data not shown).

The initial association study done in the Polish athlete group (Table 1) revealed that the genotype distributions ($P = 0.739$) and allele frequencies (47.02 % vs. 45.83 %; $P = 0.626$) of *BDKRB2* -9/+9 did not differ between athletes and sedentary controls. Any observed differences were not statistically significant when considering the frequency of the –9 allele in the four groups of athletes separately, i.e. endurance athletes (42.31%; $P = 0.616$), strength-endurance athletes (45.45%; $P = 0.933$), sprint-strength athletes (47.73%; $P = 0.601$) and strength athletes (48.50%; $P = 0.479$).

Statistically significant differences in genotype distribution were also not observed in the whole cohort of Polish athletes (+9/+9 – 26.50%, +9/–9 – 53.00%, –9/–9 – 20.50%; $P = 0.626$ ) nor in each group separately, i.e. groups of endurance athletes ($P = 0.812$), sprint-endurance athletes ($P = 0.940$), sprint-strength athletes ($P = 0.763$) and strength athletes ($P = 0.442$) when compared with controls.

The same conclusion to the initial study was obtained in the replication study (Table 2). The differences in –9 allele frequencies between all Russian athletes and controls did not reach statistical significance (46.90% vs. 45.86 %; *P* = 0.321). The differences in –9 allele frequencies were also not statistically significant in the endurance athletes (45.50%; *P* = 0.938), strength-endurance athletes (45.80%; *P* = 1.000), sprint-strength athletes (46.89%; *P* = 0.670) and strength athletes (49.48%; *P* = 0.353) compared to controls group separately.

The genotype distributions of *BDKRB2* +9/–9 in all Russian athletes (+9/+9 – 26.4%, +9/–9 – 53.4%, –9/–9 – 20.2%; *P* = 0.404) were not different to controls, nor were endurance athletes (*P* = 0.804), sprint-endurance athletes (*P* = 0.932), sprint-strength athletes (*P* = 0.257) and strength athletes *P* = 0.648) when compared with controls (+9/+9 – 29.4%; +9/–9 – 49.5%; –9/–9 – 21.1%).

Taking the results of the initial and replication studies into consideration together (Table 3), significant differences in the frequency of the –9 allele were not found in the whole cohort of Polish and Russian athletes when compared with the controls (46.93% vs. 45.84%; *P* = 0.477). The same situation was observed when comparing the differences of genotype distribution between all Polish and Russian athletes and controls (+9/+9 – 26.4%; +9/–9 – 53.3%; –9/–9 – 20.3% vs. +9/+9 – 29.1%; +9/–9 – 50.2%; –9/–9 – 20.7%; *P* = 0.278). Within the four groups of athletes, the –9 allele frequency and the genotype distribution of *BDKRB2* -9/+9 no statistical significance differences were observed when compared with controls.

To recognize the correlation between the -9/+9 *BDKRB2* polymorphism and athletic performance we investigated the genotype distribution and allele frequency in four subgroups of athletes, i.e. top elite, elite, sub-elite and non-elite athletes (Table 4). There were no significant differences in *BDKRB2* genotype and allele frequencies between each Polish and Russian subgroup, nor among controls of either ethnic group.

## DISCUSSION

The articles concerning the *BDKRB2* gene in a sport context are still unique. Moreover, till now any hypothesis referring to the role of this gene in individual capacity for physical performance has not been clearly proven.

The gene that encodes the bradykinin $\beta_2$ receptors (BDKRB2) contains a number of polymorphic loci, but in the context of sport research, the most frequently analyzed location is the polymorphic site in exon 1 (-9/+9). According to Braun et al.[5] and Lung et al.[15] the absence of a 9-base pair repeat in exon 1 of the gene encoding the bradykinin $\beta_2$ receptor is associated with higher gene transcriptional activity and higher receptor mRNA expression, which may be associated with improved performance and this effect might influence general sport ability.

The activation of the BDKRB2 by bradykinin, which is generated within exercising skeletal muscle, may have a beneficial effect on sport performance for a few reasons. First, it has been suggested bradykinin increases skeletal muscle glucose uptake during exercise.[8,18] The physiological mechanism of this process was described by Taguchi et al.[19], who indicated that bradykinin enhances insulin-stimulated tyrosine kinase activity of the insulin receptor, with subsequent GLUT-4 translocation in skeletal muscle tissue during exercise (GLUT-4 is glucose transporter type 4, a protein responsible for insulin-regulated glucose transportation into the cell).

Bradykinin is also the key element, of the kallikrein-kinin system (KKS) as one of the most important mediators, playing a critical role in the cardiovascular system and affecting blood pressure regulation.[6,20] In this case, bradykinin causes blood vessels to dilate, and therefore causes blood pressure to lower.[21,22] In humans, bradykinin is broken down into inactive fragments by three kininases: aminopeptidase P (APP) and carboxypeptidase N

(CPN) and angiotensin-converting enzyme (ACE) which is encoded by the *ACE* gene - the most frequently investigated gene in the context of genetic conditioning of sports-predispositions.[8,23] It is possible that ACE may influence performance through this system (KKS) rather than RAS (renin-angiotensin system).[2]

It is worth mentioning that interactions between RAS and KKS still do not seem to be fully recognized. The confirmation of it is inter alia the findings of Sabatini et al.[24], which indicated that expression of the bradykinin β2 receptor leads to an augmentation in ACE activity. Till this moment, despite the large number of studies describing the potentiation of bradykinin effects by ACE inhibitors, information about a possible modulation of ACE activity by kinin receptors has never been in focus.

Referring to the role of KKS in blood pressure regulation, bradykinin β2 receptors are also known to affect vasodilation by stimulating nitric oxide (NO) or eicosanoid production.[7,8] Also Venema[25] proved that bradykinin $\beta_2$ receptors interact with endothelial nitric oxide synthase (eNOS), an enzyme that produces NO – a potent local vasodilating agent. Additionally, bradykinin-induced NO generation may also modulate mitochondrial respiratory control.[1,26]

The kallikrein-kinin system, in addition to regulating blood pressure, plays a role in inflammation, coagulation and pain.[27-30] In their article Blais et al.[31] indicated that bradykinin and other kinins, acting on bradykinin β2 receptors, are powerful proinflammatory factors. They produce local edema and activate and sensitize sensory and sympathetic nerve endings, with the release of other mediators of inflammation, which mediate some of the local action of kinins and amplify their proinflammatory, nociceptive and algesic effects.[31,32] Kinins also attract leukocytes, activate the phagocytic function, and increase production and release of inflammatory mediators from neutrophils and macrophages.[31]

Additionally, bradykinin seems to play an important role in weight control. Bradykinin produces polyuria through its action on bradykinin β2 receptors to block AVP-induced re-absorption of water in the collecting ducts of the kidneys. [33,34] Given this diuretic effect of bradykinin, athletes with the –9/–9 genotype of the *BDKBR2* gene should, in theory, excrete more fluid and show the greatest weight loss.[2]

Reports regarding the connection between the *BDKRB2* +9/–9 polymorphism and sport performance level are still limited. Till this moment, only a few reports were concerned with the role of the *BDKRB2* gene for sport performance. Williams et al.[1] suggest that the –9 allele of *BDKRB2* gene is associated with higher skeletal muscle metabolic efficiency (i.e. the energy used per unit of power output during exercise or delta efficiency). What is more, the analysis revealed a linear trend of increasing –9 allele frequency with distance run in 81 Olympic standard track athletes, which seems to prove the importance of the –9 allele of *BDKRB2* gene for endurance athletic performance.

This finding seems to be supported by Saunders et al.[2], who found statistically significant differences in -9/+9 distribution between 443 male Caucasian triathletes and 203 healthy Caucasian male controls. In this case, the –9/–9 genotype of *BDKRB2* gene was over-represented in the whole cohort of athletes compare to controls (27.0% vs. 19.3%, P = 0.035). However, when divided into tertiles according to their finishing times, the –9/–9 genotype was only over-represented in the fastest tertile. There were no significant differences in the frequencies of the allele distributions between any of the triathletes and controls.

The report concerning the role of *BDKRB2* gene in sport was also published by Tsianos et al.[35] They investigated the genotype distribution and allele frequency of 8 chosen genetic polymorphisms in 438 athletes participating in the 2007 and 2008 annual running events, the Olympus Marathon (inter alia C58T *BDKRB2* polymorphism (rs1799722)). Although they evaluated only single nucleotide polymorphisms (SNPs), their findings seem to

support the reports of Williams et al.[1] and Saunders et al.[2] They found results consistent with previous studies: the high transcription allele was over-represented in this group of endurance athletes, and even more so among those who were habitual runners.

Our results and the results of Eynon et al.[36] are in opposition to observations of Williams et al.[1] and Saunders et al.[2] In our study, we have not found any statistical difference in +9/–9 genotype and allele frequencies in any of four investigated athletes group (i.e. endurance athletes, sprint-endurance athletes, sprint-strength athletes and strength athletes) compared to sedentary controls. What is important, we obtained the same results both in the Polish and Russian athletes (the same in initial and replication study – totally 938 athletes). Eynon et al.[36] found that allele frequencies and genotype distribution were similar both in athlete and control group. They also found no statistical differences between the subgroups of elite and national-level athletes.

It is necessary to point out that our report is the fourth concerning the role of -9/+9 *BDKRB2* for physical performance and the second which does not indicate the importance of this polymorphism.

Our findings indicated that the role of *BDKRB2* gene in athletic performance seems to be still unclear. Even if the -9/+9 *BDKRB2* polymorphism is not correlated with a predisposition to athletic performance, there is a second polymorphism in the *BDKRB2* gene (rs1799722) under the hypothesis that this polymorphism may influence endurance capacity.[35]

Another aspect of the +9/–9 *BDKRB2* polymorphism that warrants further studies is the possible interaction with other genetic and environmental factors. For example, it was proven that levels of bradykinin are dependent inter alia on *ACE* genotype.[37] Knowing this fact, Williams et al.[1] investigated the role of *ACE* and *BDKRB2* genotype combination for predisposition to sport performance. In their findings, *ACE* and *BDKRB2* haplotypic analysis demonstrated a significant relationship with distance run (≤5,000 vs. ≥5,000 m), both overall

(P = 0.001) and for Caucasians only (P = 0.003), with a greater proportion of "low kinin receptor activity" (*ACE* D allele, *BDKRB2* +9 allele) in events <5,000 m and, conversely, a greater proportion of "high kinin receptor activity" haplotypes (*ACE* I allele, *BDKRB2* –9 allele) competing in events >5,000 m.[1]

Another example concerning the correlation of *BDKRB2* gene with the *NOS3* gene. Saunders et al.[2] pointed out that the effect of the genotype *NOS3* GG, advantageous for endurance performance, appeared only in connection with the genotype (–9/–9) of the gene *BDKRB2*. In other combinations of genotypes of both genes (*NOS3* and *BDKRB2*), the genotype GG did not show any positive correlation with an increase in sport endurance.

In contrary to these findings, the study of Eynon et al.[36] showed no association between the polymorphism (C825T) in the gene *GNB3* coding for the guanine nucleotide binding protein β-polypeptide 3 and BDKRB2 -9/+9 polymorphic site, despite the fact that C825T polymorphism within *GNB3* gene was itself previously correlated with elite athletic performance.[38]

## CONCLUSIONS

Athletic ability is a trait that involves genes which are influenced by environmental factors. Genetic components include numerous candidate genes whose natural allelic variants occur in the general population. Identifying these polymorphisms that could have an impact on athletic performance is a matter of investigation worldwide. However, one of the main deficiencies of association studies is an inadequate number of subjects and/or a lack of replication studies. In this study, we demonstrate that there is no significant association between the +9/–9 polymorphic site in the candidate gene of *BDKRB2* and athletic performance in two independent studies of large cohorts of Polish and Russian athletes. Our results are contrary to the hypothesis that the *BDKRB2* -9/+9 polymorphism is associated with athletic ability. Our

finding does not mean that other polymorphisms in *BDKRB2* gene do not have any beneficial effect on performance parameters. There might also be possible interactions with other genetic factors, because athletic performance is a polygenic trait and more than 79 polymorphisms are suggested to influence the athletes' results.[39] In our opinion there is a need for further investigation in the field using independent cohorts of athletes of the same, as well as different ethnic backgrounds to replicate the obtained results and thus clarify the potential role of polymorphic variants of candidate genes in determining sport performance abilities.

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**Table 1.** *BDKRB2* genotype distribution and frequencies of *BDKRB2* gene –9 allele in Polish athletes stratified by the values of relative aerobic/anaerobic energy system contribution, time of competitive exercise performance and intensity of exertion in each sport (Initial study).

**Table 2.** *BDKRB2* genotype distribution and frequencies of *BDKRB2* gene –9 allele in Russian athletes stratified by the values of relative aerobic/anaerobic energy system contribution, time of competitive exercise performance and intensity of exertion in each sport (Replication study).

**Table 3.** *BDKRB2* genotype distribution and frequencies of *BDKRB2* gene –9 allele in Polish and Russian athletes stratified by the values of relative aerobic/anaerobic energy system contribution, time of competitive exercise performance and intensity of exertion in each sport (Combined study).

**Table 4.** *BDKRB2* genotype distribution and frequencies of *BDKRB2* gene –9 allele in Polish and Russian athletes stratified by sports status, i.e top elite, elite, sub-elite and non-elite (Combined study).