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Running title: *BDKRB2* gene polymorphism in European athletes

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

Introduction

The 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 (Moreau et al., 2005, Jones and Woods, 2003). One of these is the vasodilator bradykinin, an efficacious, short-lived effector of a class of peptides known as kinins, released from kininogenes by proteolytic activity of kallikreins (Kammerer et al., 1995; Prado et al., 2002). 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 (Kammerer et al., 1995; Braun et al., 1995). Regoli and Barabé (1980) suggested that bradykinin acts via two plasma membrane receptors, named the bradykinin β_1 receptor (BDKRB1) and the bradykinin β_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 (Kammerer et al., 1995).

The activation of the BDKRB2 results in increased skeletal muscle glucose uptake during exercise, muscle blood flow and endurance performance (Dietze et al., 1996, Henriksen et al., 1999). Additionally, the production of the vasodilator nitric oxide (NO) from arginine by the enzyme nitric oxide synthase (NOS) has been observed (Rett et al., 1990; Shen et al., 1995; Mayfield et al., 1996). It is indicated, that NO is one of the key substances that influences blood pressure and basal vascular tone (Quyyumi et al., 1995; Kimura et al., 2003).

The bradykinin β2 receptor is encoded by a single-copy of the *BDKRB2* gene and is expressed in most human tissues (Braun et al., 1995; Kammerer et al., 1995; Prado et al., 2002). Ma et al. (1994) localized the *BDKRB2* gene on chromosome 14q32. A three-exon structure for human *BDKRB2* gene has been revealed, with the coding region in exons 2 and 3 (Kammerer et al., 1995). 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 (Kammerer et al., 1995; Braun et al., 1995). 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 (Hallberg et al., 2003; Fu et al., 2004; Saunders et al., 2006; Williams et al., 2004). The –9 as opposed to the +9 allele, is associated with increased gene transcription and higher receptor mRNA expression (Braun et al., 1996; Lung, et al., 1997).

Individuals with the +9 /+9 genotype were characterized by the lowest levels of the BDKRB2 and showed the greatest increase in left ventricular mass as well as less left ventricular mass regression compared with other genotypes (Halberg et al., 2003). The presence of the BDKRB2 +9 allele was related with cardiovascular risk and increase in blood pressure associated with hypertension (Dhamrait et al., 2003). Increased activity of the BDKRB2 what is observed for the -9 allele carries may be involved in determining endurance performance (Saunders et al., 2006).

These conclusions seem to be supported by Williams et al. (2004), 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. (2006) have confirmed that variants of the *BDKRB2* gene which contribute to increased the KKS activity are associated with the endurance performance of South African triathletes.

Previous studies have also shown that the +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 (Hallberg et al., 2003). The aim of the study was to investigate the association between *BDKRB2* -9/+9 polymorphism and elite athletic status in two cohorts of east-European athletes. Therefore, we examined the genotype distribution of *BDKRB2* 9/+9 polymorphic site in a group of Polish athletes and confirmed the results obtained in a replication study of Russian athletes. The athletes 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

The experimental procedures were conducted in accordance with the set of guiding principles for reporting the results of genetic association studies defined by the STrengthening the REporting of Genetic Association studies (STREGA) Statement (Little et al. 2009).

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 (Bolla et al., 1995) 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 the -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 (Braun et al., 1996; Brull et al., 2001; Lung et al., 1997; Williams et al., 2004). There were no significant differences in the *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 the *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 the –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 the –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 the *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 the *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 status 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 the *BDKRB2* genotype and allele frequencies between each Polish and Russian subgroup, nor among controls of either ethnic group.

Discussion

The present report is a genetic case-control association study in which we examined the genotype distribution of the *BDKRB2* 9/+9 polymorphism in a group of Polish athletes and confirmed the results obtained in a replication study of Russian athletes. Our main findings were 1) neither the *BDKRB2* -9 and +9 alleles nor the *BDKRB2* -9/+9 genotypes were significantly more frequent among four groups of Polish and Russian athletes of different metabolic demands than in controls and 2) a lack of association between athletes of different competitive levels was observed when genotype and allele frequencies were compared among the top-elite, elite and sub-elite athletes and controls in initial and replication studies.

Reports regarding the connection between the *BDKRB2* +9/–9 polymorphism and sport performance level are still limited. Prior to this study, only a few reports were concerned with the role of the *BDKRB2* gene for sport performance. The literature data showed that the -9 allele of a *BDKRB2* gene is linked with increased gene transcription and

higher receptor mRNA expression (Braun et al., 1996; Lung, et al., 1997). Williams et al. (2004) suggested that the –9 allele of *BDKRB2* gene is associated with higher skeletal muscle metabolic 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. (2006), 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 compared to controls. 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.

A report concerning the role of the *BDKRB2* gene in sport was also published by Tsianos et al. (2010). 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. (2004) and Saunders et al. (2006). 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.

Another aspect of the +9/–9 *BDKRB2* polymorphism that warrants further study 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 (Murphey et al. 2000). Knowing this fact, Williams et al. (2004) investigated the role of the *ACE*

and *BDKRB2* genotype combination for predisposition to sport performance. In their findings, *ACE* and *BDKRB2* analysis demonstrated a significant relationship with distance run (≤5,000 vs. ≥5,000 m), both overall and for Caucasians only, 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 (Williams et al., 2004).

Another example concerned the correlation of the *BDKRB2* gene with the *NOS3* gene. Saunders et al. (2006) 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.

Contrary to these findings, Eynon et al. (2011) 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 the C825T polymorphism within the *GNB3* gene was itself previously correlated with elite athletic performance (Eynon et al., 2009).

Our results and the results of Eynon et al. (2011) are in opposition to the observations of Williams et al. (2004) and Saunders et al. (2006). In our study, we did not find any statistical difference in +9/–9 genotype and allele frequencies in any of four investigated athletes groups (i.e. endurance athletes, sprint-endurance athletes, sprint-strength athletes and strength athletes) compared to sedentary controls. Notably, we obtained the same results both in the Polish and Russian athletes (the same in the initial as in the replication study – totally 1124 athletes in total). Eynon et al. (2011) found

that allele frequencies and genotype distribution were similar both in athlete and control groups. They also found no statistical differences between the subgroups of elite and national-level athletes.

The discrepancy between results described above may be due to differences in sample size, study designs and elite athletes' phenotype classification. The positive findings have emerged from relatively smaller cohorts (Saunders et al., 2006) or from studies of a different study design (Wiliams et al., 2004). In our study, we were able to recruit large enough samples of elite athletes (over 1100 athletes) in an attempt to overcome the sample size limitation making our study unique. Moreover, all participants were of similar ethnic and geographic backgrounds as evidenced by similar Minor Allele Frequencies (MAF). In our opinion this enabled us to reach sufficient statistical power and obtain reliable conclusions. The same methodological approach was applied in our previous work (Eynon et al. 2012) and our results should be considered valid, since all STREGA criteria were met (Little et al 2009): all athletes represented an elite level of competition; participants within each cohort were ethnically-matched; genotyping was accurate and unbiased; and genotype distributions were in HWE both in athletes and the control group of the two analyzed east-European cohorts.

However, our study is not without limitations. Elite athletic status is a complex polygenic trait involving complex gene-gene interactions as well as gene-environment interactions (Lucia et al. 2010). Thus, the numerous polymorphic sites in candidate genes should be analyzed to explain individual variation of elite athletic status. It must be kept in mind that even if the -9/+9 *BDKRB2* polymorphism is not correlated with a predisposition to athletic performance, there may be other polymorphisms in the *BDKRB2* gene which could hypothetically influence elite athlete status.

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Perspectives

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 the *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 sports related phenotypes are highly polygenic and more than 79 polymorphisms are suggested to influence the athletes' results (Ahmetov and Fedotovskaya, 2012; Williams and Folland, 2008). 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.

Literature Cited

1. Ahmetov, I. I, Fedotovskaya, O. N. 2012. Sports genomics: current state of knowledge and future directions. *Cell. Mol. Exerc. Physiol*. 1:e1.
2. Braun, A., Krammerer, S., Bohme, E., et al. 1995. Identyfication of polymorphic sites of the human Bradykinin B_2 receptor gene. *Biochem. Bioph. Res. Commun.*, 211:234-240.

3. Braun, A., Kammerer, S., Maier, E., et al. 1996. Polymorphisms in the gene for the human B2-bradykinin receptor. New tools in assessing a genetic risk for bradykinin-associated diseases. *Immunopharmacology*. 33:32-35.
4. Dhamrait, S. S, Payne, J. R., Li, P., et al. 2003. Variation in bradykinin receptor genes increases the cardiovascular risk associated with hypertension. *Eur. Heart. J.* 24:1672–1680.
5. Dietze, G. J., Wicklmayr, M., Rett, K., et al. 1996. Potential role of bradykinin in forearm muscle metabolism in humans. *Diabetes* 45:S110-114.
6. Eynon, N., Meckel, Y., Alves, A. J., et al. 2001. Is there an interaction between BDKRB2 -9/+9 and GNB3 C825T polymorphisms and elite athletic performance? *Scand. J. Med. Sci. Sports*, 21:e242-246.
7. Eynon, N., Oliveira, J., Meckel, Y., et al. 2009. The guanine nucleotide binding protein beta polypeptide 3 gene C825T polymorphism is associated with elite endurance athletes. *Exp. Physiol.* 94:344-349.
8. Eynon N., Ruiz, J. R., Femia, P., et al. 2012. The ACTN3 R577X polymorphism across three groups of elite male European athletes. *PLoS One*. 7(8):e43132.
9. Fu, Y., Katsuya, T., Matsuo, A., et al. 2004. Relationship of bradykinin B2 receptor gene polymorphism with essential hypertension and left ventricular hypertrophy. *Hypertens Res.* 27:933-938.
10. Hallberg, P., Lind, L., Michaëlsson, K., et al. 2003. B2 bradykinin receptor (B2BKR) polymorphism and change in left ventricular mass in response to antihypertensive treatment: results from the Swedish Irbesartan Left Ventricular Hypertrophy Investigation versus Atenolol (SILVHIA) trial. *J. Hypertens.* 21:621–624.

11. Henriksen, E. J., Jacob, S., Kinnick, T. R., et al. 1999. ACE inhibition and glucose transport in insulinresistant muscle: roles of bradykinin and nitric oxide. *Am. J. Physiol.* 277:R332-R336.

12. Jones, A., Woods, D. R. 2003. Skeletal muscle RAS and exercise performance. *Int. J. Biochem. Cell Biol.* 35:855-866.

13. Kammerer, S., Braun, A., Arnold, N., et al. 1995. The human bradykinin B_2 receptor gene: full length cDNA, genomic organization and identyfication of the regulatory region. *Biochem. Biophys. Res. Commun.* 211:226-233.

14. Kimura, T., Yokoyama, T., Matsumura, Y., et al. 2003. NOS3 genotype–dependent correlation between blood pressure and physical activity. *Hypertension* 41:355-360.

15. Little, J., Higgins, J. P., Ioannidis, J. P., et. al. 2009. STrengthening the REporting of Genetic Association studies (STREGA)--an extension of the STROBE statement. *Eur. J. Clin. Invest.* 39:247-266.

16. Lucía, A., Morán, M., Zihong, H., et al. 2010. Elite athletes: are the genes the champions? *Int. J. Sports Physiol. Perform*. 5(1):98-102.

17. Lung, C. C., Chan, E. K., Zuraw, B. L. 1997. Analysis of an exon 1 polymorphism of the B2 bradykinin receptor gene and its transcript in normal subjects and patients with C1 inhibitor deficiency. *J. Allergy Clin. Immunol.* 99:34–46.

18. Ma, J. X., Wang, D. Z., Ward, D. C., et al. 1994. Structure and chromosomal localization of the gene (BDKRB2) encoding human bradykinin B2 receptor. *Genomics* 23:362–369.

19. Mayfield, R. K., Shimojo, N., Jaffa, A. A. 1996. Skeletal muscle kallikrein: potential role in metabolic regulation. *Diabetes* 45:S20–23.

20. Moreau, M. E., Garbacki, N., Molinaro, G., et al. 2005. The kallikrein-kinin system: current and future pharmacological targets. *J. Pharmacol. Sci*. 99:6-38.

21. Murphey, L. J., Gainer, J. V., Vaughan, D. E., et al. 2000. Angiotensin converting enzyme insertion/deletion polymorphism modulates the human in vivo metabolism of bradykinin. *Circulation* 102:829–832.

22. Prado, G. N., Taylor, L., Zhou, X., et al. 2002. Mechanisms regulating the expression, self-maintenance and signaling-function of the bradykinin B2 and B1 receptors. *J. Cell. Physiol.* 193:275-286.

23. Quyyumi, A. A., Dakak, N., Andrews, N. P, et al. 1995. Contribution of nitric oxide to metabolic coronary vasodilation in the human heart. *Circulation* 92:320–326.

24. Regoli, D., Barabé, J. 1980. Pharmacology of bradykinin and related kinins. *Pharmacol. Rev*. 32:1–46.

25. Rett, K., Wicklmayr, M., Dietze, G. J. 1990. Metabolic effects of kinins: historical and recent developments. *J. Cardiovasc. Pharmacol*. 15(6):S57-S59.

26. Saunders, C. J., de Milander, L., Hew-Butler, T., et al. 2006. Dipsogenic genes associated with weight changes during Ironman Triathlons. *Hum. Mol. Genet.* 15:2980–2987.

27. Shen, W., Zhang, X., Zhao, G., et al. 1995. Nitric oxide production and NO synthase gene expression contribute to vascular regulation during exercise. *Med. Sci. Sports Exerc.* 27:1125–1134.

28. Tsianos, G. I., Evangelou, E., Boot, A., et al. 2010. Associations of polymorphisms of eight muscle- or metabolism-related genes with performance in Mount Olympus marathon runners. *J. Appl. Physiol.* 108:567-574.

29. Williams, A. G., Dhamrait, S. S., Wootton, P. T., et al. 2004. Bradykinin receptor gene variant and human physical performance. *J. Appl. Physiol.* 96:938–942.

30. Williams, A. G., Folland, J. P. 2008. Similarity of polygenic profiles limits the potential for elite human physical performance. *J. Physiol.* 586:113–121.

Table 1. The *BDKRB2* genotype distribution and frequencies of the *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).

Sport	*n*	Genotypes (n)			*P*	-9 allele (%)	*P*
		+9/+9	+9/-9	-9/-9			
1. Endurance athletes							
Triathlon	4	2	1	1	0.554	37.50	0.637
Race walking	6	2	2	2	0.645	50.00	0.773
Road cycling	14	4	8	2	0.831	42.86	0.754
Cross-country skiing	2	1	1	0	0.697	25.00	0.403
Total	**26**	**9**	**12**	**5**	**0.812**	**42.31**	**0.616**
2. Strength-endurance athletes							
Rowing	41	11	21	9	0.953	47.56	0.761
Running 3-10 km	17	6	7	4	0.734	44.12	0.842
Swimming 800-1500 m	8	3	4	1	0.796	37.50	0.505
Total	**66**	**20**	**32**	**14**	**0.940**	**45.45**	**0.933**
3. Sprint-strength athletes							
Kayaking	10	2	3	5	0.073	65.00	0.087

Wrestling	41	8	25	8	0.369	50.00	0.462
Boxing	19	3	15	1	0.047	44.74	0.893
Fencing	17	6	8	3	0.839	41.18	0.590
Total	**110**	**28**	**59**	**23**	**0.763**	**47.73**	**0.601**
4. Strength athletes							
Running 100-400 m	29	5	18	6	0.365	51.72	0.378
Powerlifting	22	6	11	5	0.964	47.73	0.804
Weightlifting	20	3	13	4	0.353	52.50	0.404
Throwing events	14	6	6	2	0.507	35.71	0.287
Jumping events	15	3	9	3	0.723	50.00	0.650
Total	**100**	**23**	**57**	**20**	**0.422**	**48.50**	0.479
All Polish athletes	**302**	**80**	**160**	**62**	**0.739**	**47.02**	0.626
Polish controls	**684**	**197**	**347**	**140**	**1.000**	**45.83**	**1.000**

P values are calculated by χ^2 test for comparisons between groups of athletes and control group.

A Bonferroni corrected alpha level was set at 0.0021. No statistically significant differences between athletes and controls were found.

Table 2. The *BDKRB2* genotype distribution and frequencies of the *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).

Sport	n	Genotypes (n)			*P*	-9 allele %	*P*
		+9/+9	+9/-9	-9/-9			
1. Endurance athletes							
Biathlon	39	8	26	5	0.116	46.15	0.959
Cross-country skiing	44	17	18	9	0.412	40.91	0.435
Swimming 5-25 km	17	3	9	5	0.508	55.88	0.248
Total	**100**	**28**	**53**	**19**	**0.804**	**45.50**	0.938
2. Strength-endurance athletes							
Rowing	76	24	44	8	0.092	39.47	0.162
Running 3-10 km	5	0	1	4	0.006	90.00	0.005
Swimming 800-1500 m	9	1	3	5	0.042	72.20	0.032
Speed skating 5-10 km	5	2	1	2	0.387	50.00	0.793
Total	**95**	**27**	**49**	**19**	**0.932**	**45.80**	1.00
3. Sprint-strength athletes							
Kayaking	34	12	15	7	0.753	42.65	0.606
Running 800-1500 m	3	1	1	1	0.825	50.00	0.839
Swimming 200-400 m	37	8	22	7	0.477	48.65	0.641

Speed skating, 1,5-3 km	7	3	4	0	0.371	28.60	0.281
Boxing	25	5	14	6	0.600	52.00	0.468
Wrestling	112	22	58	32	0.063	54.40	0.022
Alpine skiing	19	5	14	0	0.045	36.84	0.273
Artistic gymnastics	59	16	32	11	0.785	45.76	0.984
Short track	22	5	10	7	0.468	54.55	0.257
Fencing	60	16	32	12	0.850	46.70	0.923
Football	82	22	53	7	0.011	40.50	0.238
Ice hockey	70	22	34	14	0.935	44.30	0.786
Total	**530**	**137**	**289**	**104**	**0.257**	**46.89**	**0.670**
4. Strength athletes							
Running 100-400 m	10	2	3	5	0.089	65.00	0.089
Speed skating 500-1000 m	13	3	8	2	0.691	46.15	0.976
Swimming 50-100 m	28	13	12	3	0.123	32.14	0.044
Weightlifting	34	6	17	11	0.184	57.35	0.065
Throwing events	5	0	5	0	0.087	50.00	0.793
Jumping events	7	1	3	3	0.345	64.29	0.169
Total	**97**	**25**	**48**	**24**	**0.648**	**49.48**	**0.353**
All Russian athletes	**822**	**217**	**439**	**166**	**0.357**	**46.90**	**0.629**
Russian controls	**507**	**149**	**251**	**107**	**1.000**	**45.86**	**1.000**

P values are calculated by χ^2 test for comparisons between groups of athletes and control group.

A Bonferroni corrected alpha level was set at 0.0016. No statistically significant differences between athletes and controls were found.

Table 3. The *BDKRB2* genotype distribution and frequencies of the *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).

Sport	**n**	**Genotypes (n)**			***P***	**–9 allele %**	***P***
		+9/+9	**+9/–9**	**–9/–9**			
1. Endurance athletes							
Biathlon	39	8	26	5	0.127	46.15	0.956
Triathlon	4	2	1	1	0.565	37.50	0.636
Race walking	6	2	2	2	0.659	50.00	0.773
Road cycling	14	4	8	2	0.813	42.86	0.752
Cross-country skiing 5-10 km	46	18	19	9	0.323	40.21	0.339
Swimming 5-25 km	17	3	9	5	0.500	55.88	0.243
Total	**126**	**37**	**65**	**24**	0.903	**44.84**	**0.812**
2. Strength-endurance athletes							
Rowing	117	35	65	17	0.264	42.31	0.333
Running 3-10 km	22	6	8	8	0.186	54.55	0.251
Swimming 800-1500 m	17	4	7	6	0.342	55.88	0.321
Speed skating 5-10 km	5	2	1	2	0.367	50.00	0.792
Total	**161**	**47**	**81**	**33**	**0.997**	**45.65**	**0.996**
3. Sprint-strength athletes							
Kayaking	44	14	18	12	0.425	47.73	0.727

Running 800-1500 m	10	4	4	2	0.734	40.00	0.601
Swimming 200-400 m	40	9	23	8	0.608	48.75	0.607
Speed skating. 1.5-3 km	7	3	4	0	0.373	28.6	0.305
Short track	22	5	10	7	0.435	54.55	0.251
Judo	13	5	4	4	0.371	46.15	0.974
Wrestling	153	30	83	40	0.037	53.27	0.017
Boxing	44	8	29	7	0.117	48.86	0.653
Fencing	77	22	40	15	0.949	45.45	0.992
Football	82	22	53	7	0.011	40.50	0.238
Ice hockey	70	22	34	14	0.935	44.30	0.786
Alpine skiing	19	5	14	0	0.049	36.84	0.269
Artistic gymnastics	59	16	32	11	0.829	45.76	0.986
Total	**640**	**165**	**348**	**127**	**0.206**	**47.03**	**0.514**
4. Strength athletes							
Running 100-400 m	39	7	21	11	0.256	55.13	0.105
Speed skating 500-1000 m	13	3	8	2	0.717	46.15	0.974
Swimming 50-100 m	28	13	12	3	0.108	32.14	0.041
Powerlifting	22	6	11	5	0.968	47.73	0.803
Weightlifting	54	9	30	15	0.117	55.56	0.047
Throwing events	19	6	11	2	0.546	39.47	0.434

Jumping events	22	4	12	6	0.494	54.55	0.251
Total	**197**	**48**	**105**	**44**	**0.400**	**48.98**	**0.246**
All Polish and Russian athletes	**1124**	**297**	**599**	**228**	**0.278**	**46.93**	**0.477**
Polish and Russian controls	**1191**	**346**	**598**	**247**	**1.000**	**45.84**	**1.000**

P values are calculated by χ^2 test for comparisons between groups of athletes and control group.

A Bonferroni corrected alpha level was set at 0.0014. No statistically significant differences between athletes and controls were found.

Table 4. The *BDKRB2* genotype distribution and frequencies of the *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).

Sport	**n**	**Genotypes (n)**			***P***	**-9 allele %**	***P***
		+9/+9	**+9/-9**	**-9/-9**			
1. Polish athletes							
Top elite	63	16	33	14	0.820	48.41	0.573
Elite	149	35	85	29	0.251	47.99	0.484
Sub-elite	90	26	45	19	0.996	46.11	0.945
2. Russian athletes							
Top elite	105	33	49	23	0782	45.24	0.866
Elite	259	70	127	62	0.500	48.45	0.302
Sub-elite	272	65	154	53	0.133	47.79	0.438
3. Polish and Russian athletes							
Top elite	168	49	82	37	0.916	46.43	0.840
Elite	408	105	212	91	0.423	48.28	0.244
Sub-elite	362	91	199	72	0.242	47.37	0.495
Polish and Russian controls	**1191**	**346**	**598**	**247**	**1.000**	**45.84**	**1.000**

P values are calculated by χ^2 test for comparisons between groups of athletes and control group.

A Bonferroni corrected alpha level was set at 0.0056. No statistically significant differences between athletes and controls were found.