Original Paper DOI: 10.5604/20831862.1096047 Biol. Sport 2014;31:109-113

BDKRB2 GENE -9/+9 POLYMORPHISM AND SWIMMING PERFORMANCE

Accepted for publication 24.03.2014

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ABSTRACT: The aim of the study was to evaluate the association between swimming performance and the -9/+9 (rs5810761) polymorphism within the BDKRB2 gene in successful competitive swimmers.Best individual swimming results expressed in FINA points achieved at short, middle and long distance events of 157 well-trained Polish swimmers were incorporated into an analysis. Athletes' genotype and allele distributions were analysed in comparison to 230 unrelated sedentary subjects who served as controls with the χ^2 test. All samples were genotyped for the BDKRB2 -9/+9 polymorphism using the polymerase chain reaction (PCR). The effects of genotype on swimming performance were analysed with two-way (3 x 2; genotype x gender) analysis of variance with metrical age as a covariate for each distance specialization. No statistical differences in the genotype and allele frequencies were found in long distance swimmers when compared with the total group of swimmers or controls. The BDKRB2 +9/-9 genotype had no significant effect on swimming performance at short, middle or long distance, regardless of gender. The results of this study do not support the hypothesis that the BDKRB2 -9/+9 polymorphism is associated with swimming performance in Polish swimmers.

KEY WORDS: endurance, athletic, exercise, genetics, performance, swimmers

INTRODUCTION

The renin-angiotensin system (RAS) plays a key role in human circulatory homeostasis. One of the main components of RAS is the angiotensin-converting enzyme (ACE), which catalyses production of angiotensin II (ANG II), consequently increasing blood pressure from angiotensin I (ANG I). Furthermore, ACE is an essential part of the kallikrein-kinin system (KKS), where it degrades kinins into inactive fragments, thus reducing blood pressure [24,29].

One of the peptides known as kinins is bradykinin (BK) – a significant vasodilator, released from kininogens by proteolytic activity of kallikreins [13,22]. The protein is involved in various biological processes, including vascular regulation, inflammation, oedema, pain, neurotransmission, cell proliferation, smooth muscle contraction, and modulation of glucose metabolism [1,5,13,23]. In 1980 Regoli and Barabé proposed that BK acts via specific two-cell surface receptors that are classified as the bradykinin β_1 receptor (BDKRB1) and the bradykinin β_2 receptor (BDKRB2) [23]. However, the majority of physiological activities are mainly mediated by BDKRB2, which exhibits high affinity for kallidin (Lys-BK) and BK [5,13]. The receptors are located on the plasma membrane of the skeletal muscle cells and the vascular endothelium [24]. The activation of BDKRB2 results in increased skeletal muscle glucose uptake during physical activity, blood flow in muscles, and as a result higher endurance performance [29]. Furthermore, the synthesis of the vasodilator nitric oxide (NO) from arginine by the enzyme nitric oxide synthase (NOS) has been described [17,27,29].

The human BDKRB2 is composed of 359 amino acids with a molecular weight of 41 kDa [22]. Analyses have shown that BDKRB2 is encoded by a single-copy gene, which is localized to chromosome 14q32 and is expressed in most human tissues. A three-exon structure for the human *BDKRB2* gene has been proposed, with the coding region in exon 2 and 3 [13,17]. Numerous studies on the sequence of the gene have revealed 1 polymorphism in the promoter region and 3 located in each exon [2,13]. The insertion/deletion polymorphism (-9/+9, rs5810761) in exon 1 is the most frequently investigated polymorphism in the context of relationships between genotypes and athlete status, as well as cardiovascular diseases and hypertension [11,12,24,29]. In contrast to the presence of a 9 base pair (bp) repeat (+9), the absence of

a 9 bp (-9) repeat in exon 1 of the *BDKRB2* gene is associated with higher gene transcriptional activity, higher mRNA expression, and increased receptor activity [3,16]. As a result, the -9 allele may be connected with higher skeletal muscle metabolic efficiency and endurance athletic performance [29]. Therefore, in this study it was hypostatized that swimmers with the -9/-9 genotype perform better in long distance than swimmers with other genotypes of the *BDKRB2* gene.

The aim of the study was to evaluate the association between swimming performance and the -9/+9 (rs5810761) polymorphism within the *BDKRB2* gene in well-trained Polish swimmers.

MATERIALS AND METHODS

Participants. One hundred and fifty-seven Polish swimmers (84 males and 73 females, 20.31 ± 2.67 years), who competed in national and international events, were recruited for this study. Level of ability was varied. Mean swimming performance treated as personal best result at any distance was 683 ± 119 FINA points. There were one European record holder and World Champion, two World Champions and three Olympic Games finalists at long distance events. More than 50% of the participants were finalists of National Championships. The range of number of training sessions per week was from 5 to 13. As a control group, samples were prepared from 230 unrelated volunteers with no background in sport (male students from the University of Szczecin). The athletes and controls were all Caucasians to ensure there was no ethnicity skew and to overcome any potential problems of population stratification.

Ethics Committee

The Pomeranian Medical University Ethics Committee, Poland, approved the study and an informed consent form was completed by each participant. The study complied with the guidelines set out in the Declaration of Helsinki and the ethics policy of the Szczecin University [14].

Swimming Performance

For each swimmer, the best ever performances for short (50–100 m), middle (200 m) and long (400–1500 m) distance events at long course (Olympic-size swimming pool) in true competitions were retrieved and converted into International Swimming Federation (FINA) points, based on the FINA 2013 tables.

Endurance ratio was calculated by dividing the personal best result at long distances or middle by the personal best results at short distance events.

Participants' metric age, when the best result was achieved, was noted and implemented into statistical analysis. Metric age was used as a covariate to detrend any possible confounding effect of subjects' age on performance results, as age significantly correlates ($r=0.61$; $p<0.0001$) with competitive swimming performance.

Genetic Analyses

Genomic DNA was extracted from the buccal cells using the GenElute Mammalian Genomic DNA Miniprep Kit (Sigma, Germany), according to the producer protocol.

The 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. [29]. PCR mixture and thermal-time profile were coequal as described by Sawczuk et al. [26]. The amplified DNA fragments were visualized by 7.5% polyacrylamide gel electrophoresis.

Statistical Analysis

Genotype distribution and allele frequencies between the groups of athletes and controls were compared with the χ^2 test. Two-way (3 x 2; genotype x gender) analysis of variance with metric age as a covariate was conducted separately for each distance event (short, middle, long). The participants' swimming performances, treated as personal ever best in each distance category, were taken as the dependent variable. Data distribution was tested with the Kolmogorov-Smirnov test; Levene's test was used for testing homogeneity of variance. Values of endurance ratio were log transformed prior to analysis to improve linearity and homoscedasticity. The logarithmic mean of the transformed ratios were back transformed for presentation of results. STATISTICA 8 statistical package was used for calculations. P values of < 0.05 were considered statistically significant.

RESULTS

The genotype distribution of -9/+9 *BDKRB2* in Polish swimmers and controls followed the Hardy-Weinberg equilibrium ($p>0.05$ for each group). The *BDKRB2* genotype distribution results of the Polish control group (+9/+9 – 29%; +9/-9 – 52%; -9/-9 – 19%) were similar to those reported in previous studies on Caucasian populations [3,4,16,29]. The genotype distributions of +9/-9 *BDKRB2* in all swimmers as well as in long distance swimmers were not significantly different to controls ($p=0.19$ and $p=0.26$). There were no significant differences in the genotype and allele frequencies between males an d females amongst both athletes and controls (data not shown).

Differences in the -9 allele frequencies between all Polish swimmers and controls did not reach statistical significance (45% vs. 40%; $p=0.20$). Similarly, differences in the -9 allele frequencies were also not statistically significant in long distance swimmers, when compared to controls or all swimmers separately (47% vs. 40%; $p=0.26$ and 47% vs. 45%; $p=0.79$) (Table 1).

The mean swimming performance was not statistically different in male and female participants, regardless of the *BDKRB2* +9/-9 polymorphism. The *BDKRB2* +9/-9 genotype had no significant

TABLE 1. *BDKRB2* +9/-9 GENOTYPE DISTRIBUTION AND FREQUENCIES OF -9 ALLELE IN POLISH SWIMMERS AND CONTROL GROUP

	Genotype frequency, n(%)					Allele frequency, n(%)			
Participants	+9/+9	+9/-9	-9/-9	Chi	p	+9	-9	Chi	P
All swimmers (n=157)	45 (29%)	82 (52%)	30 (19%)	1.48	0.48	172 (55%)	142 (45%)	0.90	0.34
Long distance swimmers (n=47)	13 (28%)	24 (51%)	10 (21%)	0.21	0.90	50 (53%)	44 (47%)	0.11	0.74
Controls (n=230)	62 (27%)	112 (49%)	56 (24%)			236 (51%)	224 (49%)		

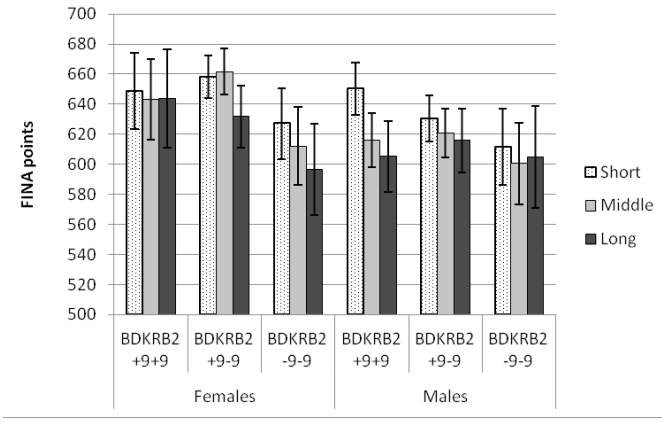

FIG. 1. MEAN (± SE) PERSONAL BEST SWIMMING PERFORMANCE IN SHORT, MIDDLE AND LONG DISTANCE EVENTS EXPRESSED AS FINA POINTS, ADJUSTED TO SWIMMERS' METRIC AGE, WHEN THE RESULT WAS REACHED IN SUBJECTS OF DIFFERENT *BDKRB2* GENOTYPES.

effect on swimming performance either at short, middle or long distance, expressed in FINA points and adjusted to swimmers' age, either in female or male athletes (Figure 1). Only a tendency to higher results was noted at short distance events than at long distance events in male athletes of +9/+9 genotype of the *BDKRB2* gene, but the differences were statistically insignificant. Also, the endurance ratios were not significantly different among swimmers of different *BDKRB2* +9/-9 genotype, both in males and females (Table 2).

No interaction effect of gender x *BDKRB2* +9/-9 genotype was found for each of the swimming performance indices.

TABLE 2. ENDURANCE RATIO IN FEMALE AND MALE ATHLETES OF DIFFERENT *BDKRB2* GENOTYPE

	Female		Male	
+9/+9	1.04	(0.96 - 1.13)	1.02	(0.96 - 1.08)
+9/-9	1.04	(0.98 - 1.09)	1.04	(0.99 - 1.09)
-9/-9	1.03	(0.95 - 1.11)	1.06	(0.97 - 1.15)

Note: Note: the values are means and 95% coefficient intervals

DISCUSSION

Athletic performance is dependent on a combination of many environmental factors such as diet, physical training, and sociocultural factors, as well as genetic factors. Still, the most important factors that could solely be a predictor of performance are being sought. Within the group of genetic components that are believed to play an important role in physical performance, there are key gene variants that have a significant impact on human body composition and metabolism. One of the main potential genetic markers for athletic performance seems to be the group of genes involved in human circulatory regulation such as *ACE*, *NOS3*, and *BDKRB2,* which have been previously mostly analysed alone [7, 20, 21].

In this study, the *BDKRB2* gene, which encodes the bradykinin β_2 receptor whose activation results in increased skeletal muscle glucose uptake, blood flow in muscles, and production of NO, was taken into consideration alone as a genetic marker of sport ability. The gene has been shown to be associated with higher skeletal muscle metabolic efficiency and with endurance athletic ability [24,25,29]. However, its role in determining individual capacity for physical performance has not been clearly established [10,26].

To the authors' best knowledge, this is the first study aimed at investigating the association between the *BDKRB2* -9/+9 polymorphism and swimming performance in competitive athletes. It was hypothesized that the *BDKRB2* -9/-9 genotype should be more frequent in long distance swimming, or at least swimming performance of athletes with the *BDKRB2* -9/-9 genotype should be higher at long distance events than any other. This study failed to confirm this hypothesis as no statistically significant differences were observed in genotype distribution in either long distance swimmers or the group of all swimmers when compared to controls. Additionally, there was no significant overrepresentation of the -9 allele frequency in either group of swimmers when compared with controls. Moreover, the *BDKRB2* +9/-9 polymorphism did not significantly differentiate swimming performance regardless of distance and gender.

Reports regarding the connection between the *BDKRB2* +9/-9 polymorphism and sport performance level are still limited. To date, only a few reports have been concerned with the role of the *BDKRB2* gene in sport performance. Our findings are in agreement with some previous results. Eynon et al. found that allele frequencies and genotype distribution of the *BDKRB2* -9/+9 polymorphism were similar

both in Israeli Caucasian athletes and the control group [10]. They also revealed no statistical differences between endurance athletes and sprinters, as well as athletes of different competitive levels. Moreover, Sawczuk et al. found no statistical differences in the +9/-9 genotype or allele frequencies in any of the four investigated Polish and Russian athlete groups (i.e. endurance athletes, sprint-endurance athletes, sprint-strength athletes and strength athletes), compared to sedentary controls. They also analysed differences in the -9/+9 distribution between swimmers of different distances (5-25 km, 800-1500 m, 200-400 m, 50-100 m), but no significant disparities were found [26].

Nevertheless, the obtained results are in disagreement with previous study that revealed statistically significant differences in the -9/+9 distribution between Caucasian triathletes who completed South African Ironman Triathlons and controls [24,25]. The -9/-9 genotype 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. Williams et al. [29] also 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 Olympic standard track athletes, which seems to prove the importance of the -9 allele of the *BDKRB2* gene in endurance athletic performance.

The results are not consistent with previous studies for several possible reasons. One of them may be the fact that the investigated group was not large enough, owing to limitations imposed by the small number of elite swimmers available in Poland and their willingness to participate in our research. Unfortunately, the relatively small size of the group in this study may cause ambiguity of the obtained results (relatively low statistical test power). In addition, athletic performance is a polygenic trait; over 20 polymorphisms have been associated with elite endurance performance [15,28]. The other polymorphisms in this gene may also play an important role in endurance abilities and should be taken into consideration in further studies. Moreover, it was proven that levels of BK are dependent inter alia on other genes such as *ACE* [19]. Williams et al. [29] studied the role of the *ACE* and the *BDKRB2* genotype combination for predisposition to sport ability. The genetic analysis showed a significant association with distance run (<5,000 vs. ≥5,000 m), with a greater proportion of "low kinin receptor activity" (*ACE* D allele, *BDKRB2* +9 allele) in events <5,000 m and, in opposition, a greater proportion of "high kinin receptor activity" haplotypes (*ACE* I allele, *BDKRB2* -9 allele) competing in events ≥5,000 m. It is an additional example concerning the relationship of the *BDKRB2* gene with the *NOS3* gene. Saunders et al. [24,25] reported that the effect of the *NOS3* GG polymorphism, beneficial for endurance performance, appeared only in connection with the *BDKRB2* -9/-9 genotype. In other combinations of genotypes of the investigated genes, the genotype GG did not show any positive association with the increase in sport endurance [24].

In opposition to these findings, the study of Eynon et al. [8-10] revealed no correlation between the C825T polymorphism in the gene *GNB3* coding for the guanine nucleotide binding protein β-polypeptide 3 and the *BDKRB2* -9/+9 polymorphism, despite the fact that the *GNB3* C825T polymorphism was previously associated with elite athletic performance.

Taking only one genotype into the analysis is an important limitation of this study. For this reason, in further studies it is necessary to analyse the interaction of other genes such as *ACE* and *NOS3*, which also play an important role in physical performance and have an impact on the level of BK.

CONCLUSIONS

It was found that the insertion/deletion polymorphism (-9/+9, rs5810761) within the *BDKRB2* gene is not associated with swimming performance, regardless of distance specialization and gender, in Polish swimmer cohorts. It is more likely that several gene loci, each with a small but significant contribution, are responsible for the genetic component of swimming performance. Thus, further research should be aimed at evaluating the significance of a set of selected genes in determining physical conditioning.

Conflict of interest

The authors report no conflicts of interest

REFERENCES

1. Bhoola K.D., Figueroa C.D., Worthy K. Bioregulation of kinins: kallikreins, kininogens, and kininases. Pharmacol Rev. 1992;44:1-80.
2. Braun A., Krammerer S., Bohme E., Müller B., Roscher A.A. Identification of polymorphic sites of the human Bradykinin B2 receptor gene. Biochem Bioph Res Commun. 1995;211:234-240.
3. Braun A., Kammerer S., Maier E., Bohme E., Roscher A.A. Polymorphisms in the gene for the human B2-bradykinin receptor. New tools in assessing a genetic risk for bradykinin-associated diseases. Immunopharmacology 1996;33:32-35.
4. Brull D., Dhamrait S., Myerson S., Erdmann J., Woods D., World M., Pennell D., Humphries S., Regitz-Zagrosek V., Montgomery H.. Bradykinin B2BKR receptor polymorphism and left-ventricular growth response. Lancet 2001;358:1155-1156.
5. Burch R.M., Kyle D.J. Minireview: recent development in the understanding of bradykinin receptors. Life Sci. 1992;50:829-838.
6. Costa A.M., Silva A.J., Garrido N.D., Louro H., de Oliveira R.J., Breitenfeld L. Association between ACE D allele and elite short distance swimming. Eur J Appl Physiol. 2009;106:785-90.
7. Drozdovska S.B., Dosenko V.E., Ahmetov I.I., Ilyin V.N. The association of gene polymorphisms with athlete status in Ukrainians. Biol. Sport 2013;30:163-167.
8. Eynon N., Meckel Y., Alves A.J. Nemet D., Eliakim A. Is there an interaction between BDKRB2 -9/+9 and GNB3

C825T polymorphisms and elite athletic performance? Scand. J. Med. Sci. Sports 2001;21:e242-246.
9. Eynon, N., Oliveira, J., Meckel, Y., Sagiv M., Yamin C., Sagiv M., Amir R., Duarte J.A. The guanine nucleotide binding protein beta polypeptide 3-gene C825T polymorphism is associated with elite endurance athletes. Exp. Physiol. 2009;94:344-349.
10. Eynon N., Meckel Y., Alves A.J., Nemet D., Eliakim A. Is there an interaction between BDKRB2 -9/+9 and GNB3 C825T polymorphisms and elite athletic performance? Scand J Med Sci Sports. 2011; 21:e242-246.
11. Fu Y., Katsuya T., Matsuo A., Yamamoto K., Akasaka H., Takami Y., Iwashima Y., Sugimoto K., Ishikawa K., Ohishi M., Rakugi H., Ogihara T. Relationship of bradykinin B2 receptor gene polymorphism with essential hypertension and left ventricular hypertrophy. Hypertens Res. 2004;27:933-938.
12. Hallberg P., Lind L., Michaëlsson K., Karlsson J., Kurland L., Kahan T., Malmqvist K., Ohman K.P., Nyström F., Melhus H. 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. 2003;21:621-624.
13. Kammerer S., Braun A., Arnold N., Roscher A.A. The human bradykinin B2 receptor gene: full length cDNA, genomic organization and identyfication of the regulatory region. Biochem. Biophys. Res. Commun. 1995;211:226-233.
14. Kruk J., Good scientific practice and ethical principles in scientific research and higher education. Centr. Eur. J. Sports Sci. Med. 2013;1:25-29.
15. Leońska-Duniec A. Genetic research in modern sport. Centr. Eur. J. Sport Sci. Med. 2013;3:19-26.
16. Lung C.C., Chan E.K., Zuraw B.L. 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. 1997;99:34-46.
17. Ma J.X., Wang D.Z., Ward D.C., Chen L., Dessai T., Chao J., Chao L. Structure and chromosomal localization of the gene (BDKRB2) encoding human bradykinin B2 receptor. Genomics 1994;23:362-369.
18. Mayfield R.K., Shimojo N., Jaffa A.A. Skeletal muscle kallikrein: potential role in metabolic regulation. Diabetes 1996;45:20-23.
19. Murphey L.J., Gainer J.V., Vaughan D.E., Brown N.J. Angiotensin converting enzyme insertion/deletion polymorphism modulates the human in vivo metabolism of bradykinin. Circulation 2000;102:829-832.
20. Orysiak J., Zmijewski P., Klusiewicz A., Kaliszewski P., Malczewska-Lenczowska J., Gajewski J., Pokrywka A. The association between ACE gene variation and aerobic capacity in winter endurance disciplines. Biol. Sport 2013;30:249-253.
21. Pokrywka A., Kaliszewski P., Majorczyk E., Zembron-Lacny A. Genes in sport and doping. Biol. Sport 2013;30:155-61.
22. Prado G.N., Taylor L., Zhou X., Ricupero D., Mierke D.F., Polgar P. Mechanisms regulating the expression, self-maintenance and signaling-function of the bradykinin B2 and B1 receptors. J. Cell. Physiol. 2002;193:275-286.
23. Regoli D., Barabé J. Pharmacology of bradykinin and related kinins. Pharmacol. Rev. 1980;32:1-46.
24. Saunders C.J., de Milander L., Hew-Butler T., Xenophontos S.L., Cariolou M.A., Anastassiades L.C., Noakes T.D., Collins M. Dipsogenic genes associated with weight changes during Ironman Triathlons. Hum. Mol. Genet. 2006;15:2980-2987.
25. Saunders C.J., Xenophontos S.L., Cariolou M.A., Anastassiades L.C., Noakes T.D., Collins M. The bradykinin beta 2 receptor (BDKRB2) and endothelial nitric oxide synthase 3 (NOS3) genes and endurance performance during Ironman Triathlons. Hum Mol Genet. 2006 Mar 15;15:979-87.
26. Sawczuk M., Timshina Y.I., Astratenkova I.V., Maciejewska-Karłowska A., Leońska-Duniec A., Ficek K., Mustafina L.J., Cięszczyk P., Klocek T., Ahmetov I.I. The -9/+9 polymorphism of the bradykinin receptor beta 2-gene and athlete status: A study involving two European cohorts. Human Biology in press 2013.
27. Shen W., Zhang X., Zhao G., Wolin M.S., Sessa W., Hintze T.H. Nitric oxide production and NO synthase gene expression contribute to vascular regulation during exercise. Med. Sci. Sports Exerc. 1995;27:1125-1134.
28. Wang G., Padmanabhan S., Wolfarth B., Fuku N., Lucia A., Ahmetov I. I., Cieszczyk P., Collins M., Eynon N., Klissouras V., Williams A., Pitsiladis Y. Genomics of Elite Sporting Performance: What Little We Know And Necessary Advances. In: Friedmann T., Dunlap J. C., Goodwin S. F., editors. Advances in genetics. Oxford: Elsevier; 2013. p 125-151.
29. Williams A.G., Dhamrait S.S., Wootton P.T., Day S.H., Hawe E., Payne J.R., Myerson S.G., World M., Budgett R., Humphries S.E., Montgomery H.E. Bradykinin receptor gene variant and human physical performance. J. Appl. Physiol. 2004;96:938-942.