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Effect of BDKRB2 Gene -9/+9 polymorphism on training improvements in competitive swimmers

Effect of *BDKRB2* gene -9/+9 polymorphism on training improvements in competitive swimmers

Abstract

The aim of the study was to investigate the possible association between the *BDKRB2* gene and training-induced improvements in swimming performance in well-trained swimmers.

One hundred Polish swimmers (52 males and 48 females, aged 18.1 ± 1.9 years), who competed in national and international competitions at middle (200 m) and long distance events (≥400 m), were included in the study. Athletes' genotype and allele distributions were analyzed 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 by polymerase chain reaction (PCR). The effects of genotype on swimming performance improvements were analyzed with two-way (3 x 2; genotype x time) analysis of variance with metrical age as a covariate. Training period of 1.9 ± 0.4 years had a significant ($p<0.01$) effect on swimming performance, both in female and male athletes. Both in female and male athletes the *BDKRB2* gene -9/+9 polymorphism had no significant effect on swimming performance. Only in male athletes was an interaction effect of the *BDKRB2* gene +9/-9 polymorphism x time found for swimming performance. Post hoc analyses indicated that swimmers with the +9/+9 *BDKRB2* genotype had a greater improvement in swimming performance than swimmers with +9/-9 polymorphism ($p<0.05$). No interaction effects for gender x *BDKRB2* gene -9/+9 polymorphism were found for either swimming performance or improvement in swimming performance.

Conclusions: These results suggest that the response to long-term exercise training could be modulated by *BDKRB2* gene +9/-9 polymorphism in male athletes. In well-trained swimmers *BDKRB2* gene variation was not found to be an independent determinant of swimming performance.

Introduction

A number of studies suggest that genetic markers may explain, in part, interindividual variability of aerobic performance characteristics in response to endurance training. In recent years several candidates for predicators of athletic predisposition have been proposed (6,27). One of these genetic markers associated with athletic exercise performance is the bradykinin β2 receptor gene (*BDKRB2*) (22).

Bradykinin is one of the peptides known as kinins. This peptide was recognized as a significant vasodilator, released from kininogens by proteolytic activity of kallikreins (16,23). It was determined that bradykinin is involved in various biological processes: numerous inflammatory processes, vasodilation, and cell growth (34).

The main biological effects of kinins are mediated by bradykinin receptors. Bradykinin receptors are prevalent in a wide range of tissues. BDKRB2 is a subtype of receptor constitutively expressed by endothelial cells, smooth muscle vascular cells, nociceptive neurons, and macrophages (15,17).

Lu J. et al. (19) found that heightened *BDKRB2* expression in the sensory nerves contributes to the exaggerated muscle mechanoreflex in rats with femoral artery occlusion and concluded that BDKRB2 is a primary contributor to the over-reactive muscle mechanoreflex observed in peripheral artery disease. The increase in bradykinin in response to exercise may result in modulation of exercise-induced glucose transport through elevated GLUT-4 translocation, as well as enhancement of the insulin signal pathway, during the post-exercise period in skeletal muscle (33).

The *BDKRB2* gene contains a number of polymorphic loci, including a nine-base insertion/deletion in the first exon of the gene (+9/-9, rs5810761) and a C to T transition in the promoter region (C −58T, rs1799722) (29,35). In exon 1 of the gene encoding BDKRB2, the deletion variant (-9), rather than the presence (+9), is related to higher gene transcriptional activity and higher receptor mRNA expression (4). The +9/+9 *BDKRB2* genotype, which has a significant role in coronary artery disease, systemic hypertension, and increased left ventricular mass related to hypertension and pulmonary artery pressure, was also found (9,26).

It is also thought that +9/-9 polymorphism is linked with a physiological left-ventricular growth response to exercise training (5). The activation of BDKRB2 may also result in increased skeletal muscle glucose uptake during physical activity, blood flow in muscles, and as a result higher endurance performance (37). Studies have shown that the -9 allele is associated with greater metabolic efficiency of skeletal muscle and physical performance during endurance training and the -9 allele may be involved in determining endurance performance (30,37).

Since some studies have proved the association between athletic endurance status and polymorphism of *BDKRB2*, we decided to select a group of well-trained endurance-discipline athletes and designed a study to investigate the possible association between *BDKRB2* and training-induced changes in swimming performance. Hence, we hypothesized that long-term

effects of training in swimming performance would be greater in subjects with the -9/-9 polymorphism of the *BDKRB2* gene.

Materials and Methods

Experimental Approach to the Problem

We used a repeated measures field study design to examine the association between the BDKRB2 gene -9/+9 polymorphism and pre-/post-training changes in swimming performance among 100 well-trained competitive swimmers. The study period was two years including the dates when swimmers' best ever performance was achieved and the seasonal best results from two years previously. For each subject, performance in middle (200 m) and long (400–1500 m) distance events in real competitions was analyzed and times were converted into FINA points. All of the subjects had extensive competitive experience and were Caucasian to reduce the possibility of racial gene skew and to overcome any potential problems because of population stratification.

Subjects. One hundred Polish swimmers (52 males and 48 females, aged 18.1 ± 1.9 years), who competed in national and international competition at middle (200 m) and long distance events (≥400 m), were recruited for this study. Only subjects who achieved a result higher than 600 FINA (International Swimming Federation) points were included in this study. Mean swimming performance treated as the personal best result at any distance was 734 ± 86 FINA points. To reduce the confounding factors of health status, we selected only those athletes who obtained a Polish Swimming Federation license after the obligatory medical examination.

More than 50% of the subjects were finalists of National Championships. The range of number of training units per week was from 5 to 13. As a control group, samples were prepared from 230 unrelated volunteers with no previous involvement in processional 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 Szczecin University (18).

Swimming performance

Swimmers' best ever performance for middle (200 m) and long (400–1500 m) distance events in real competitions was retrieved and converted into FINA points, based on the FINA 2013 tables. Following that, seasonal best results achieved two years earlier at the same events in a competition were incorporated into the analysis. Mean (± SD) time difference between gaining two analyzed results was 1.9 ± 0.4 years. Improvements in performance were expressed as absolute (points) and relative (percentage) changes in swimming results, and they were treated as training effects. Absolute pre-/post-training changes in swimming performance were calculated as the difference between two results expressed in FINA points. Relative pre-/post-training changes in swimming performance were calculated as the ratio of two results.

Genetic analyses

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

The samples were genotyped for the -9/+9 polymorphism within exon 1 of the *BDKRB2* gene using 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. (38). PCR mixture and thermal-time profile were coequal as described by Sawczuk et al. (31). The amplified DNA fragments were visualized by virtue of 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. Bivariate correlation analyses of quantitative data (pre- and post-training performance, pre-/post-training absolute and relative performance differences) showed significant correlations with metrical age; therefore, associations between the *BDKRB2* gene and dependent variables were assessed by analysis of covariance (ANCOVA). Subjects' metrical age when the best results were achieved was noted and incorporated into the statistical analysis as a covariate. Normality of each quantitative trait was tested with the Kolmogorov-Smirnov test, and Levene's test was used for testing homogeneity of variance.

The possible association between swimming performance improvements in response to training and the *BDKRB2* gene -9/+9 polymorphism was analyzed with a two-factor (3x2; genotype x time) analysis of variance with repeated measures on training and with subjects' age at post-training events as a covariate. All significant associations from the main ANCOVA model were subjected to pairwise statistical tests among each of the three genotype groups (+9/+9, +9/-9, -9/-9). Linear tests were performed between each of the genotype groups to determine which genotype groups were significantly different from one another. The resulting P values from these linear tests were adjusted for multiple comparisons by using the Bonferroni post hoc multiple-comparison test.

Results

The genotype distribution of -9/+9 *BDKRB2* in swimmers and controls met Hardy-Weinberg equilibrium ($p>0.05$ for each group). The *BDKRB2* genotype frequency of the Polish control group (+9/+9 – 29%; +9/-9 – 52%; -9/-9 – 19%) was similar to those reported in previous studies on Caucasian populations (4,5,20,38). The genotype distributions of +9/-9 *BDKRB2* in all swimmers as well as in male and female swimmers were not significantly different to controls ($p>0.05$) (Table 1). There were no significant differences in the genotype and allele frequencies between males and females amongst both the athletes and controls ($p>0.05$ for each subgroup). Differences in the -9 allele frequencies between swimmers and controls did not reach statistical significance (44% vs. 49%; $p>0.05$).

Mean (±SD) unadjusted pre-training as well as post-training swimming results in females were insignificantly ($p>0.05$) lower than in male athletes (638 ± 87 vs. 649 ± 115 points and 725 ± 75 vs. 743 ± 94 points, respectively). However, swimming results were strongly related to age ($r=0.57$; $p<0.00001$). When adjusted to age, the mean swimming performance was not statistically different in male and female subjects regardless of the *BDKRB2* +9/-9 polymorphism, both at pre-training and post-training. Training period of 1.9 ± 0.4 years had a significant ($p<0.01$) effect on swimming performance, both in female and male athletes. Mean improvements in swimming performance were similar in female and male swimmers (71 ± 49 vs. 75 ± 49 points).

Both in female and male athletes the *BDKRB2* +9/-9 polymorphism had no significant effect on swimming performance. Nevertheless, only in male athletes was an interaction effect of training x *BDKRB2* +9/-9 polymorphism found for swimming performance (Figure 1). As a follow-up to the significant interaction, ANCOVA and Bonferroni's post hoc test were performed with the adjusted significance level, to control for type I error. Post hoc analyses

indicated that swimmers with the +9/+9 genotype had a greater improvement in swimming performance than swimmers with the +9/-9 genotype ($p<0.05$). No other pairwise comparisons were significant in terms of improving swimming results (Figure 2).

No interaction effect of gender x *BDKRB2* +9/-9 polymorphism was found for either swimming performance or improving swimming performance.

Discussion

Different swimming training protocols have been shown to present a variety of effects on swimming performance (3,13). One might expect to observe positive exercise adaptation and a significant improvement in swimming results within 2 years of swimming training in young competitive swimmers. The results of our study are consistent with most exercise interventions in swimmers (24), as we observed a significant improvement in swimming performance in the whole study group.

The first important finding in this study is that we observed an interaction effect of training x +9/-9 genotype in male athletes for the 2-year improvement in swimming performance. It was found that swimmers with the +9/+9 genotype had a greater improvement in swimming performance than swimmers with +9/-9 polymorphism ($p<0.05$). Secondly, our data showed that there is no association between *BDKRB2* genotype and swimming performance in well-trained swimmers (post-training results). To date, training effects in endurance performance in relation to +9/-9 genotype have not been sufficiently analyzed. Only a few reports on the impact of bradykinin receptor genes and exercise performance in humans have been published. The results of this study do not support the hypothesis that the -9/-9 genotype will be favorable for improvements in swimming performance at middle and long-distance events. The results are also in contradiction to some previous studies involving endurance athletes. In a study of 453 athletes who completed the South Africa Ironman Triathlon, -9/-9 genotype of the *BDKRB2* gene was over-represented in the entire field of consenting male Caucasians when compared to controls (30). More recently, Sawczuk et al. (31) reported that Russian long-distance (0.8-25 km) swimmers had significantly greater (61.5%) frequency of the -9 allele than controls (45.9%, $P=0.0271$) or short-distance (50-100 m) swimmers (32.1%, $P=0.0022$). Conversely, the frequency of the +9 allele was overrepresented in Russian short-distance swimmers compared to controls (67.9% vs. 54.1%) (31). However, similarly to our previous study (14), Sounders et al. also found that the -9 allele was not significantly different in three groups of athletes who completed the event in fast, medium, and slow times (30). Our findings are also in disagreement with the results of the study by Williams et al. (38), who

found that common genetic variation in *BDKRB2* is associated with efficiency of skeletal muscle contraction and with distance event performance of elite track athletes. They also observed that the I allele of the *ACE* (angiotensin-converting enzyme) gene together with the -9 allele of the *BDKRB2* gene was associated with endurance performance of elite athletes (38). This suggests that at least part of the associations of *ACE* and fitness phenotypes is through elevation of kinin activity. So, kallikrein-kinin system components could have been involved as an element determining athletic performance. Moreover, Gacesa et al. (28) found that the muscle morphological response to strength training was related to polymorphisms of *BDKRB2*. It was demonstrated that -9 allele individuals exhibited significantly higher hypertrophy compared to those with one or two +9 alleles after six-week maximal strength training. These findings suggest that *BDKRB2* may participate in the modulation of exercise.

On the other hand, Eynon et al. (10) found no significant differences in the frequencies of the -9 allele and -9/-9 genotype between endurance athletes and controls. Also, lack of a relationship between *BDKRB2* polymorphism and best marathon times in male and female Israeli runners has been reported. This is in agreement with the results from our study. The same negative results were obtained in our previous study incorporating 157 well-trained swimmers (14). In the Gacesa et al. study (28), simultaneously to the observed differences of training response in muscle morphology changes with respect to *BDKRB2* polymorphism, *BDKRB2* genotype did not affect functional muscle properties – pre- and post-training and improvements in maximal and endurance strength and fatigue rate showed no significant correlation with *BDKRB2* genotype.

Interestingly, we found an association between *BDKRB2* and training-induced changes in endurance exercise performance only in men, which suggests possible sex-related effects. Nunes et al. (25) reported that the *BDKRB2* polymorphism (rs5810761) influenced exercise systolic blood pressure. However, this relationship was also found only in men. On the other hand, results obtained by Sgourou et al. suggest that the co-existence of *ACE* (D/D), *BDKRB2* (+9/-9) or *LEP* (G/A) genotypes in female athletes might be correlated with a superior level of physical performance (32). Furthermore, results of the Brull et al. (5) study showed that heart rate muscle hypertrophy could be greater in male army recruits with *BDKRB2* +9/+9 genotype after 10 weeks of physical training. It was suggested that kinins regulate left-ventricular growth, mediating some of the effects of *ACE* in this regard. It could be assumed that improvements in swimming at middle and long distance events could be related to heart properties and this is mediated by *BDKRB2* +9/+9 genotype. That could be one reason why we did not observe greater training-induced effects among

athletes carrying the -9/-9 genotype, as was hypothesized. Similarly to our results, Sgourou et al. found that *BDKRB2* genotype alone is not a strong predictor of athlete success, but the co-existence of *ACE* (D/D) with *BDKRB2* (+9/-9) or *LEP* (G/A) genotypes and the reduction of I allele frequency and of both IG+9A and IG-9A allelic combinations were proven to be significant, compared to the female control group (32).

Limitations

The present study has limitations that must be addressed. The multiple variables that impact on swimming performance in a competition could mask a single gene effect. Swimming performance is a multifactorial phenotype in which a number of effects of both genetic and environmental factors determine the results. Competitive swimming is strongly influenced by training and recovery programs and training conditions (e.g. altitude vs sea level), as well as psychological and technological (e.g. swimsuit materials) factors during competition.

The low sample size could also limit the power of the statistical analysis. Furthermore, lack of physiological measurements limits the discussion on the adaptation mechanism. The association that was observed may not indicate a direct relationship between the analyzed polymorphism and exercise phenotypes, since these variants may be in strong linkage disequilibrium with many other genetic loci not analyzed in the present study. It is more likely that several gene loci, each with a small but significant contribution, are responsible for genetic component improvements in swimming performance. Indeed, at least seven genetic markers located on the *ACE*, *ACTN3*, *AMPD1*, *MCT1*, *PPARA*, *PPARGC1A* and *VEGFR2* genes have been reported to be associated with competitive swimmer performance (1,2,7,8,11,12,21,36).

In conclusion, the collected data suggest that the response to long-term exercise training could be modulated by *BDKRB2* +9/-9 polymorphism in male athletes. However, in well-trained swimmers *BDKRB2* gene variation alone was not found to be an independent determinant of swimming performance.

Practical application

Identifying genetic polymorphisms that enhance endurance performance could be supportive in understanding individual variations in health- and exercise-related phenotypes. Further investigations on larger cohorts of competitive athletes are needed to clarify the potential role

of polymorphic variants of candidate genes in determining sport performance abilities. The data on the effects of *BDKRB2* gene variation on various aspects of endurance performance in general are far from clear. Despite positive results of some previous studies, the use of *BDKRB2* genotyping as an independent factor in talent identification programs could be questioned. Probably, broader and more homogeneous sampling of athletes would demonstrate how strong the results of this study are and allow examination of the effects of multiple genetic variants and allele combinations on superior physical performance. New approaches should be identified to evaluate the impact of DNA polymorphisms in human fitness and high-level performance.

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Table 1. *BDKRB2* +9/-9 genotype distribution and frequencies of -9 allele in Polish swimmers and control group

	Genotype frequency, n (%)					Allele frequency, n (%)			
Subjects	+9/+9	+9/-9	-9/-9	Chi	p	+9	-9	Chi	p
All swimmers (n=100)	29 (0.29)	52 (0.52)	19 (0.19)	1.1	p=0.52	110 (0.55)	90 (0.45)	0.8	p=0.38
Male swimmers (n=52)	19 (0.37)	25 (0.48)	8 (0.15)	2.9	p=0.24	63 (0.61)	41 (0.39)	2.9	p=0.09
Female swimmers (n=48)	10 (0.21)	27 (0.56)	11 (0.23)	1.1	p=0.59	47 (0.49)	49 (0.51)	0.2	p=0.68
Controls (n=230)	62 (0.27)	112 (0.49)	56 (0.24)	-	-	236 (0.51	224 (0.49)	-	-

Note: Genotype and allele frequency compared to control group

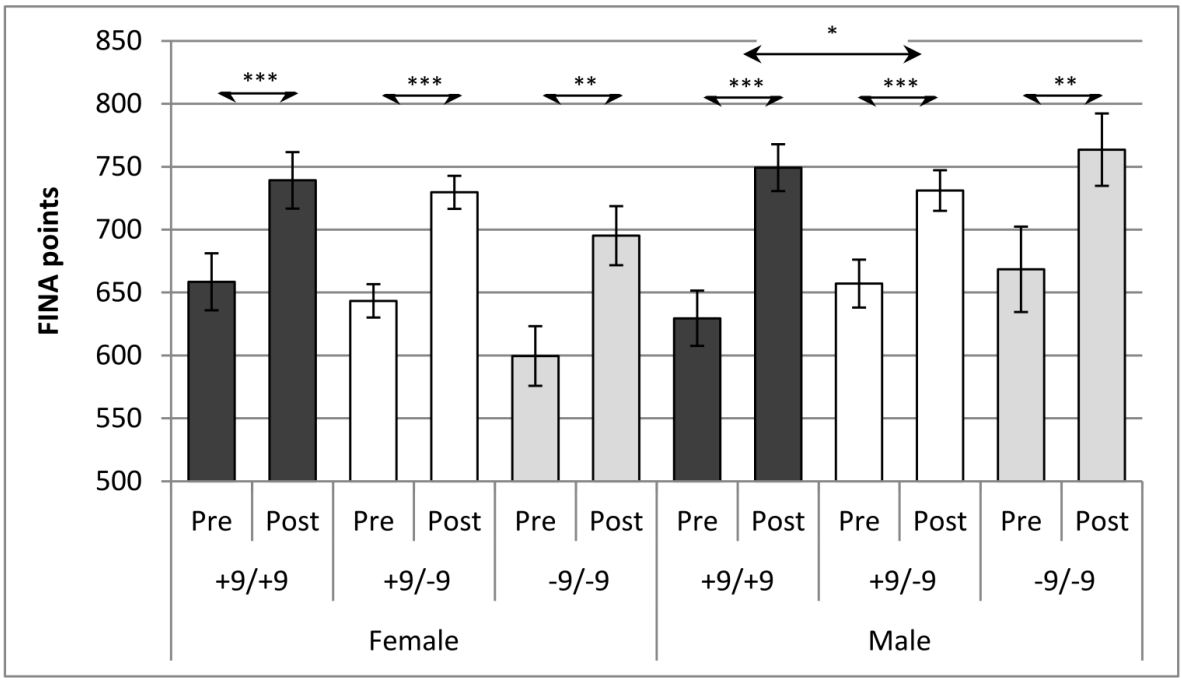

Figure 1. Pre- and post-training mean (±SE) values of results expressed in FINA points, adjusted to subjects' age, among male and female swimmers of different +9/-9 polymorphisms of *BDKRB2* gene.

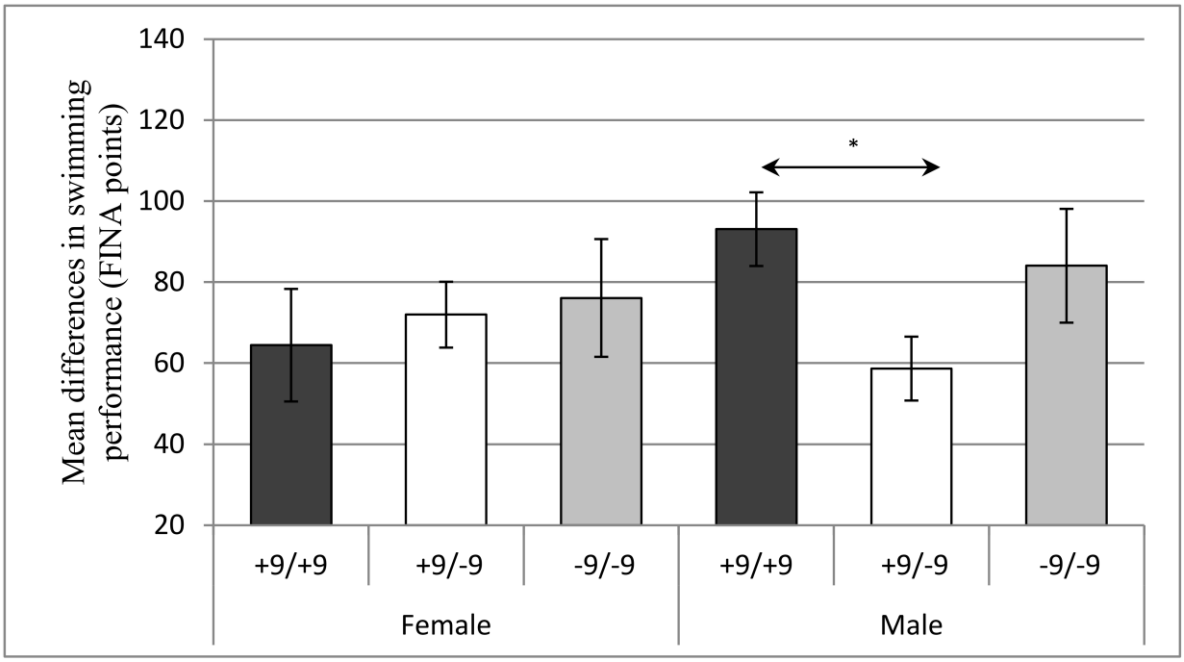

Figure 2. Pre-/Post-training differences in swimming performance expressed in FINA points, adjusted to subjects' age, among male and female swimmers of different +9/-9 polymorphisms of *BDKRB2* gene.