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Is there an interaction between *BDKRB2* -9/+9-*GNB3* C825T polymorphisms and elite athletic performance?

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Running head: *BDKRB2* and *GNB3* genes and elite athletes

Abstract

The -9 deletion allele in the -9/+9 *BDKRB2* polymorphism was previously associated with improved endurance performance. We compared the frequency distribution of the *BDKRB2* -9/+9 (rs5810761) polymorphism between athletes (n=155) of sports with different demands (endurance runners; n=74 vs. sprinters; n=81) as well as between athletes of different competitive levels (elite level; n=46 vs. national level; n=109). These results were compared to those of 240 non-athletic healthy individuals. We also tested the influence of the interaction between the *BDKRB2* -9/+9 and the *GNB3* C825T (rs5443) genotypes in relation to endurance performance. Genotype distribution and allele frequencies were found to be similar in the endurance athlete, sprinter, and control groups (P=0.83 for genotype distribution and P=0.9 for allele frequencies). Similarly, no statistical differences were found between the subgroups of elite-level endurance athletes and national-level endurance athletes, or between elite-level and national-level sprinters (P>0.09 for all comparisons). There was no interaction between *BDKRB2* -9/+9 and *GNB3* C825T polymorphisms in relation to endurance performance (P=0.16 for interaction effect). In conclusion, the *BDKRB2* +9/-9 polymorphism is not associated with endurance performance, at least among Israeli athletes, and the *GNB3*TT+ *BDKRB2* -9/-9 "optimal genotype" is not associated with endurance performance.

Key words: genetics, top-level athletes, bradikinin, G-proteins

Introduction

Kinins are endogenous ligands for G-protein coupled inducible B_1 and constitutive B_2 receptors (B_{1R} and B_{2R}, respectively). The kallikrein kinin system (KKS) plays a vital role in the cardiovascular system, affecting blood pressure regulation, cell proliferation, and matrix synthesis by fibroblasts (Burch and Kyle 1992). Activation of KKS induces coronary vasodilatation (Su et al., 2000), and increases the production of the vasodilator, nitric oxide (NO) from arginine by the enzyme nitric oxide synthase (NOS) (Dietze et al., 1996; Shen et al., 1995). Bradikinin, a component of the skeletal muscles' kallikrein kinin system (KKS). acts via the bradykinin b_2 receptor, which is encoded by the *BDKRB2* gene, to augment skeletal muscle glucose uptake during exercise (Dietze, 1982; Mayfield et al., 1996).

A common repeat sequence variation of 9bp (-9/+9 alleles) in exon 1 of the *BDKRB2* gene has been identified, in which the -9 deletion allele, is associated with higher transcription activity (Braun et al., 1996). Only a few studies have tested the potential role of this genetic variant in athletic performance. Williams and colleagues have suggested that the -9 allele is associated with efficiency of skeletal muscle contraction and with distance events of elite track athletes (Williams et al., 2004). Furthermore, the *BDKRB2* -9/-9 genotype was associated with the actual performance of 701 males who completed an Ironman Triathlon (Saunders et al., 2006).

The guanine nucleotide binding protein beta polypeptide 3 (*GNB3*), which encodes the Gβ3 subunit of G protein-coupled receptors, was considered a potential candidate gene for endurance performance, because a large number of hormones, neurotransmitters, chemokines, and local mediators exert their effects on cells by binding to G protein-coupled receptors (Hamm, 1998). The C825T polymorphism, a substitution of

cytosine (C) for thymine (T) at nucleotide 825 of *GNB3* cDNA, activates a splice site that results in alternative splicing of exon 9, leading to the deletion of 41 amino acids in the β3 subunit of the GTP-binding protein (Siffert et al., 1998). This splice variant, referred to as Gβ3, is a biologically active protein that enhances signal transduction via pertussis toxin-sensitive G proteins (Siffert et al., 1998).

Rankinen and colleagues (2002b) found that the *GNB3* C825T polymorphism plays a minor role in heart rate and body fatness regulation in African Americans, as well as in responsiveness of resting blood pressure to endurance training in African American women. This polymorphism was also associated with VO_{2max} in non-athletes (Faruque et al., 2009), and our group recently reported a higher frequency of the TT genotype in Israeli elite endurance athletes than in sprinters of the same origin (Eynon et al., 2009).

Since both polymorphisms are functional and were previously associated with elite athletic performance, the purposes of the present study were twofold: 1) to compare the frequency distribution of the *BDKRB2* -9/+9 (rs5810761) polymorphism between athletes of sports with different demands (endurance runners vs. sprinters) as well as between athletes of different competitive levels (elite level vs. national level), and 2) to test the interaction between the *BDKRB2* -9/+9 and the *GNB3* C825T (rs5443) genotypes in relation to endurance performance.

Material and Methods

Participants

One hundred and fifty-five current and former track and field athletes (119 men and 36 women, age=35.9±12.2 yrs) volunteered to participate in the study. The personal best times of the endurance and sprint athletes are reported in Table 1. We included athletes in the study sample only if they had participated in national/international track and field championships. The control group consisted of 240 non-athletic healthy individuals (170 men and 70 women) who were randomly selected from the Israeli population. Controls were not engaged in physical activity on a regular basis. We divided the athletes into two groups: 1) An endurance-type group that included 74 long distance runners (60 men and 14 women) whose main events were the 10000m run and the marathon; and 2) A sprint-type group that included 81 sprinters (59 men and 22 women) whose main events were the 100-200m dash and the long jump. According to their individual best performances, we further divided the athletes within each group into two subgroups: elite level (those who had represented Israel in track and field world championships or in the Olympic Games; 28 men and 18 women) and national level (91 men and 18 women). All participants, athletes and non-athletes, were Israeli Caucasians for ≥ 3 generations. The study was approved by the Helsinki Committee, the formal ethics committee of the Hillel Yaffe Medical Center, Hadera, Israel, according to the Declaration of Helsinki. Written informed consent was obtained from each participant.

Genotyping

We extracted genomic DNA from peripheral EDTA-treated anti-coagulated blood using a standard protocol. Genotyping of the *BDKRB2* -9/+9 (rs5810761) and *GNB3* C825T (rs5443) was performed using polymerase chain reaction (PCR). Genotype analyses were performed as explained below in the Genetics and Molecular Biology Laboratory of the Zinman College of Physical Education and Sport Sciences at the Wingate Institute, Netanya, Israel.

Information on the primers, PCR annealing temperature, restriction enzyme, and fragments obtained for each allele, respectively, for the studied polymorphisms is shown in Table 2.

Genotyping for the *GNB3* C825T polymorphism was performed according to a previously-described method (Eynon et al., 2009). To ensure proper internal control, for each genotype analysis we used positive and negative controls from different DNA aliquots that were previously genotyped with the same method, according to recent recommendations for replicating genotype-phenotype association studies (Chanock et al., 2007). The genotypes' results were scored by two experienced and independent investigators who were blind to the participants' data.

Data analysis

The SPSS statistical package, version 17.0, was used to perform all statistical evaluations (SPSS Inc., Chicago, IL, USA). A Pearson χ^2 test, a Yates corrected χ^2 test, or a Fischer exact test was used to confirm that the observed genotype frequencies were in Hardy-Weinberg equilibrium, and to compare the *BDKRB2* -9/+9 and *GNB3* C825T alleles and genotype frequencies between athletes and controls. One of these tests was also

used to examine the interaction between the *BDKRB2* -9/+9 and *GNB3* C825T genotypes in relation to endurance performance, as well as in relation to the endurances athletes' level of performance. The level of significance was set at $P< 0.05$.

Results

The complete data on allele and genotype frequencies of the *BDKRB2* -9/+9 polymorphism are shown in Table 3. The genotype subtype did not differ by gender in the athletes' group (χ^2 = 0.17, d.f=2, P=0.91) or in the control group (χ^2 = 1.88, d.f=2, P=0.4). *BDKRB2* -9/+9 genotype distribution was in agreement with the Hardy-Weinberg equilibrium within all groups ($P > 0.05$).

Genotype distribution and allele frequencies (Table 3) were similar in the endurance athlete, sprinter, and control groups. The *GNB3* TT genotype was previously associated with elite athletic status in the same group of athletes used in the current study (χ^2 = 6.1, d.f=2, P=0.046 for genotype frequencies in elite endurance athletes vs. controls, and χ^2 = 6.2, d.f=2, P=0.045 for genotype frequencies in elite endurance athletes vs. elite sprinters). Although this should be interpreted with caution because of the small sample size, there were no associations of the investigated polymorphisms with the athletic ability of either group of elite athletes.

There was also no interaction between *BDKRB2* -9/+9 and *GNB3* C825T polymorphisms in relation to endurance performance (Table 4).

Discussion

The main finding of the present study was the lack of association between *BDKRB2* -9/+9 polymorphism and elite endurance performance. This is in disagreement with previous studies that revealed a close association between the -9/-9 genotype and South African Ironman triathletes (Saunders et al., 2006), and between the -9 allele and elite endurance athletic status (Williams et al., 2004). Furthermore, our results do not support a linkage analysis, performed by the HERITAGE study group, which suggests an effect of a locus in close proximity to the *BDKRB2* gene on performance-related phenotypes, such as cardiac output and stroke volume (Rankinen et al., 2002a).

The *BDKRB2* gene that encodes the bradikinins b_2 receptor was proposed as a genetic marker for endurance performance, due to the bradikinins b_2 receptor's potential function in increasing skeletal muscle glucose uptake during exercise (Dietze, 1982; Mayfield et al., 1996), as well as in generating vasodilatation via the production of nitric oxide (NO) (Dietze et al., 1996; Shen et al., 1995). In fact, through the b_2 receptor, bradykinin enhances insulin-stimulated tyrosine kinase activity of the insulin receptor, with subsequent GLUT-4 translocation in skeletal muscle tissue during exercise (Taguchi et al., 2000).

The genotype distribution of the rare -9/-9 genotype in our control group was similar to other reports of 115 British (Williams et al., 2004) and 203 South African controls (Saunders et al., 2006). However, only 16% of our endurance athletes harbored the -9/-9 genotype, as opposed to 30% of the fast (148 athletes who finished the event within 11.8h) South African triathletes (Saunders et al., 2006), and the minor -9 of our endurance athletes was also much lower than the frequency reported by Williams and colleagues (Williams et al., 2004). Such data is

important since it reinforces the hypothesis that the genetic endowment of elite athletes is probably different than that of athletes who are not from the same origin.

The second main finding was that there is no interaction between the *BDKRB2* -9/+9 and the *GNB3* C825T polymorphisms in relation to endurance performance. Elite athletic performance is a polygenic trait, with over 20 polymorphisms suggested to influence the result of endurance and/or power-oriented athletes (Williams and Folland 2008). Among these polymorphisms is the *GNB3* C825T, with overrepresentation of the T allele in elite endurance athletes (Eynon et al., 2009). The T allele was also associated with maximal oxygen consumption (VO_{2max}), which is influenced by autonomic modulation of heart rate, in a cohort of African-American university students (Faruque et al., 2009). The explanation for this confirmed association perhaps comes from the theory that supports the role of the T allele in higher adrenergic activation, and thus in increased mobilization of circulating fatty acids and glucose that can be oxidized by muscle fibers (Eynon et al., 2009). This study was based on the hypothesis that not only several *individual* polymorphisms, but also the *combination* between several polymorphisms, may play a role in the determination of athletic performance. In this case, in opposition to our hypothesis, the *BDKRB2* -9/+9 polymorphism was not associated with endurance performance when we tested it separately. Thus, we tried to perform an interaction between two functional polymorphisms (e.g; *BDKRB2* -9/+9 and *GNB3* C825T), comparing the presence of the *GNB3*TT+ *BDKRB2* -9/-9 "optimal genotype" between endurance athletes and sprinters and between endurance athletes at different levels. However, this interaction was not statistically significant.

Our study was not without limitations. The group of athletes was relatively small, owing to the small number of available athletes who met the criteria. Nevertheless, it consisted of highly-selected endurance and sprint athletes having a unique phenotype. Also, genetic association studies must always be interpreted with caution. As with any statistical analysis, there is a non-trivial possibility of a false positive result, especially when detecting the interaction between several polymorphisms and such an "extreme" phenotype. Therefore, it is important to corroborate these and other findings in the field with other ethnic groups.

To summarize, among Israeli athletes the *BDKRB2* +9/-9 polymorphism is not associated with endurance performance. Furthermore, no association was found between the theoretical *GNB3*TT+ *BDKRB2* -9/-9 "optimal genotype" and endurance performance. Further investigations are needed to clarify the possible role of other polymorphisms, and combinations of polymorphisms, in determining athletic performance.

Perspectives

Identifying candidate polymorphisms that might influence athletic performance is an ongoing project. The *BDKRB2* -9/-9 and the *GNB3* C825T were selected as candidate polymorphisms due to previous positive reports in cohorts of elite athletes (Eynon et al., 2009; Saunders et al., 2006; Williams et al., 2004). Athletic champion status is a complex polygenic trait in which numerous candidate genes, complex gene-

gene interactions and environment-gene interactions are involved (Lucia et al., 2010). We believed that studies involved in gene-gene interactions are needed using new approaches, such as total genotype score (Williams and Folland 2008), taking into account the complexity of the problem.

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Tables:

Table 1. Personal best times of the endurance and sprint athletes. Data are mean±SD.

	Endurance athletes (n=74): Personal best marathon time		**Sprinters (n=81): Personal best 100m time**	
	Male (n=60)	Female (n=14)	Male (n=59)	Female (n=22)
Elite-level (n=46)	2h 19 min 57 s ± 2 min (n=14)	2 h 44 min 20 s ± 3 min (n=6)	10.43 ±0.15 s (n=15)	11.80±0.1 s (n=11)
National-level (n=109)	2 h 44 min 6 s ± 25 min (n=46)	3 h 5min 20 s ± 35 min (n=8)	10.85± 0.26 s (n=44)	12.22±0.34 s (n=11)

*Best marathon time.

**Best 100 m sprint time

Table 2. Information on genotyping methods for each polymorphism

SNP name	Referense SNP ID	Primers 5' →3'	Annealing temperature	Restriction enzyme	Obtained fragment
BDKRB2 -9/+9	rs5810761	F- TAAAATGAATAAAGGTGGGGGT	53°	_____	+9 allele → 100 bp
		R- TAAGAGTGGAAGGGTGGAGAA			-9 allele →91 bp
GNB3 C825T	rs5443	F- TGACCCACTTGCCACCCGTGC	62°	*BsaJI*	825T allele → 268 bp
		R- GCAGCAGCCAGGGCTGGC			825C allele →152 and 116 bp

Table 3. The *BDKRB2* +9/-9 genotype and allele frequencies distribution in all groups.

		Genotype			Allele frequencies	
Athlete groups	n	+9/+9	+9/-9	-9/-9	Allele +9	Allele -9
Endurance	74	22 (29.7)	40 (54.1)	12 (16.2)	84 (0.57)	64 (0.43)
Sprinters	81	23 (28.4)	40 (49.4)	18 (22.2)	86 (0.53)	76 (0.47)
Control	240	63 (26.3)	133 (55.4)	44 (18.3)	259 (0.54)	221 (0.46)

χ^2=1.49, d.f.=4, P=0.83 for genotype frequencies between endurance athletes, sprinters and controls

χ^2=0.33, d.f.=2, P=0.85 for allele frequency between endurance athletes, sprinters and controls

Table 4. Combined *GNB3* C825T and *BDKRB2* +9/-9 polymorphisms genotype frequencies within the endurance athletes, the sprinters, and the control group.

***GNB3* genotype**	***BDKRB* Genotype**	**Endurance athletes (n=74)**	**Sprinters (n=81)**	**Controls (n=240)**
CC	+9/+9	5 (6.8)	7 (8.6)	27 (11.3)
CC	+9/-9	15 (20.3)	18 (22.2)	42 (17.5)
CC	-9/-9	5 (6.8)	4 (4.9)	21 (8.8)
CT	+9/+9	11 (14.9)	15 (18.5)	29 (12.1)
CT	+9/-9	18 (24.3)	20 (24.7)	77 (32.1)
CT	-9/-9	6 (8.1)	13 (16)	18 (7.5)
TT	+9/+9	6 (8.1)	1 (1.2)	7 (2.9)
TT	+9/-9	7 (9.5)	2 (2.5)	14 (5.8)
TT	-9/-9	1 (1.4)	1 (1.2)	5 (2.1)

Data is presented as absolute and relative values (within parentheses)

χ^2 = 21.4, d.f=16, P=0.16 for overall combined genotype distribution

χ^2 = 0.34, d.f=2, P=0.84 for "optimal endurance" genotype frequencies *GNB3* TT+ *BDKRB2* -9/-9 vs. other genotypes between endurance athletes, sprinters, and controls.