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IS *GNB3* C825T POLYMORPHISM ASSOCIATED WITH ELITE STATUS OF POLISH ATHLETES?

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ABSTRACT: The *GNB3* gene encodes the beta 3 subunit of heterotrimeric G-proteins that are key components of intracellular signal transduction between G protein-coupled receptors (GPCR) and intracellular effectors and might be considered as a potential candidate gene for physical performance. Objectives: The aim of this study was to compare frequency distribution of the common C to T polymorphism at position 825 (C825T) of the *GNB3* gene between athletes and nonathletic controls of the Polish population as well as to compare the genotype distribution and allele frequency of C825T variants within a group of athletes, i.e. between athletes of sports of different metabolic demands and competitive levels. Methods: The study was performed in a group of 223 Polish athletes of the highest nationally competitive standard (123 endurance-oriented athletes and 100 strength/power athletes). Control samples were prepared from 354 unrelated, sedentary volunteers. Results: The χ^2 test revealed no statistical differences between the endurance-oriented athletes and the control group or between sprint/strength athletes and the control group across the *GNB3* 825C/T genotypes. There were no male-female genotype or allele frequency differences in controls or in either strength/power or endurance-oriented athletes. No statistically significant differences in either allele frequencies or genotype distribution were noted between the top-elite, elite or sub-elite of endurance-oriented and strength/power athletes and the control group. Conclusions: No association between elite status of Polish athletes and the *GNB3* C825T polymorphic site has been found.

KEY WORDS: *GNB3* C825T, gene polymorphism, performance, elite athletes status

INTRODUCTION

A significant number of studies have been published in the last decade indicating that genetic variants have an influence on human physical performance and/or elite athlete status [3,5,12,16]. One gene that might be considered as a potential candidate for physical performance is the guanine nucleotide binding protein beta polypeptide 3 (*GNB3*) gene. The *GNB3* gene encodes the beta 3 subunit of heterotrimeric G-proteins, which integrate signal transduction between G protein-coupled receptors (GPCR) and intracellular effectors in almost all cells of the human body [4]. The functional cytosine (C) to thymine (T) polymorphism (rs5443) at position 825 of the cDNA in exon 10 of the *GNB3* gene was first described by Siffert et al. [26]. This C to T transition results in alternative splicing of exon 9 and leads to the deletion of 41 amino acids in the beta 3 subunit of the GTP-binding protein [26]. It has been suggested that the shorter isoform encoded by the 825T allele is biologically active and is associated with enhanced G protein activation [25,26]. The C825T polymorphic site has been linked with many disorders, e.g. elevated diastolic blood pressure (DBP) and increased risk of left ventricular hypertrophy [15,24]. Other studies have reported that the *GNB3* C825T polymorphism has an influence on body fat, heart rate regulation and responsiveness of blood pressure to endurance training in African Americans [17] as well as being associated with $\dot{V}O_2$max in non-athletes [10]. The study of Eynon et al. [8] showed a higher frequency of TT genotype in elite endurance athletes than in sprinters of the same Israeli origin. However, this observation was not confirmed in another population when a replication study was conducted by Ruiz et al. [20]. Considering the conflicting results, the aim of this study was to compare the frequency distribution of the *GNB3* C825T (rs5443) polymorphic site between athletes and nonathletic controls of the Polish population. To maximize the statistical power of the presented genetic association study, the other goal was to compare the frequency distribution of C825T variants within a group of athletes, i.e. between athletes of sports of different metabolic demands and competitive levels.

MATERIALS AND METHODS

Athletes and controls. The procedures followed in the study were approved by the local Ethics Committee. All participants gave informed consent for the genotyping with the understanding that it was

anonymous and that the obtained results would be confidential.

This study was performed in a group of 223 Polish current and former professional athletes, aged 19-41, of the highest nationally competitive standard (male n=156 and female n=67). The group of athletes comprised two subgroups:

1. endurance-oriented athletes (n=123; male=100, female=23) characterised by predominantly aerobic energy production (duration of exertion over 5 minutes, intensity of exertion moderate to high): triathletes (n=4), race walkers (n=6), road cyclists (n=14), 15-50 km cross-country skiers (n=6), marathon runners (n=12), rowers (n=53), 3-10 km runners (n=17) and 800-1500 m swimmers (n=11);
2. strength/power athletes (n=100; male=57, female=43) with predominantly anaerobic energy production (duration of exertion < 1 minute, intensity of exertion sub-maximal 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 48 athletes classified as 'top-elite' (gold medallists in the World and European Championships, World Cups or Olympic Games) and 106 athletes classified as 'elite' (silver or bronze medallists in the World and European Championships, World Cups or Olympic Games). The others (n=69) 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.

The control samples were prepared from 354 unrelated, sedentary volunteers (university students, aged 19–23; 81 females and 273 males). All athletes and controls were Caucasian to reduce the possibility of racial gene skewing and to overcome any potential problems due to population stratification.

Genetic analyses

The buccal cells donated by the participants were collected in Resuspension Solution (GenElute Mammalian Genomic DNA Miniprep Kit, Sigma, Germany) using sterile foam-tipped applicators (Puritan, USA). DNA was extracted from the buccal cells using a GenElute Mammalian Genomic DNA Miniprep Kit according to the manufacturer's protocol. All samples were genotyped using an allelic discrimination assay on a StepOne real-time polymerase chain reaction (PCR) instrument (Applied Biosystems, USA) with TaqMan probes. For discrimination between the *GNB3* C825 and 825T alleles (rs5443) TaqMan Pre-Designed SNP Genotyping Assays were used (Applied Biosystems, USA), including primers and fluorescently labelled (FAM and VIC) MGB probes for detection of the alleles.

Statistical analysis

The STATISTICA software package, version 8.0, was used to perform all statistical evaluations. Allele frequencies were determined by gene counting. A χ^2 test was used to confirm that the observed genotype frequencies were in Hardy-Weinberg equilibrium as well as to compare the *GNB3* C825T alleles and the genotype frequencies among the athletes and control participants and among athletes from different sports and competitive levels. The level of statistical significance was set at $p<0.05$.

RESULTS

All genotype distributions for all groups of athletes and controls met Hardy-Weinberg expectations ($p>0.05$) in all groups tested separately. The results of the distribution of alleles and genotypes for the *GNB3* 825C/T polymorphic site in Polish athletes compared with the non-athletic controls (all subjects stratified by gender) are presented in Table 1. There were no statistical differences between the endurance-oriented athletes and the control group or between sprint/

TABLE 1. GENOTYPE DISTRIBUTION AND ALLELE FREQUENCIES OF *GNB3* GENE C825T POLYMORPHISM IN POLISH ATHLETES AND CONTROL GROUP WITH GENDER

Group	n	GNB3 C825T genotypes				Minor T allele frequency	
		CC	CT	TT	p		P
ALL ATHLETES	223	100 (44.8)	104 (46.6)	19 (8.5)	0.947	142 (31.8)	0.896
Endurance-oriented athletes	123	59 (48.0)	55 (44.7)	9 (7.3)	0.721	73 (29.7)	0.462
Strength/power athletes	100	41 (41)	49 (49)	10 (10)	0.798	69 (34.5)	0.540
ALL CONTROLS	354	155 (43.8)	170 (48.0)	29 (8.2)	1.000	228 (32.2)	1.000
Male athletes	156	72 (46.2)	72 (46.2)	12 (7.7)	0.934	96 (30.4)	0.739
Endurance-oriented athletes	99	46 (46.5)	46 (46.5)	7 (7.0)	0.911	60 (30.3)	0.685
Strength/power athletes	57	22 (38.6)	29 (50.9)	6 (10.5)	0.670	41 (36.0)	0.396
Male controls	273	121 (44.3)	130 (47.6)	22 (8.0)	1.000	174 (32.1)	1.000
Female athletes	67	28 (41.8)	32 (47.8)	7 (10.4)	0.929	46 (34.3)	0.858
Endurance-oriented athletes	24	13 (54.2)	9 (37.5)	2 (8.3)	0.555	13 (27.1)	0.414
Strength/power athletes	43	19 (44.2)	20 (46.5)	4 (9.3)	0.954	28 (32.6)	0.903
Female controls	81	34 (42.0)	40 (49.4)	7 (8.6)	1.000	54 (33.3)	1.000

Note: Data are presented as absolute and relative values (in parentheses)

strength athletes and the control group across the *GNB3* 825C/T genotypes (genotype percentage frequency for CC, CT, TT = 48, 44.7, 7.3 vs 43.8, 48.0, 8.2, p = 0.721, and 41.0, 49.0, 10.0 vs 43.8, 48.0, 8.2, p = 0.798 respectively). The allele frequency of the control group (C/T = 67.8/32.2) was similar to that of endurance-oriented athletes (C/T = 70.3/29.7, p = 0.462) and strength/power (C/T = 65.5/34.5, p = 0.540) athletes. The data showed no male-female genotype or allele frequency differences in controls or in either strength/power or endurance-oriented athletes (Table 1). No statistically significant differences in genotype or allele frequencies were observed when we compared genotype and allele frequencies among the top-elite, elite and sub-elite endurance-oriented athletes and controls. Similarly, no difference in either allele frequencies or genotype distribution was noted among the top-elite, elite or sub-elite strength/power athletes and the control group (Table 2).

We did not observe any significant differences in genotype distribution when all endurance-oriented athletes and all strength/power athletes were compared (genotype percentage frequency for CC, CT, TT = 48, 44.7, 7.3 vs 41.0, 49.0, 10.0, p = 0.673, respectively). No significant differences were also observed when groups of athletes stratified by gender were compared (male endurance-oriented and strength/power athletes genotype percentage frequency for CC, CT, TT = 46.5, 46.5, 7.0 vs 38.6, 50.9, 10.5, p = 0.739, respectively; female endurance-oriented and strength/power athletes genotype percentage frequency for CC, CT, TT = 54.2, 37.5, 8.3 vs 44.2, 46.5, 9.3, p = 0.838, respectively). The allele frequency of the all endurance-oriented group (C/T = 70.3/29.7) was similar to that of strength/power athletes (C/T = 65.5/34.5, p = 0.324). The data also showed no allele frequency differences between endurance-oriented and strength/power athletes, both in males (endurance-oriented C/T = 69.7/30.3 vs. strength/power C/T = 64.0/36.0, p = 0.366) and females (endurance-oriented C/T = 72.9/27.1 vs. strength/power C/T = 67.4/32.6, p= 0.643).

DISCUSSION

Sports genomics, describing the organization and functioning of the genome of athletes, is a relatively new scientific discipline [1]. The first genetic marker associated with athletic performance was identified in the late 1990s [13]. From that time, the importance of allelic variants of genes, and their associations with the athletic status and athletic performance, have been widely discussed in the literature of sports sciences [14,18,21,27] and over 130 articles in relation to sport genomics have been published [1]. Several methodological approaches have been proposed to find an association between gene polymorphism and athlete performance/status. Cross-sectional studies examine whether athletes with a particular genotype or allele show different measures of a trait (e.g. VO_2max) compared to the rest of the athletes [1]. Another approach is genome-wide association studies that examine polymorphic DNA markers at many positions of the whole genome, to enable genetic markers to be linked with particular physical characteristics [2,9]. Case-control association studies remain the most common study design in the field of sports genomics. These studies rely upon the assumption that one allele of a gene, referred to as candidate because of its known function presumed to be relevant to the trait under study, is more or less common in a group of elite athletes than it is in the general population and therefore enhances performance [1].

The present report is a genetic case-control association study in which the frequencies of alleles as well as genotype distribution of the C825T SNP polymorphism of the *GNB3* gene among athletes of

TABLE 2. THE *GNB3* C825T GENOTYPES AND ALLELE FREQUENCIES IN POLISH ATHLETES STRATIFIED ACCORDING TO THEIR LEVEL OF COMPETITION AND CONTROL GROUP

Group	n	GNB3 C825T				Minor T allele frequency	p
		CC	CT	TT	p		
Endurance-oriented	123	59 (48.0)	55 (44.7)	9 (7.3)	0.721	73 (29.7)	0.462
top-elite	28	14 (50.0)	13 (46.4)	1 (3.6)	0.623	15 (26.8)	0.402
elite	60	29 (48.3)	26 (43.3)	5 (8.4)	0.787	36 (30.0)	0.632
sub-elite	35	16 (45.7)	16 (45.7)	3 (8.6)	0.967	22 (31.4)	0.893
Strength/Power	100	41 (41.0)	49 (49.0)	10 (10.0)	0.798	69 (34.5)	0.540
top-elite	20	8 (40.0)	10 (50.0)	2 (10.0)	0.927	14 (35.0)	0.713
elite	46	19 (41.3)	22 (47.8)	5 (10.9)	0.818	32 (34.8)	0.619
sub-elite	34	14 (41.2)	17 (50.0)	3 (8.8)	0.956	23 (33.8)	0.786
All athletes	223	100 (44.8)	104 (46.6)	19 (8.5)	0.947	142 (31.8)	0.896
top-elite	48	22 (45.8)	23 (47.9)	3 (6.3)	0.886	29 (30.2)	0.694
elite	106	48 (45.3)	48 (45.3)	10 (9.4)	0.853	68 (32.1)	0.975
sub-elite	69	30 (43.5)	33 (47.8)	6 (8.7)	0.991	45 (32.6)	0.924
Controls	354	155 (43.8)	170 (48.0)	29 (8.2)	1.000	228 (32.2)	1.000

Note: Data are presented as absolute and relative values (in parentheses)

sports of different metabolic demands as well as competitive levels in Poland and non-athletic controls were compared. Our main findings were that 1) neither the *GNB3* T nor C allele, nor the *GNB3* C825T genotype, was significantly more frequent among either group of athletes or controls, 2) the *GNB3* C825T genotype as well as C/T allele frequencies were not more frequent in endurance-oriented athletes or in the strength/power group with respect to their level of competition.

In the study of Eynon et al. [8] the *GNB3* TT genotype was more frequent in endurance athletes than in sprinters, and within the endurance group it was higher in top level athletes. The authors suggested a positive association between the TT genotype and the likelihood of being an elite endurance athlete because of increased G protein activity of the T homozygotes (increased adrenergic activation and in consequence increased mobilisation of circulating fatty acids and glucose that can be oxidised by the muscles) [8,26]. However, the study of Ruiz et al. [20] did not corroborate the aforementioned findings of Eynon et al. [8] in a cohort that included endurance as well as power athletes of another ethnic/geographic origin. It is worth mentioning that if a significant association with physical performance is observed in one population for some polymorphic variants, not necessarily the same association or even lack of an association is noted in another population [11,23]. Thus we cannot exclude the possibility that our negative results are distinctive for the Polish population of elite athletes.

It should also be remembered that achieving elite athletic endurance or strength/power status is a complex trait. It is thus very likely that the combined influence of several genetic loci with a significant contribution, complex interaction of multiple genetic polymorphisms (with or without an individual contribution) and/or gene-environment interactions rather than an individual effect of a single polymorphic site have an influence on individual variation of human athletic performance [19-21,27]. To test the potential interaction of two polymorphisms that have been previously associated with elite athletic performance and their combined influence on athletic status of Israeli elite athletes, Eynon et al. [6] investigated the common variation of 9bp-9/+9 (rs5810761) of the bradykinin receptor b2 gene (*BDKRB2*) and the *GNB3* C825T polymorphism. The authors, however, observed no association in relation to endurance performance. Thus, research in sports genetics should focus on polygenic profiles of sports related phenotypes as well as epigenetic mechanisms that change the gene function and role of miRNAs as post-transcriptional regulators of protein synthesis in the phenotypic change and individual variability in response to exercise training [9,20].

Finally, because the main limiting factor in the field of sports genetics is to recruit large enough samples of elite athletes of different ethnic backgrounds, genotype-phenotype association studies should be corroborated with the largest possible population sizes to obtain valid results [7,9,20].

CONCLUSIONS

In our study we did not find an association between elite status of Polish athletes and the *GNB3* C825 polymorphic site.

REFERENCES

1. Ahmetov I.I., Fedotovskaya O.N. Sports genomics: Current state of knowledge and future directions. Cell. Mol. Exerc. Physiol. 2012;1:e1.
2. Bouchard C., Sarzynski M.A., Rice T.K., Kraus W.E., Church T.S., Sung Y.J., Rao D.C., Rankinen T. Genomic predictors of maximal oxygen uptake response to standardized exercise training programs. J. Appl. Physiol. 2010;110:1160–1170
3. Bray M.S., Hagberg J.M., Pérusse L., Rankinen T., Roth S.M., Wolfarth B., Bouchard C. The human gene map for performance and health-related fitness phenotypes: the 2006-2007 update. Med. Sci. Sports Exerc. 2009;41:35-73.
4. Cabrera-Vera T.M., Vanhauwe J., Thomas T.O., Medkova M., Preininger A., Mazzoni M.R., Hamm H.E. Insights into G protein structure, function, and regulation. Endocr. Rev. 2003;24:765–781.
5. 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.
6. 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.
7. Eynon N., Nasibulina E.S., Banting L.K., Cieszczyk P., Maciejewska-Karlowska A., Sawczuk M., Bondareva E.A., Shagimardanova R.R., Raz M., Sharon Y., Williams A.G., Ahmetov I.I., Lucia A., Birk R. The FTO A/T polymorphism and elite athletic performance: a study involving three groups of European athletes. PLoS One 2013;8:e60570.
8. 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.
9. Eynon N., Ruiz J.R., Oliveira J., Duarte J.A., Birk R., Lucia A. Genes and elite athletes: a roadmap for future research. J. Physiol. 2011;589:3063-3070.
10. Faruque M.U., Millis R.M., Dunston G.M., Kwagyan J., Bond V. Jr, Rotimi C.N., Davis T., Christie R., Campbell A.L. Association of GNB3 C825T polymorphism with peak oxygen consumption. Int. J. Sports. Med. 2009;30:315-319.
11. Ginevičienė V., Pranculis A., Jakaitienė A., Milašius K., Kučinskas V. Genetic variation of the human ACE and ACTN3 genes and their association with functional muscle properties in Lithuanian elite athletes. Medicina (Kaunas). 2011;47: 284-290.
12. Macarthur D.G, North K.N. Genes and human elite athletic performance. Hum. Genet. 2005;116:331-339.
13. Montgomery H.E., Marshall R., Hemingway H., Myerson S., Clarkson P., Dollery C., Hayward M., Holliman D.E., Jubb M., World M., Thomas E.L., Brynes A.E., Saeed N., Barnard M., Bell J.D., Prasad K., Rayson M., Talmud P.J., Humphries S.E. Human gene for physical performance. Nature 1998;393:221-222.
14. Ostrander E.A., Huson H.J., Ostrander G.K. Genetics of athletic

performance. Annu. Rev. Genomics Hum. Genet. 2009;10:407-429.
15. Poch E., Gonzalez D., Gomez-Angelats E., Enjuto M., Pare JC., Rivera F., de La Sierra A. G-protein beta(3) subunit gene variant and left ventricular hypertrophy in essential hypertension. Hypertension 2000;35:214-218.
16. Pokrywka A., Kaliszewski P., Majorczyk E., Zembroń-Łacny A. Genes in sport and doping. Biol. Sport 2013;30:155-161.
17. Rankinen T., Rice T., Leon A.S., Skinner J.S., Wilmore J.H., Rao D.C., Bouchard C. G protein beta 3 polymorphism and hemodynamic and body composition phenotypes in the HERITAGE Family Study. Physiol. Genomics 2002;8:151-157.
18. Roth S.M., Rankinen T., Hagberg J.M., Loos R.J., Pérusse L., Sarzynski M.A., Wolfarth B., Bouchard C. Advances in exercise, fitness, and performance genomics in 2011. Med. Sci. Sports Exerc. 2012;44: 809-817.
19. Ruiz J.R., Arteta D., Buxens A., Artieda M., Gómez-Gallego F., Santiago C., Yvert T., Morán M., Lucia A. Can we identify a power-oriented polygenic profile? J. Appl. Physiol. 2010;108:561-566.
20. Ruiz J.R., Eynon N., Meckel Y., Fiuza-Luces C., Santiago C., Gómez-Gallego F., Oliveira J., Lucia A. GNB3 C825T Polymorphism and elite athletic status: A replication study with two ethnic groups. Int. J. Sports Med. 2011;32:151-153.
21. Ruiz J.R., Gómez-Gallego F., Santiago C., González-Freire M., Verde Z., Foster C., Lucia A. Is there an optimum endurance polygenic profile? J. Physiol. 2009;587:1527-1534.
22. Sarpeshkar V., Bentley D.J. Adrenergic-beta(2) receptor polymorphism and athletic performance. J. Hum. Genet. 2010;55:479-485.
23. Sawczuk M., Maciejewska-Karłowska A., Cięszczyk P. A single nucleotide polymorphism rs553668 in the ADRA2A gene and the status of Polish elite endurance athletes. Trends Sport Sci. 2013;1:30-35.
24. Schunkert H., Hense H.W., Doring A., Riegger GA., Siffert W. Association between a polymorphism in the G protein beta3 subunit gene and lower renin and elevated diastolic blood pressure levels. Hypertension 1998;32:510-513.
25. Siffert W., Rosskopf D., Moritz A., Wieland T., Kaldenberg-Stasch S., Kettler N., Hartung K., Beckmann S., Jakobs K.H. Enhanced G protein activation in immortalized lymphoblasts from patients with essential hypertension. J. Clin. Invest. 1995;96:759-766.
26. Siffert W., Rosskopf D., Siffert G., Busch S., Moritz A., Erbel R., Sharma A.M., Ritz E., Wichmann H.E., Jakobs K.H., Horsthemke B. Association of a human G-protein beta3 subunit variant with hypertension. Nat. Genet. 1998;18:45-48.
27. Williams A.G., Folland J.P. Similarity of polygenic profiles limits the potential for elite human physical performance. J. Physiol. 2008;586:113-121.