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Section II - Exercise Physiology & Sports Medicine

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GALNTL6 rs558129: A Novel Polymorphism for Swimming Performance?

by

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The enzyme polypeptide N-acetylgalactosaminyltransferase like 6, encoded by the GALANTL6 gene, plays a role in the gut microbiome regarding regulation of short-chain fatty acids and their anti-inflammatory and resynthesis functions. It was hypothesized that the T allele of the GALNTL6 rs558129 polymorphism could have a positive effect on anaerobic metabolism. Thus, this study was performed to investigate the association between GALNTL6 rs558129 polymorphism and athletic performance in swimmers. A total of 147 Polish short distance (SDS) and 49 long distance swimmers (LDS) of national or international competitive levels and 379 controls were genotyped using the real-time polymerase chain reaction (real-time PCR). We found that the carriers of the T allele (CT+TT) had a 1.56 times higher chance of being SDS (odds ratio (OR): 95%CI 1.06-2.29) than the CC homozygotes. The T allele was overrepresented in the SDS compared with controls (33.7% vs. 25.7%, p = 0.025, OR 1.40, 95% CI 1.04-1.87), but no statistically significant differences were found for LDS. This study provides evidence for an association between the GALNTL6 rs558129 polymorphism and short distance swimming athlete status. Although more replication studies are needed, the preliminary data suggest an opportunity to use the analysis of GALNTL6 polymorphism along with other variants of candidate genes and standard phenotypic assessment in power-oriented sports selection.

***Key words**: sport, swimming, performance, gene, athlete status, gut microbiome.*

Introduction

Physical performance is a complex phenotype with a well-confirmed strong genetic basis. Accordingly, it has been shown that the heritability of athlete status is around 66% (De Moor et al., 2007). It is a highly polygenic trait. More than 200 genetic markers (situated within autosomal genes, mitochondrial DNA, X and Y chromosomes) have been linked to sport predispositions (Ahmetov et al., 2016). Recently, in a study comparing elite athletes and sedentary controls from seven cohorts – Australia, Ethiopia, Japan, Kenya, Poland, Russia, and Spain – an international consortium (GAMES) found a novel relationship between the N-acetylgalactosaminyltransferase like 6 gene (*GALNTL6*) and elite endurance performance (Rankinen et al., 2016). Next, Ramirez et al. (2020) observed its association with anaerobic performance in sprint/power athletes. Although the *GALNTL6* gene may be a very promising candidate gene for physical performance, which may underlie differences in the potential to be an elite athlete, little is known about the gene and its role in development of sports abilities; thus more experimental studies are required.

The *GALNTL6* gene encodes the membrane-bound protein N-acetylgalactosaminyltransferase like 6 (protein symbol: Q49A17-GLTL6_HUMAN; size: 601 amino acids; molecular mass: 69788 Da), which belongs to a large family of enzymes (ppGalNAc-

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Ts; EC 2.4.1.41). It has a significant role in the pathway of protein glycosylation, which is part of polypeptides' post-translational modification. The enzyme catalyses the initial step in mucin-type O-glycosylation in the Golgi apparatus. Its main role is transfer of N-acetylgalactosamine (GalNAc) from UDP-GalNAc to the hydroxyl group of serine or threonine in specific target proteins. The enzyme requires manganese (Mn^{2+}) as a cofactor (Peng et al., 2010). The O-glycosylation activity has a role in the host-microbiota interactions, since the glycans are nutrient sources for commensal bacteria and the site of attachment (Arike et al., 2017). This relationship affects human health and physiology, providing benefits such as immune development modulation, dietary nutrients digestion, inhibition of pathogen colonization, and energy metabolism regulation (Arike et al., 2017; Díaz Ramírez et al., 2020; Flint et al., 2008; McNeil, 1984; Peng et al., 2010).

The *GALNTL6* gene (4q34.1) has 21 exons and is expressed mainly in adult testis, brain, spinal cord, cerebellum, and skeletal muscle tissue (Peng et al., 2010). A human C/T polymorphism (rs558129), located in the last intron of *GALNTL6*, has been positively associated with sport performance (Díaz Ramírez et al., 2020; Rankinen et al., 2016). Firstly, Rankinen et al. (2016) found that the carriers of the C allele had a 1.23 times higher chance of being endurance athletes. Next, Ramírez et al. (2020) reported that the T allele could be favourable for anaerobic performance, implying that sprint/strength athletes may benefit from carrying this allele. Considering that many previous studies have demonstrated that one polymorphism can be associated with both endurance and sprint/power performance such as angiotensin I converting enzyme gene (*ACE*) I/D or alpha-actinin-3 gene (*ACTN3*) R/X polymorphisms (Cięszczyk et al., 2011; Grenda et al., 2014; Jastrzebski et al., 2014; Petr et al., 2022; Youn et al., 2021), it is possible that the C/T variation within *GALNTL6* is one of them.

The main aim of the present study was to compare the genotype distributions and the allele frequencies of the *GALNTL6* C/T polymorphism (rs558129) between elite short- (SDS) and long-distance swimmers (LDS), as well as sedentary controls. In light of the evidence, we hypothesized that: 1) the polymorphism would be associated with elite athletic status, 2) the frequency of the T allele and the TT genotype would be higher among SDS compared with controls, 3) the frequency of the C allele and the CC genotype would be higher among LDS. To our knowledge, this is the first study to investigate the *GALNTL6* gene and elite swimming performance in a Caucasian population. Thus, codominant, dominant, recessive, and overdominant genetic models were assessed to determine differences amongst athlete phenotypes.

Methods

Ethics statement

The Pomeranian Medical University Ethics Committee, Poland, approved the procedures. The experimental protocols were conducted ethically according to the World Medical Association Declaration of Helsinki and Strengthening the Reporting of Genetic Association studies statement (STREGA). All participants were informed of the risks and benefits of the experiment and a written consent form was completed by each participant or their parents if the participant was under 18 years of age. All individual data were anonymous.

Participants

The study group included 197 swimmers (20.31 ± 2.67 years) from Poland, who competed in national and international competitions and achieved a result of more than 600 FINA points. The division of the study group, based on their competitive distance and values of relative contribution of the aerobic or anaerobic energy systems, is presented in Table 1. Detailed characteristics of Polish swimmers were described previously by Zmijewski and Leońska-Duniec (2021).

The control group included 379 (22.6 ± 2.8 years) unrelated, sedentary students (Table 1). All participants were Caucasians.

Genetic analyses

Total DNA was isolated from the buccal cells by a GenElute Mammalian Genomic DNA Miniprep Kit (Sigma, Germany) according to the producer's protocol. All samples were genotyped in duplicate. An allelic discrimination assay on a C1000 Touch Thermal Cycler (Bio-Rad, Germany) instrument with TaqMan probes was used. To identify the *GALNTL6* rs558129 alleles, we used TaqMan Pre-Designed SNP Genotyping Assays (Applied Biosystems, USA) (assay ID:

C____968950_10), containing fluorescently labelled (FAM and VIC) minor groove binder (MGB) probes and two specific primers.

Statistical analyses

Hardy-Weinberg equilibrium expectations were compared with observed counts using the chi-square test with 1 degree of freedom. Allele frequencies were estimated using genotype counts. Genotype and allele distribution between groups were compared using the chi-square test and the analysis of sex differences was conducted. The models of inheritance, i.e., codominant, dominant, recessive and overdominant, were constructed assuming a minor allele as the risk allele. Odds ratios with 95% confidence intervals (95% CI) were used as a measure of the strength of the association. All analyses were performed using STATISTICA version 13 (TIBCO Software Inc., http://statistica.io).

Results

The *GALNTL6* gene rs558129 polymorphism was in Hardy-Weinberg equilibrium in controls ($p = 0.422$) as well as in the entire cohort ($p = 0.256$). There were no significant differences in genotypic and allelic distribution between male and female athletes, so we decided to pool the sample, as this allowed the statistical power to be increased. We found a significant difference in genotype (under the dominant models) and allele frequencies between SDS and controls (Table 2).

Table 1

Size and division of the study and control groups

Group		Females	Males	Total
Swimmers	SDS (50 m - 200 m)	67	80	147
	LDS (≥ 400 m)	25	24	49
	LDS + SDS	93	104	197
Controls		157	222	379

Table 2

Comparison of SDS and control individuals (genotypes and alleles)

Genotype	Controls (n=379)	SDS (n=147)	OR (95% CI)	*p*†
		Dominant		
CC	212 (55.9%)	66(44.9%)	1	
CT-TT	167 (44.1%)	81(55.1%)	1.56 (1.06-2.29)	**0.023**
		Recessive		
CC-CT	351 (92.6%)	129(87.8%)	1	
TT	28 (7.4%)	18(12.2%)	1.75 (0.92-3.25)	0.080
		Overdominant		
CC-TT	240 (63.3%)	84(57.1%)	1	
CT	139 (36.7%)	63(42.9%)	1.29 (0.88-1.91)	0.191
C	536 (74.3%)	195 (66.3%)	1	
T	195 (25.7%)	99(33.7%)	1.40 (1.04-1.87)	0.025

OR – odds ratio (95%CI – confidence intervals), † - chi-square test

Table 3

Comparison of LDS and control individuals (genotypes and alleles)

Genotype	Controls (n=379)	LDS (n=49)	OR (95% CI)	*p*†
			DOM	
CC	212 55.9%)	25 (51.0%)	1	0.515
CT-TT	167 (44.1%)	24 (49.0%)	1.22 (0.67-2.22)	
			REC	
CC-CT	351 (92.6%)	44 (89.8%)	1	0.489
TT	28 (7.4%)	5 (10.2%)	1.42 (0.47-3.60)	
			OVER	
CC-TT	240 (63.3%)	30 (61.2%)	1	0.774
CT	139 (36.7%)	19 (38.8%)	1.09 (0.58-2.00)	
C	536 (74.3%)	69 (70.4%)	1	
T	195 (25.7%)	29 (29.6%)	1.16 (0.73-1.84)	0.542

OR – odds ratio (95%CI – confidence intervals), † - chi-square test

Specifically, under the dominant model, the carriers of the T allele (CT+TT) had a 1.56 times higher chance of being a swimmer (95%CI 1.06-2.29) than the CC homozygotes. The finding was confirmed in an allelic association, where the T allele was overrepresented in the SDS compared with controls (33.7% vs. 25.7%, $p = 0.025$, OR 1.40, 95% CI 1.04-1.87). In contrast, when LDS were compared against control individuals, no significant differences were observed in genotypic and allelic distribution (Table 3) regardless of the model of inheritance.

Discussion

In the present study, we assessed the genotype distributions and the allele frequencies of the *GALNTL6* C/T polymorphism (rs558129) between Caucasian swimmers divided into two groups based on their competitive distance and values of relative contribution of the anaerobic or aerobic energy systems (SDS and LDS), as well as sedentary controls. The main finding of the study concerned a relationship between the *GALNTL6* rs558129 polymorphism and short distance swimming athlete status. We confirmed the second hypothesis and observed higher frequency of the T allele among SDS compared with controls. Specifically, the carriers of the T allele (carriers of the CT and TT genotypes) had a 1.56 times higher chance of being elite SDS than the CC homozygotes. Unfortunately, when LDS were compared against sedentary individuals, no significant differences were observed in genotypic and allelic distribution, thus rejecting our third hypothesis. Additionally, we did not find significant differences in the genotype distributions and the allele frequencies between male and female athletes.

The current status of research shows that the endurance athlete status remains the most studied trait in sports genomics. A literature search revealed at least 100 endurance-related genetic markers. Less is known on genes

responsible for power, sprint and short distance traits. The *GALNTL6* rs558129 polymorphism among others genetic markers has been identified and it has shown positive associations with athlete status in at least two studies (Semenova et al., 2019). However, only this paper reports the results of a study conducted on swimmers.

An international consortium (GAMES) conducted the largest genome-wide association study connected with a meta-analysis in an attempt to establish promising genetic variants of endurance performance. Firstly, genome-wide association studies (GWAS) were performed on two cohorts of elite endurance athletes and controls (GENATHLETE and Japanese endurance runners), from which 45 molecular markers were chosen. Secondly, the authors conducted a meta-analysis of available studies for replication of the obtained results in seven added cohorts of 1520 endurance athletes and 2760 controls from Australia, Ethiopia, Japan, Kenya, Poland, Russia, and Spain. The one novel finding of the study was a statistically significant association between the *GALNTL6* polymorphism rs558129 (p = 0.0002) and endurance performance, even after correcting for multiple testing. The C allele (rs558129) was overrepresented in each studied population in elite endurance athletes compared with the control group. Specifically, the carriers of the C allele had a 1.23 times higher chance of being endurance athletes (Rankinen et al., 2016). Inspired by these results, in a study including 85 Caucasian physically active participants from Spain who performed a Wingate anaerobic test (WAnT), as well as 173 power athletes, 169 endurance athletes, and 201 controls from Russia, Ramirez et al. (2020) found the relevance of the T allele to significantly higher power values in a WAnT compared to the CC genotype (5–7% higher absolute and relative mean power and peak power). Additionally, significantly higher frequency of the T allele was observed in Russian power athletes compared with endurance athletes and the control group (p < 0.05), suggesting a dominant model of inheritance. However, they did not find differences in the C allele frequency among endurance athletes and controls, as was observed in the study of Rankinen et al. (2016). The results obtained in the present study are in accordance with the findings of Ramirez et al. (2020). We also found that the carriers of the T allele had a 1.56 times higher chance to achieve outstanding results in short-distance events promoting strength and speed. The higher frequency of the C allele in endurance athletes, observed by Rankinen et al. (2016), was also not confirmed.

To date, a possible biological mechanism underlying the association between the *GALNTL6* polymorphism rs558129 and human physiology, and, consequently, elite athletic performance, is still mostly unknown. It was shown that adaptation to physical activity may be affected by the gut microbiota and factors influencing its composition and activity, since the commensal bacteria play a role in the production, storage, and expenditure of energy as well as in redox reactions, inflammation, and status of hydration (Barton et al., 2018; Mach and Fuster-Botella, 2017). O-glycosylation, which is catalysed by the GALANT6 enzyme, has a key role in regulation of the gut microbiota, since it could facilitate the digestion of glycans by commensal bacteria to short-chain fatty acids (SCFAs). These SCFAs have an influence on metabolic, immunological, and developmental processes, and, consequently, have been associated with enhanced fitness and health (Arike et al., 2017; Barton et al., 2018; Díaz Ramírez et al., 2020; Flint et al., 2008; McNeil, 1984). In a study including elite professional athletes and healthy controls, Barton et al. (2018) found a clear relationship between the physical fitness level and the functional potential of the gut microbiota and its metabolites. Additionally, results obtained by Estaki et al. (2016) revealed that cardiorespiratory fitness was linked to higher microbial diversity in healthy participants. There was also reported to be an association between greater microbial diversity and compositional alterations in the gut and higher maximal oxygen uptake (VO_{2max}), which in professional athletes can be twice as high as in sedentary individuals (Joyner and Coyle, 2008). Kulecka et al. (2020) reported that 20 and 5 taxa differentiated controls from marathon runners and cross-country skiers, respectively, suggesting that excessive training was associated with differences in composition and promotion of greater commensal bacteria diversity. Taxa enriched in professional athletes are known to take part in fermentation of fibre (Kulecka et al., 2020). In light of the evidence that gut microbiota may have a key role in controlling

oxidative stress, inflammatory responses as well as improving metabolism and energy expenditure during physical activity (Mach and Fuster-Botella, 2017), it was hypothesized that the expression of the *GALNT* family genes can be significant for physical performance and athlete status (Díaz Ramírez et al., 2020). *GALNTL6* gene expression could have an important function in anaerobic performance in some individuals which has benefits for energy utilization, by acidosis homeostasis due to lactate recycling and a role in the intestines regarding regulation of SCFAs and their anti-inflammatory and resynthesis functions. It was also found that *GALNTL6* is highly expressed in brain and testis tissues, but the functional role of the gene is not fully known (Fagerberg et al., 2014). Elucidating the mechanism of the association between the *GALNTL6* rs558129 polymorphism and elite athletic performance status will help understand the genetic background of performance and therefore can contribute to the improvement of talent identification and development or training methodology.

In summary, this study provides evidence for an association between the *GALNTL6* rs558129 polymorphism and short distance swimming athlete status. We observed higher frequency of the T allele in the SDS group, implying that sprint/strength athletes may benefit from carrying this allele. We assessed that individuals with at least one T allele (carriers of the CT and TT genotypes) had a 1.56 times higher chance to achieve outstanding results in short-distance events promoting strength and speed. Although more replication studies are needed, the preliminary data suggest an opportunity to use the analysis of the *GALNTL6* polymorphism along with other variants of candidate genes and standard phenotypic assessment in power-oriented sports selection.

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