

THE *GSTP1* c.313A>G POLYMORPHISM MODULATES THE CARDIORESPIRATORY RESPONSE TO AEROBIC TRAINING

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ABSTRACT: The *GSTP1* c.313A>G polymorphism is a candidate to explain some of the individual differences in cardiorespiratory fitness phenotypes' responses to aerobic exercise training. We aim to explore the association between the *GSTP1* c.313A>G polymorphism and the response to low-high impact aerobic exercise training. Sixty-six Polish Caucasian women were genotyped for the *GSTP1* c.313A>G polymorphism; 62 of them completed 12-week aerobic (50-75% HR_{max}) exercise training and were measured for selected somatic features (body mass and BMI) and cardiorespiratory fitness indices – maximal oxygen uptake (VO_{2max}), maximum heart rate (HR_{max}), maximum ventilation (V_{Emax}) and anaerobic threshold (AT) – before and after the training period. Two-factor analysis of variance revealed a main training effect for body mass reduction (p=0.007) and BMI reduction (p=0.013), improvements of absolute and relative VO_{2max} (both p<0.001), and increased V_{Emax} (p=0.005), but not for changes in fat-free mass (FFM) (p=0.162). However, a significant training x *GSTP1* c.313A>G interaction was found only for FFM (p=0.042), absolute and relative VO_{2max} (p=0.029 and p=0.026), and V_{Emax} (p=0.005). As the result of training, significantly greater improvements in VO_{2max}, V_{Emax} and FFM were gained by the GG+GA group compared to the AA genotype group. The results support the hypothesis that heterogeneity in individual response to training stimuli is at least in part determined by genetics, and *GSTP1* c.313A>G may be considered as one (of what appear to be many) target polymorphisms to influence these changes.

KEY WORDS: genes, exercise, athletic performance, polymorphism

INTRODUCTION

Aerobic dance refers to a variety of activities such as high-low impact aerobics and jazz dancing [1]. This aerobic-oriented physical activity has become one of the most popular forms of non-competitive free-time group exercise, among women in particular, to improve cardiorespiratory fitness and body composition [2,3]. When structured to comply with the recommendations of the American College of Sports Medicine (ACSM) for the quantity and quality of training, aerobic dance training provides significant improvements in cardiorespiratory fitness results in maximal oxygen uptake (VO_{2max}) gains [1,4–6].

VO_{2max} is one of the main physiological variables used to indicate cardiorespiratory fitness, and an increase in VO_{2max} is the most common method of demonstrating the inter-individual differences in the response to exercise training [7]. Studies have shown considerable individual differences in VO_{2max} and other cardiorespiratory fitness

phenotypes' responses to exercise training [8–11], which allows for the classification of individuals as non-responders, low responders and high responders with respect to changes in cardiorespiratory fitness phenotypes [9].

Human twin and family intervention experiments confirmed that there may be a substantial genetic component in determining the individual differences in training-induced VO_{2max} changes [12,13]. More recent data from the HERITAGE family study suggested that the heritability of changes in VO_{2max} with exercise training is ~47% in sedentary subjects [14]. To date, genetic association studies have revealed over 200 single nucleotide polymorphisms (SNPs) associated with exercise and health-related phenotypes, such as VO_{2max} [15–17].

One candidate gene that may explain some of the inter-individual response to exercise training is the glutathione S-transferase

P1 (*GSTP1*) gene that encodes the GSTP1 enzyme. Glutathione S-transferases (GSTs) are a family of phase II enzymes which play crucial roles in cellular protection against oxidative stress by exhibiting detoxification and reactive oxygen species (ROS) scavenging activities [18]. GSTP1 enzyme is a member of the GST family. GSTP1 not only catalyses the conjugation reaction with reduced glutathione (GSH), but also exhibits non-enzymatic ligand-binding capacity, thereby modulating cellular signal transduction [19]. Studies have shown that cytosolic GSTs may bind to other intracellular proteins [20–22]. For example, *in vitro* studies showed that the c-Jun N-terminal kinase (JNK) involved in the regulation of the cardiac hypertrophy process [23] can be inhibited in a dose-dependent manner with the addition of purified GSTP1 [21].

One of the most extensively studied genetic polymorphisms in the *GSTP1* gene, first described by Board et al. [24], results in substitutions at amino acids 105 (Ile to Val) with nucleotide sequence changes A to G in the 313 position of exon 5 (c.313A>G, rs1695) [19]. Homozygotes for the *GSTP1* A allele (Ile105) experienced decreased enzyme activity and greater risk for developing GST-mediated resistance to thiotepa (an anti-cancer drug) [25].

Carrying at least one copy of the *GSTP1* G allele, on the other hand, is thought to be beneficial for exercise and health-related phenotypes. The GG genotype is associated with better survival in children with acute lymphoblastic leukaemia [26], and children without a protective G allele (carriers of the AA genotype) showed an increased risk for new onset asthma with increasing participation in team sports [26].

Given the role of GSTP1 in the antioxidant defence system and the evidence to suggest that *GSTP1* c.313A>G polymorphism may be beneficial for exercise, we aimed to explore the association between *GSTP1* c.313A>G polymorphism and the response to a 12-week programme of aerobic exercise training. We hypothesised that carriers of the *GSTP1* G allele would have better improvement in VO_{2max} and other cardiorespiratory fitness measurements compared to A allele carriers following the aerobic training programme.

MATERIALS AND METHODS

Participants. Sixty-six Polish Caucasian women aged 21 ± 1 years (range 19–24) met the inclusion criteria and were included in the study. None of these individuals had engaged in regular physical activity in the previous 6 months. They had no history of any metabolic or cardiovascular diseases. Participants were non-smokers and refrained from taking any medications or supplements known to affect metabolism. Prior to the start of the training phase participants were asked to keep a balanced diet of approximately 2000 kilocalories a day. The subjects were fully informed of any risks and discomfort associated with the experimental procedures before giving their consent to participate. The study was approved by the Pomeranian Medical University Ethics Committee in accordance with the Helsinki Declaration and proceeded according to good scientific practice and ethical principles in scientific research [27].

Design

All participants were measured for selected cardiorespiratory fitness parameters – VO_{2max} , maximum heart rate (HR_{max}), maximum ventilation (VE_{max}), anaerobic threshold (AT), and body composition (body mass, BMI) variables – before and after the completion of a 12-week training period. Sixty-two individuals completed both physical examinations and their results were incorporated into a phenotype-related analysis.

Aerobic capacity test (VO_{2max})

Subjects performed a continuous graded exercise test on an electronically braked cycle ergometer (Oxycon Pro, Erich JAEGER GmbH, Hoechberg, Germany) to determine their VO_{2max} . The test began with 5 minutes of continuous pedalling, with a frequency of 60 revolutions per minute (RPM) and a relative load of $1.2 \text{ W} \cdot \text{kg}^{-1}$. After this phase, the workload was systematically increased by 15 watts every minute until exhaustion. The effort was interrupted when pedalling frequency declined by 10%, that is, when the pedalling frequency fell below 54 RPM. The highest value of the oxygen uptake maintained for 15 s was considered to be the VO_{2max} . The anaerobic threshold values were obtained using the V-slope method [28].

Training phase

The training stage was preceded by a week-long familiarization stage, when the examined women exercised 3 times a week for 30 minutes, at an intensity of about 50% of their HR_{max} . After the week-long familiarization stage, the proper training was started. Each training unit consisted of a warm-up routine (10 minutes), the main aerobic routine (43 minutes) and stretching and breathing exercise (7 minutes). The main aerobic routine was a combination of two alternating styles – low and high impact. The low impact style comprises movements with at least one foot on the floor at all times, whereas high impact styles include running, hopping and jumping with a variety of flight phases [2]. Music of variable rhythm, intensity and tempo was incorporated into both styles. A 12-week programme of low-high impact aerobics was divided as follows: (i) 3 weeks (9 training units), 60 minutes each, at about 50–60% of HR_{max} , tempo 135–140 BPM, (ii) 3 weeks (9 training units), 60 minutes each, at 50–60% of HR_{max} , tempo 135–140 BPM, (iii) 3 weeks (9 training units), 60 minutes with the intensity of 60%–70% of HR_{max} , tempo 140–152 BPM, and (iv) 3 weeks (9 unit training), 60 minutes with an intensity of 65%–75% of HR_{max} , tempo 140–152 BPM. All 36 training units were administered and supervised by the same instructor.

Genotyping

DNA was extracted from buccal cells using a GenElute Mammalian Genomic DNA Miniprep Kit (Sigma, Germany) according to the manufacturer's protocol. All samples were genotyped using an allelic discrimination assay on a StepOne Real-Time Polymerase Chain Reaction (RT-PCR) instrument (Applied Biosystems, USA) with TaqMan probes. To discriminate *GSTP1* A and G alleles (rs1695),

a TaqMan Pre-Designed SNP Genotyping Assay was used (Applied Biosystems, USA) (assay ID: C__3237198_20), including primers and fluorescently labelled (FAM and VIC) MGB probes to detect both alleles. All sample were analysed in duplicate, and there was 100% agreement in genotype detection between samples.

Statistical analyses

Deviations from Hardy–Weinberg equilibrium (HWE) in genotype distributions were assessed using the HWE exact test. Unpaired Student t-tests were used to analyse the difference between groups (GG+AG and GG group) prior to training. Two-way analysis of variance (ANOVA) with two factors (training x *GSTP1* c.313A>G polymorphism: GG+AG and GG group) was used to determine training and gene effects. Effect sizes were reported as eta-squared (η^2). According to the classification, a large (strong) effect is determined when η^2 is greater than 0.14, a moderate-sized effect is determined when η^2 is 0.06–0.14, and a small effect is determined when η^2 is smaller than 0.06 [29]. Statistical significance was assigned if $P < 0.05$. Data processing and statistical evaluations were performed using SPSS version 19.0 for WINDOWS (SPSS Inc, Chicago, IL).

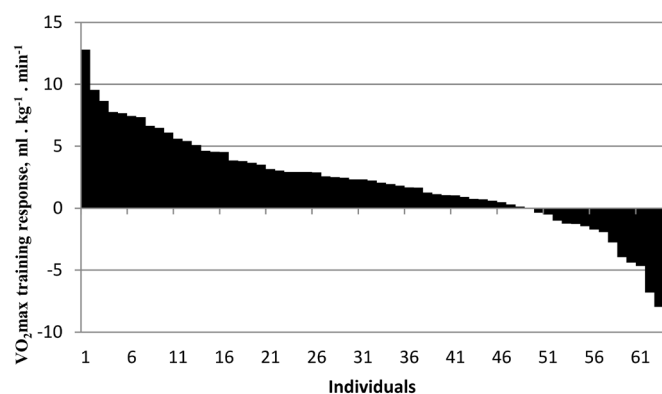


FIG. I. PARTICIPANTS' DISTRIBUTION IN VO_{2MAX} TRAINING RESPONSE

RESULTS

GSTP1 c.313A>G polymorphism was in agreement with HWE ($p=0.760$). Overall, there were 36 (55%) participants homozygous for the A allele, 25 (38%) participants were heterozygous, and 5 participants (8%) were homozygous for the G allele. Allele frequency was 73% and 27% for the A and the G alleles, respectively. Allele frequency was similar to those in European-Americans (66% and 33% for A and G alleles, respectively) [30] and Spanish Caucasians (70% and 30% for A and G alleles, respectively) [31].

All women completed the prescribed 12-week low-high impact aerobic exercise training programme. The participant's distribution in VO_{2max} training response is presented in Figure 1. The study group experienced a modest but significant decrease in total body mass ($p=0.007$) and BMI ($p=0.013$) during the course of the training programme. With respect to cardiorespiratory fitness variables, significant improvements were found in absolute VO_{2max} ($p < 0.001$), VO_{2max} relative to body mass ($p < 0.001$) and VE_{max} ($p=0.005$), but not for HR_{max} ($p=0.94$) or FFM ($p=0.162$). Increase in VO_2/AT did not reach statistical significance, but a tendency was shown ($p=0.053$).

Next, the study group was divided according to *GSTP1* genotypes. Owing to the low number of GG homozygotes ($n=5$), they were combined with AG heterozygotes (GG+AG group, $n=30$), and compared to AA homozygotes (AA group, $n=36$). Nevertheless, data of the physical examination from 26 GG+AG and 36 AA homozygotes were analysed, because four heterozygous carriers did not complete the second test correctly. Comparison of pre-and-post training values with respect to the *GSTP1* genotypes is summarized in Table 1. A pre-training values comparison revealed that GG+AG and AA groups did not differ in age (21 ± 1 vs 21 ± 1 , $p=0.29$), body mass ($p=0.98$), BMI ($p=0.95$), HR_{max} ($p=0.40$), or VE_{max} ($p=0.92$). There were, however, significant differences in baseline VO_{2max} and tendency to a small increase in VO_2/AT index. The carriers of the G allele had higher pre-training VO_{2max} (34.7 ± 4.3) and VO_2/AT (26.9 ± 3.8) than

TABLE I. EFFECT OF AEROBIC DANCE TRAINING AND *GSTP1* POLYMORPHISM ON BODY COMPOSITION AND CARDIORESPIRATORY FITNESS

	Pre-test		Post-test		Time (training) effect	Interaction time*genotype effect		
	AA	GG+AG	AA	GG+AG		Alpha level	Alpha level	η^2
Body mass (kg)	60.6 ± 6.2	60.6 ± 7.4	59.8 ± 6.1	60.4 ± 7.5	$p=0.007$	$p=0.075$	0.05	0.43
Fat-free mass (kg)	45.4 ± 2.5	45.4 ± 2.8	45.3 ± 2.5	45.9 ± 2.7	$p=0.162$	$p=0.042$	0.07	0.53
BMI	21.8 ± 2.1	21.7 ± 3.0	21.5 ± 2.0	21.7 ± 3.0	$p=0.013$	$p=0.162$	0.03	0.29
VO_{2max} (ml · min ⁻¹)	1977 ± 247	2086 ± 256	2027 ± 293	2271 ± 323	$p < 0.001$	$p=0.029$	0.06	0.60
VO_{2max} (ml · kg ⁻¹ · min ⁻¹)	32.7 ± 3.6	34.7 ± 4.3	34.1 ± 5	38.2 ± 5.2	$p < 0.001$	$p=0.026$	0.06	0.61
VE_{max} (l · min ⁻¹)	76.9 ± 14.6	76.5 ± 16.7	77.1 ± 15.6	86.5 ± 12	$p=0.005$	$p=0.007$	0.10	0.79
VO_2/AT (ml · kg ⁻¹ · min ⁻¹)	25.2 ± 3.3	26.9 ± 3.8	26.4 ± 3.9	27.8 ± 3.5	$p=0.053$	$p=0.819$	<0.001	0.06
HR_{max} (beats · min ⁻¹)	189.9 ± 8	188.2 ± 7.9	188.4 ± 8.1	189.9 ± 7.2	$p=0.940$	$p=0.026$	0.08	0.61

Note: BMI – body mass index; VO_{2max} – maximum oxygen uptake; VE_{max} – maximum minute ventilation; HR_{max} – maximum heart rate; VO_2/AT (ml · kg⁻¹ · min⁻¹) oxygen consumption at anaerobic threshold; VO_2/AT (% VO_{2max}) – percentage of VO_{2max} at anaerobic threshold, η^2 = effect size

the AA homozygotes (32.7 ± 3.6 , $p=0.046$ and 25.2 ± 3.3 , $p=0.07$, respectively).

When changes in body composition and cardiorespiratory fitness following the 12-week training period were considered in the context of the *GSTP1* genotypes (Table 1), a moderate-sized interaction gene x time effect was noted for VO_{2max} , VE_{max} and HR_{max} . Significantly greater improvements in VO_{2max} and VE_{max} were seen in the GG+GA group, while a decrease in HR_{max} was noted in the AA group. No significant changes in VO_2/AT , body mass or BMI were found with respect to *GSTP1* genotypes.

DISCUSSION

We examined the association between *GSTP1* c.313A>G polymorphism and changes in cardiorespiratory fitness following 12 weeks of supervised aerobic exercise training. We found that the *GSTP1* G allele (Val105) was associated with gains in VO_{2max} and VE_{max} . This supports the hypothesis that heterogeneity in individual response to training stimuli is at least in part determined by genetics [9,32], and the *GSTP1* c.313A>G polymorphism may be considered as one (of what appears to be many) target polymorphism to influence these changes.

In the present study, women with at least one copy of the G allele (GG+AG) showed a significantly greater increase in VO_{2max} in response to training. A significant difference in baseline VO_{2max} between GG+AG and AA was also noted; carriers of the G allele had greater VO_{2max} (by 6.0%) than the AA homozygotes at baseline. Improvements in VO_{2max} in response to training are a common occurrence and are influenced by genetic components. This has been previously demonstrated in the HERITAGE family study [9]. Evidence for a genetic influence on pre-training (baseline) performance has also been presented recently, as in the case of *ACTN3* R577X, the most investigated polymorphism in athletic performance, the alpha-actinin-3 deficient mice (*ACTN3* XX) had a pre-training lower grip strength (lower muscle strength) and were able to run 33% further on a treadmill when run to exhaustion (improved endurance performance) than their wild-type (*ACTN3* RR) littermates [33,34].

Thus, there is a reasonable scientific basis to explain why our GG+AG cohort not only responds better to training but also appears to be "pre-trained" for endurance performance.

There are several reasons for us to hypothesise that people with the *GSTP1* AG+GG genotypes will be predisposed to a better response to exercise training. First, the A to G substitution at position 313 has been shown to be functional, and results in a miscoded *GSTP1* protein. The Val105 variant (G allele) with lower enzymatic activity represents impaired *GSTP1* functions in catalytic reactions to remove excessive ROS [35], which may be beneficial for exercise. Second, aerobic exercise-induced reactive oxygen radicals are recognized as an important regulator of the adaptations in skeletal muscles in response to aerobic exercise by triggering or affecting many cell signalling pathways [26,36,37].

Low levels of ROS have been shown to activate numerous key signalling molecules such as peroxisome proliferator-activated recep-

tor-gamma coactivator-1 α (PGC-1 α), AMP-activated protein kinase (AMPK), mitogen-activated protein kinase (MAPK) and insulin growth factor 1 (IGF-1), which control cellular mechanisms for muscle adaptation. For example, ROS generated during exercise may promote PGC-1 α -dependent mitochondrial biogenesis [38,39], an important peripheral skeletal muscle adaptation to endurance training [40].

Another possible reason for humans with *GSTP1* AG+GG genotype to be predisposed to enhanced training adaptations is the possible indirect involvement of *GSTP1* in cardiac function, leading to changes in VO_{2max} . Animal *in vitro* studies provided evidence that the activation of JNK alone is sufficient to induce features of cardiac hypertrophy [23]. *GSTP1* was shown to inhibit c-Jun N-terminal kinase [22] and the suppression of *GSTP1* in a model of *GSTP1* knockout mice led to elevated JNK activity, increased proliferation and reduced apoptosis in mouse embryonic fibroblasts [41]. Holley et al. [42] conducted a series of experiments in which two *GSTP1* haplotypes consisting of c.313A>G and another functional variant, c.341C>T, were compared with regard to cellular proliferation, apoptosis and influence on the JNK pathway. Induced expression of the wild-type haplotype (*GSTP1**A) with two wild-type alleles (c.313A and c.341C) led to slower cellular growth, and the variant haplotype (*GSTP1**C) consisting of c.313G and c.341T reduced the JNK activity by 69% compared to 35% for *GSTP1**A [42]. Given the central role of JNK in cardiac hypertrophy, one could hypothesise that the c.313G allele, alone or in combination with the c.341T allele, may have a beneficial effect on the cardiac hypertrophic response to regular physical activity.

The strength of the present study comes from applying a controlled, 12-week training study that generated robust pre-and-post training cardiorespiratory fitness phenotypes. While progress with exercise intervention studies, such as the present study, is slow because of resource requirements, such studies are desperately needed to fully understand the genetics as well as the exercise biology of complex traits and to confirm the gene-exercise interactions derived from observational studies [43]. Our training programme was constructed in accordance with ACSM guidelines with sufficient quantity (3 days a week, intensity of 50%-75% of HR_{max} , 60 minutes per session) and quality (high and low aerobics) to improve cardiorespiratory fitness. Indeed, the training programme significantly improved body composition parameters (body mass, BMI), and significantly altered most of the cardiorespiratory fitness measures, namely relative and absolute VO_{2max} , and VE_{max} . There was no significant difference in pre-post training values in HR_{max} , however small but statistically significant changes with respect to genotypes were noted (interaction time x genotype effect; $p=0.026$). The response of HR_{max} to endurance training remains controversial due to the modest, minimal or lack of change which has been reported in literature [44]. Comparing previous studies of low-high impact aerobic exercise training conducted with similar conditions (young women, duration 12-14 weeks) revealed no changes [45] or a slight decrease [2] in HR_{max} . Zavorsky et al. [44] suggested that the conflicting data may result

from differences in how researchers report HR_{max} or the lack of training adaptation. It is therefore not surprising that HR_{max} slightly changed in a contrary direction, decreased in AA and increased in GG+AG by approx. 1-2 beats per minute, but *de facto* HR_{max} remained similar after training in our cohort, as this magnitude of changes lack of clinical or practical significance.

A primary limiting factor in our study, as in any gene-exercise training studies, is the recruitment of a large enough training cohort. However, monitoring a cohort's physical activity over a long period of time is both costly and tedious. Despite the inadequate number of subjects who completed the study and although the analysis is underpowered, we believe that the results of this study are promising and could add insight into further, wider investigations. Also, we followed the latest genotype:phenotype study recommendations [46], and all of the following criteria have been met: participants within the cohort were both age and ethnically matched (all European Cau-

casians), and genetic assessment was accurate and unbiased, with genotype distribution being in Hardy-Weinberg equilibrium (HWE).

CONCLUSIONS

In conclusion, the *GSTP1* G allele (Val105) was associated with gains in VO_{2max} and VE_{max} in response to aerobic exercise training. Replication training studies are needed to verify this association in other cohorts.

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