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Interindividual Variation in Cardiorespiratory Fitness: A Candidate Gene Study in Han Chinese People

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Received: 1 April 2020; Accepted: 11 May 2020; Published: 15 May 2020

Abstract: Cardiorespiratory fitness, as assessed through peak oxygen uptake (VO_{2peak}), is a powerful health indicator. We aimed to evaluate the influence of several candidate causal genetic variants on VO_{2peak} level in untrained Han Chinese people. A total of 1009 participants (566 women; age [mean ± SD] 40 ± 14 years, VO_{2peak} 29.9 ± 7.1 mL/kg/min) performed a maximal incremental cycling test for VO_{2peak} determination. Genomic DNA was extracted from peripheral whole blood, and genotyping analysis was performed on 125 gene variants. Using age, sex, and body mass as covariates, and setting a stringent threshold p-value of 0.0004, only one single nucleotide polymorphism (SNP), located in the gene encoding angiotensin-converting enzyme (rs4295), was associated with VO_{2peak} ($\beta = 0.87$; $p < 2.9 \times 10^{-4}$). Stepwise multiple regression analysis identified a panel of three SNPs (rs4295 = 1.1%, angiotensin II receptor type 1 rs275652 = 0.6%, and myostatin rs7570532 = 0.5%) that together accounted for 2.2% ($p = 0.0007$) of the interindividual variance in VO_{2peak}. Participants carrying six 'favorable' alleles had a higher VO_{2peak} (32.3 ± 8.1 mL/kg/min) than those carrying only one favorable allele (24.6 ± 5.2 mL/kg/min, $p < 0.0001$). In summary, VO_{2peak} at the pre-trained state is partly influenced by several polymorphic variations in candidate genes, but they represent a minor portion of the variance.

Keywords: VO_{2max}; maximal oxygen uptake; single nucleotide polymorphism; genomics; endurance performance

1. Introduction

Cardiorespiratory fitness (CRF) is positively associated with endurance exercise performance [1] and is a strong prognostic factor of morbidity and mortality from all causes and, particularly, from cardiovascular disease (CVD) [2,3]. While both physical activity (PA) and exercise training can modify CRF and are inversely associated with morbidity and mortality rates [4], CRF per se is a much stronger predictor of prognosis in CVD and metabolic disorders [5,6]. The measure of an individual's peak capacity to perform dynamic aerobic exercise is dependent on the synergistic action of pulmonary, cardiovascular and muscle tissue via a suite of physiological actions that effectively transport and

deliver oxygen from the atmosphere to mitochondria in working muscles [7,8]. Accordingly, CRF can be assessed by directly measuring the peak oxygen uptake (VO_{2peak}) reached during a graded dynamic exercise test until exhaustion, involving large muscle masses (e.g., running or bicycling), or by indirectly estimating this variable from the peak workload achieved. Nevertheless, direct evaluation of VO_{2peak} is considered the gold standard measure of CRF and, indeed, the American Heart Association recently advocated for the routine assessment of this measure as a clinical vital sign [9].

VO_{2peak} is characterized by a high interindividual variability even in people of the same sex, age and with the same level of PA and exercise training. This variability is believed to be related, at least partly, to heredity. A seminal study by Claude Bouchard and colleagues found comparable VO_{2peak} values in brothers of the same sibship, and the similarities in VO_{2peak} were even greater in dizygotic and monozygotic twins [10]. The authors suggested that the genetic effect on VO_{2peak} reached ~40%. In a similar study of 170 individuals and their offspring (n = 259), it was found that about 50% of the interindividual variance in VO_{2peak} corresponded to heritable factors after adjusting for age, sex, body mass, and body composition [11]. These findings have been replicated in subsequent studies with siblings and twins [12] and, to date, it is commonly accepted that VO_{2peak} is influenced by both genetic (~50–60%) and environmental factors. It has also been reported that twins with similar VO_{2peak} values present with comparable levels of a variety of PA indices [13], suggesting that part of the heritability of VO_{2peak} in twins might be due to the similarity of their PA levels. In fact, in a recent analysis of 123,545 single nucleotide polymorphisms (SNPs), only nine were associated with VO_{2peak} [14]. The authors of this study found that those individuals whose genotype was associated with a high VO_{2peak} value had a lower CVD risk (e.g., less visceral fat or lower total blood cholesterol), but they did not calculate the additive effect that the nine SNPs had on the interindividual variability of VO_{2peak}. There is therefore controversy on the influence of genetics on VO_{2peak}, which mostly likely stems from the discrepancies between studies conducted on siblings/twins vs those conducted on individuals with no familial connection. In this regard, determining the actual genetic contribution to the interindividual variability in VO_{2peak} would be of major importance to inform how environmental factors—including lifestyle—might contribute to heightened VO_{2peak} values. It is possible that if the influence of genetics on VO_{2peak} is low, exercise training might be a determining factor to enhance 'innate' VO_{2peak} even in those less genetically predisposed, with obvious subsequent benefits for cardiovascular health. Indeed, previous research has reported VO_{2peak} increases of up to 44% after strenuous training interventions, which would support a strong influence of environmental factors on CRF [15].

Aerobic/endurance exercise-based training appears to be the most effective way to augment VO_{2peak}. Exercise training increases rather than decreases the individual differences seen at baseline VO_{2peak} because the response to training itself shows large variation [16]. A genome-wide association study based on 324,611 SNPs found that only 21 SNPs could explain 48.6% of the change in VO_{2peak} induced by a 20-week exercise training program [17]. Among them, rs6552828, located in the acyl-CoA synthase long-chain member 1 (*ACSL1*) gene, accounted by itself for 6% of the training-induced enhancement in $VO2_{peak}$. In a recent meta-analysis of 35 articles on the genetic influence on VO_{2peak} trainability, a total of 97 genes were associated with this phenotype, although only 13 genetic variants were reproduced by more than two investigations [18].

Knowledge on the genetic influence on baseline VO_{2peak} (i.e., in isolation from training) is mainly based on studies conducted on siblings/twins or in individuals of Caucasian descent, and it remains to be determined whether the genetic variants that might be associated with baseline VO_{2peak} are similar or different in individuals of other ethnicities. Thus, the aim of the present study was to assess the influence of several candidate genetic variants in the interindividual variation of baseline CRF measured as $VO_{2peak,}$ in Han Chinese individuals.

2. Materials and Methods

2.1. Participants

A total of 1047 participants (56% women) volunteered to participate in the study. The sample was recruited from five cities in China: Beijing, Xi'an, Guangzhou, Shenyang, and Tianjin. Inclusion criteria were the following: male/female aged 18–69 years; being of Chinese (Han) descent and unrelated to the other participants; having no CVD, diabetes or abnormal glucose tolerance, or any other acute or chronic disease; and being untrained (i.e., ≤ 2 sessions/week of ≤ 30min of regular physical exercise in the previous 12 months). One week before the start of the investigation, participants were fully informed of the experimental procedures and signed an informed written consent to participate in the investigation. The study protocol was approved by the Institutional Review Board of the China Institute of Sport Science.

2.2. Experimental Design

This is an observational cross-sectional study aimed at determining the genetic influence of target genes on the interindividual variability in VO_{2peak} values in untrained Han Chinese individuals. We selected untrained individuals to avoid any influence of exercise training or planned PA in the analysis.

2.3. Experimental Protocol

The day of the first experimental trial, participants underwent a medical examination (including medical history and other routine physical examinations) carried out by a licensed physician, to ensure the suitability of all participants to take part in the research protocols. On the same day, whole body dual-energy X-ray absorptiometry (GE Lunar DPX system, Madison, WI, USA) assessments were performed and used to calculate body fat and fat-free mass following previous recommendations [19]. VO_{2peak} (in mL/kg/min) was determined during a continuous incremental exercise test to volitional fatigue performed on a bicycle ergometer (Ergoselect 100, Ergoline GmbH, Bitz, Germany). Before tests, participants were familiarized with the ergometer and with the rating of perceived exertion (RPE), as measured by the Borg 6–20 scale [20]. Participants performed a standardized warm-up (5 min cycling at 20 W and 60 rpm), and the workload (starting at 20 W) was then increased by 25 W (men) or 20 W (women) every 2 minutes until volitional exhaustion. In participants >60 years of age, the workload was increased by 20 W (men) or 15 W (women) every 2 minutes. During the test, gas exchange data were measured 'breath-by-breath' with a metabolic cart (MetaMax 3B, Cortex Biophysik GmbH, Leipzig, Germany). Certified calibration gases (16.0% O_2, 5.0% CO_2, Cortex Biophysik) and a 3-L syringe were used to calibrate the gas analyzer and the flow meter, respectively, before each test. VO_{2peak} was defined as the highest VO_2 value (60-s average) obtained during the test. VO_{2peak} was considered valid when participants achieved at least two of the following criteria: (i) RPE >17, (ii) VO_2 difference between the last two consecutive loads <0.15 L/min, (iii) respiratory exchange ratio >1.1, and (iv) peak heart rate >85% of the age-adjusted estimate [21]. Heart rate was recorded with a chest strap transmitter (Polar RS400, Polar Electro, Kempele, Finland). The environmental temperature was similar in all measurement centers (~22 °C, 40% relative humidity).

On a different day during the week of testing, genomic DNA was extracted from peripheral whole blood samples using the Wizard Genomic DNA Purification Kit (Promega; Madison, WI, USA). Genotyping was performed at Shanghai Benegene Biotechnology, LTD (Shanghai, China). For analysis, a list of 125 SNPs (Tables A1 and A2, Tables A3 and A4) for the Han population of Beijing, China (CHB) was obtained from the International HapMap Project database. Haplotype-tag SNPs were selected using the following criteria: minor allele frequency ≥0.01 and measure of linkage disequilibrium ($r^2 > 0.8$). Initially, genes associated with cardiovascular responses to exercise were chosen, and genes associated with endurance performance, muscle performance, or body composition were then added as all of these factors might contribute to the value of VO_{2peak} (Table 1).

Table 1. Target genes selected for the investigation.

Gene	Numbers of SNPs	Chromosome Location	References
ACE	3	chr17:58,908,166-58,928,711	[22]
ACE2	2	chrX:15,489,077-15,529,058	[23,24]
ACSL1	15	chr4: 185,911,544-185,986,209	[17,25]
ACTN3	1	chr11:66,313,866-66,330,800	[26,27]
AGT	13	chr1:228,902,892-228,918,564	[28,29]
AGTR1	9	chr3:149,898,348- 149,943,480	[30,31]
AGTR2	3	chrX:115,214,031-115,221,847	[32]
BDKRB2	28	chr14:95,738,950-95,782,536	[33]
FGF21	2	chr19:53,949,156-53,955,394	[34]
FGFR2	1	chr10:123,237,848-123,357,972	[34]
FNDC5	3	chr1:33,327,869-33,338,083	[35]
FST	3	chr5: 52,812,352-52,817,659	[36,37]
FTO	3	chr16:53,737,875-54,155,853	[38]
GDF8	4	chr2:190,920,423-190,927,455	[39,40]
IL-6	7	chr7:22,733,345-22,738,141	[41]
IL-15	2	chr4:142,557,752-142,665,140	[42,43]
ITLN1	5	chr1:160,846,329-160,854,960	[44]
PGC-1α	6	chr4: 23,756,664-23,905,712	[45]
PGC-1β	1	chr5:149,109,861-149,234,585	[45]
PPRC1	1	chr10: 103, 880, 777-103, 902, 078	[45]
PRDM16	2	chr1: 2,985,732-3,355,185	[46]
PYY	5	chr17:39,385,633-39,437,363	[47]
REN	5	chr1: 202,390,571-202,402,088	[48]
RETN	1	chr19:7,639,972-7,641,340	[49]

Abbreviations: SNP, single nucleotide polymorphism. Abbreviations for gene names: *ACE*, angiotensin-converting enzyme; *ACE2*, angiotensin-converting enzyme 2; *ACSL1*, acyl-CoA synthase long-chain member 1; *ACTN3*, alpha-actinin-3; *AGT*, angiotensinogen; *AGTR1*, angiotensin II receptor type 1; *AGTR2*, angiotensin II receptor type 2; *BDKRB2*, bradykinin receptor B2; *FGF21*, Fibroblast growth factor 21; *FGFR2*, fibroblast growth factor receptor 2; *FNDC5*, fibronectin type III domain-containing protein 5; *FST*, follistatin; FTO, fat mass and obesity-associated protein (also known as 'alpha-ketoglutarate-dependent dioxygenase'; *GDF8*, growth differentiation factor 8 (also known as 'myostatin'); *IL-6*, interleukin 6; *IL-15*, interleukin 15; *ITLN1*, intelectin 1; PGC-1α, peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1alpha; PGC-1β, peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1beta; peroxisome *PPRC1*, proliferator-activated receptor gamma, coactivator-related 1; *PRDM16*, PR domain containing 16; *PYY*, peptide YY; *REN*, renin; *RETN*, resistin.

For high-throughput genotyping of SNPs, we used a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) platform (Agena, San Diego, CA, USA). Primers for the polymerase chain reaction (PCR) and single-base extension were designed using the Assay Designer software package (Assay Design Suite V2.0, Agena, San Diego, CA, USA). Genotyping was performed as described elsewhere [50].

2.4. Statistical Analysis

All statistical analyses were performed using SAS 9.4 statistical package (SAS institute, Inc., Cary, NC, USA) and PLINK (v1.07). Hardy–Weinberg Equilibrium (HWE) was tested using χ^2 tests. Linear regression analyses were conducted to assess the association—expressed as standardized regression coefficients (β)—between each SNP and VO_{2peak}, with age, body mass and sex as covariates. The Bonferroni correction for multiple comparisons was applied to test for statistically significant associations between SNPs and VO_{2peak}, thereby setting the minimum level of significance at $p < 0.0004$ (i.e., 0.05 divided by the number of SNPs, i.e., 125). A multivariable regression analysis was then conducted to assess the overall contribution of the most significant SNPs to the interindividual variability of VO_{2peak}. All SNPs with $p < 0.05$ were included, and a regression model with backward elimination was used to filter-out redundant SNPs. By using a threshold of 5.0 points in the variance inflation factor, we avoided multicollinearity. SNPs that were retained in the final backward elimination model were then analyzed with a multivariate regression model using forward selection. The produced

regression equation was accepted at a significance level of $p < 0.01$. The values of R^2 were adjusted for the number of cases and parameters in the analysis. The relative contribution (R^2) of each SNP in relation to the explained variance in VO_{2peak} was calculated as follows (Equation (1)):

$$\text{Partial contribution } (R^2 \text{ adjusted}) = ([\beta \text{ for parameter}] / \Sigma [\text{of all } \beta \text{ in equation}]), \quad (1)$$

In the SNPs retained in multiple regression, VO_{2peak} values were compared among genotypes by using one-way analysis of variance (ANOVA). When the ANOVA showed a significant F value, pairwise differences were assessed using the Tukey post-hoc test. By using the SNPs retained in multiple regression analyses, we calculated a weighted genotype score to assess the combined influence of the SNPs on VO_{2peak} following the procedure of Williams and Folland [51]. First, each genotype was scored within each SNP by assigning 0 arbitrary units (a.u.) to homozygotes for the allele theoretically associated with low VO_{2peak}, 1 a.u. to heterozygotes, and 2 a.u. to homozygotes for the allele associated with high VO_{2peak}, following an additive model. Each SNP was then weighted by its β-coefficient (allele effect) based on the assumption that all SNPs of interest have independent effects and contribute in an additive manner to VO_{2peak}. Finally, the scores obtained for each SNP were summed to obtain a unique weighted genotype score for each participant (theoretical range: 0–6 a.u.). For clarity, we merged data of participants by using intervals of 1 a.u. Differences in VO_{2peak} between participants in the different groups were assessed by one-way analysis of variance and using the least significant difference post hoc test. Finally, the ability of weighted genotype score to distinguish individuals with low or intermediate CRF (i.e., below or above 28 mL/kg/min, as proposed by Kodama et al. [52]) was assessed using a receiver operating characteristic (ROC) curve and by determining the area under the ROC curve (AUC).

3. Results

Of the initial 1047 individuals recruited, valid VO_{2peak} measurements were obtained for 1009 individuals (566 women), and thus only these participants were included in the analyses. The main characteristics of the participants are shown in Table 2.

Table 2. Main characteristics of the study participants (N = 1009) and their association with peak oxygen uptake.

Variable	Mean ± SD	Range	β	*p*-Value
Age (year)	40 ± 14	19–69	−0.27	<0.001
Height (cm)	165.3 ± 8.3	146.2–187.0	0.31	<0.001
Body mass (kg)	64.3 ± 11.6	39–104	−0.01	0.523
Body mass index (kg/m^2)	23.4 ± 3.1	15.6–34.8	−0.64	<0.001
Body fat (%)	27.1 ± 8.8	4.5–44.5	−0.58	<0.001
Fat-free mass (kg)	43.8 ± 9.5	24.8–70.1	0.31	<0.001

Genotyping was successful (i.e., successful determinations for all SNPs) in 1006 of 1009 participants (99.7%). From the 125 SNPs analyzed, 10 were discarded because they deviated from HWE (Table A2), 10 because they had a MAF <5% (Table A3), and two because only one genotype was detected across the group of participants (Table A4). From the remaining pool of 103 SNPs, only rs4295, located in the angiotensin-converting enzyme (*ACE*) gene, was significantly associated with VO_{2peak} ($p < 2.9 \times 10^{-4}$, $\beta = 0.87$; minor allele (G) frequency, 38.1%, heterozygosity frequency, 47.4%).

Figure 1 shows the distribution of VO_{2peak} values in the study sample. Approximately 2.5% of all participants had a VO_{2peak} <20 mL/kg/min and 1.4% had a VO_{2peak} level >50 mL/kg/min.

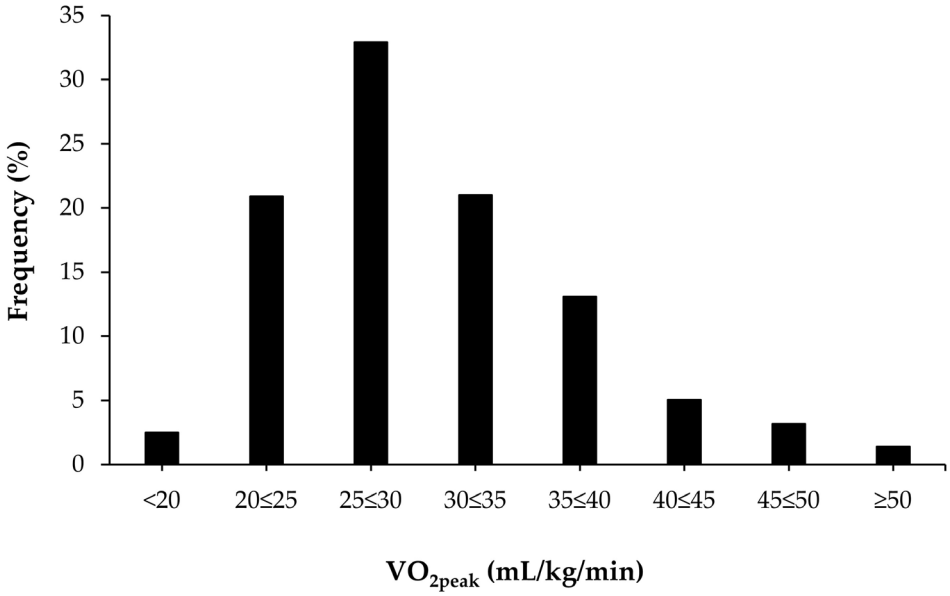

Figure 1. Distribution of peak oxygen uptake (VO_{2peak}) data in the study participants.

In multiple regression analysis, and after excluding those SNPs with collinearity, only three were retained in the final model (*ACE* rs4295, *AGTR1* rs275652, *GDF8* rs7570532), which explained together 2.2% ($p = 0.0007$) of the variance in VO_{2peak} (Figure 2a, statistical power = 0.987). The partial contribution of each SNP to the variance in VO_{2peak} is shown in Table 3. The explained variance of VO_{2peak} increased to 50.1% ($p < 0.0001$) when including covariates such as age, sex and weight in the model (Figure 2b, statistical power = 1.00).

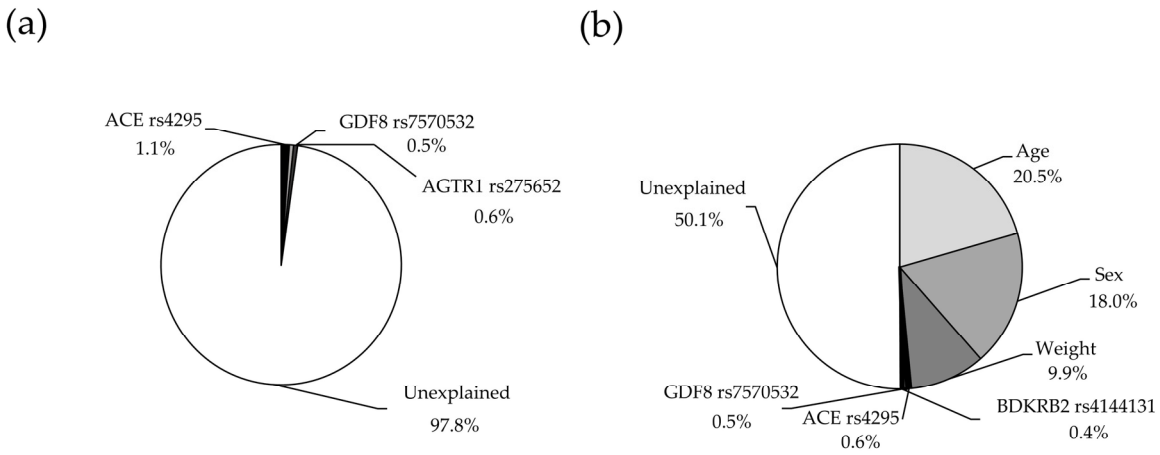

Figure 2. Variance in peak oxygen uptake in the study participants explained by genetic variants alone (**a**) and by genetic variants plus anthropometric covariates (**b**). Abbreviations for gene names: *ACE*, angiotensin-converting enzyme; *AGTR1*, angiotensin II receptor type 1; *BDKRB2*, bradykinin receptor B2; *GDF8*, growth differentiation factor 8 (also known as 'myostatin').

Individual VO_{2peak} values for each genotype of the *ACE* rs4295, *AGTR1* rs275652, and *GDF8* rs7570532 polymorphisms are shown in Figure 3. The one-way ANOVA revealed statistically significant differences in *ACE* rs4295 ($F = 4.95$, $p = 0.007$) and *AGTR1* rs275652 ($F = 3.90$, $p = 0.021$) polymorphisms, while the ANOVA did not show differences for *GDF8* rs7570532 ($F = 1.64$, $p = 0.194$) polymorphism. Specifically, GG homozygotes in *ACE* rs4295 had a mean VO_{2peak} of 31.1 ± 7.9 mL/kg/min, which was higher than that found in heterozygotes (GC, 29.8±6.9 mL/kg/min; $p = 0.049$) or in homozygotes for the common allele (CC, 28.9 ± 6.7 mL/kg/min; $p = 0.013$). In addition, AA homozygotes in *AGTR1* rs275652

had a mean VO_{2peak} of 30.0 ± 7.3 mL/kg/min, which was higher than that found in homozygotes for the minor allele (CC, 25.5 ± 5.3 mL/kg/min; $p = 0.024$).

Table 3. List of single nucleotide polymorphisms associated with peak oxygen uptake in the study participants. Model 1, with genetic-only influence; model 2 with covariates.

	SNP	Partial R^2	*p*-Value
Model 1	*ACE* rs4295	0.0110	0.0024
	AGTR1 rs275652	0.0056	0.0293
	GDF8 rs7570532	0.0053	0.0342
Model 2	Age	0.2052	<0.0001
	Sex	0.1800	<0.0001
	Weight	0.0994	<0.0001
	ACE rs4295	0.0063	0.0015
	GDF8 rs7570532	0.0046	0.0058
	BDKRB2 rs4144131	0.0037	0.0135

Abbreviation: SNP, single nucleotide polymorphism; Abbreviations for gene names: *ACE*, angiotensin-converting enzyme; *AGTR1*, angiotensin II receptor type 1; *BDKRB2*, bradykinin receptor B2; *GDF8*, growth differentiation factor 8 (also known as 'myostatin').

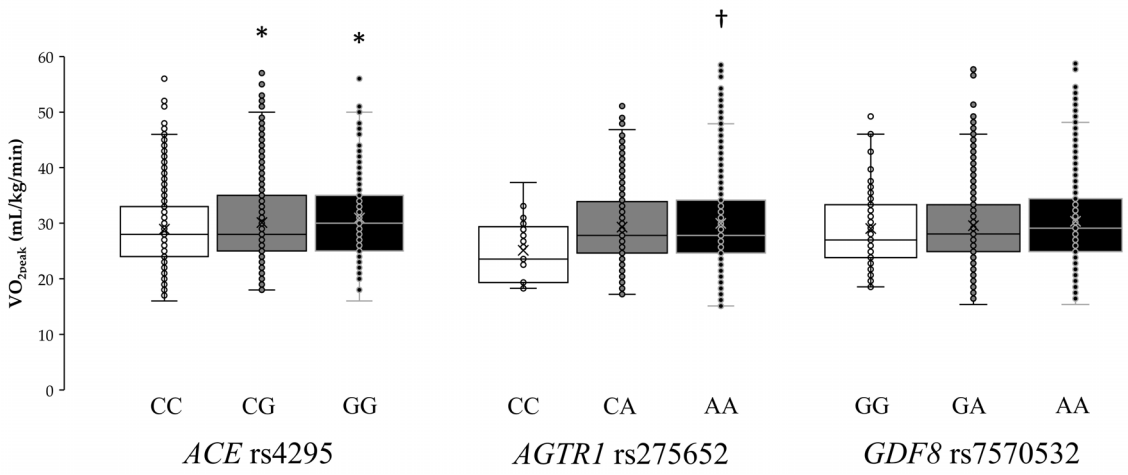

Figure 3. Box-and-whisker plots showing peak oxygen uptake (VO_{2peak}) in the study participants according to genetic variations in the genes for angiotensin-converting enzyme (*ACE*; rs4295), angiotensin II receptor type 1 (*AGTR1*; rs275652), and growth differentiation factor 8 (*GDF8*, also known as 'myostatin'; rs7570532). The lines in the box represent the first, second (median) and third quartiles, and the whiskers represent 1.5 × interquartile ranges. Each dot represents one individual within the specified genotype. (*) Depicts a statistically significant difference from CC genotype in *ACE* rs4295 polymorphism at $p < 0.05$. (†) Depicts a statistically significant difference from CC genotype in *AGTR1* rs275652 polymorphism at $p < 0.05$.

A weighted genotype score was constructed using the three SNPs shown in model 1 of genetic-only influence. Participants were categorized with a genotype score from 0 a.u., indicating the presence of homozygosity for all the alleles associated with a lower VO_{2peak} in *ACE* (rs4295), *AGTR1* (rs275652) and *GDF8* (rs7570532), to 6 a.u., indicating the presence of homozygosity for all the alleles associated with a higher VO_{2peak} in the aforementioned SNPs. A linear effect was found for genotype score on VO_{2peak} (Figure 4). Specifically, the individuals with 6 a.u. had a higher VO_{2peak} than those with scores up to 4.0 a.u. ($p < 0.05$). In addition, participants with scores >2 a.u. had a higher VO_{2peak} than those with scores <1.0 a.u. ($p < 0.05$). ROC analysis showed significant discriminatory accuracy of the weighted genotype score in the identification of individuals with low/intermediate CRF (AUC = 0.542) with a sensitivity of 0.733 and a specificity of 0.305.

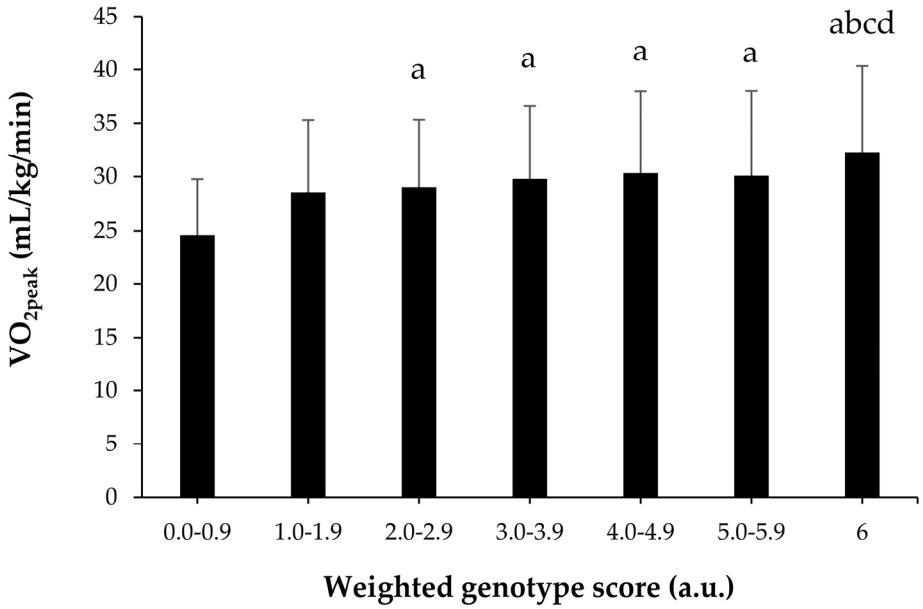

Figure 4. Peak oxygen uptake (VO_{2peak}) levels in the study participants according to the genotype score (computed by using a weighted score of angiotensin-converting enzyme rs4295, angiotensin II receptor type 1 rs275652 and growth differentiation factor 8 rs7570532 genotypes). Abbreviations/symbols: a.u., arbitrary units; a, difference from 0–0.9 a.u. at $p<0.05$; b, difference from 1.0–1.9 a.u. at $p < 0.05$; c, difference from 2.0–2.9 a.u. at $p < 0.05$; d, difference from 3.0–3.9 a.u. at $p < 0.05$.

4. Discussion

CRF, particularly when objectively determined as VO_{2peak}, is strongly associated with endurance performance and health outcomes. Indeed, VO_{2peak} reflects the peak integrative ability of the organism to deliver oxygen from the atmosphere to the mitochondria of working muscles. The VO_{2peak} is thus determined, among other factors, by peak cardiac output and pulmonary ventilation, lung diffusion capacity, blood and plasma volume, hemoglobin mass, and muscle capillary density and oxidative capacity [53]. Importantly, the mean values of VO_{2peak} of our participants (29.9 ± 7.1 mL/kg/min or 8.5 metabolic equivalents, i.e., METs) were barely above the minimum healthy threshold for all-cause and CVD mortality in middle-aged men/women (i.e., 8 METs [52]). It is thus of medical importance to determine whether genetic factors (including specific gene variants) are associated with variability of CRF around (i.e., above vs below) the 8-MET cutoff. Previous research in siblings/twins suggests that 50%–60% of the variance of VO_{2peak} is associated with heredity [10,12]. These values seem surprisingly high given the variety of physiological processes and body tissues involved in the uptake and utilization of oxygen in muscle mitochondria. Indeed, there is open debate about the limits of the evidence that support the relative influence of genetics on the variability and trainability of CRF [54,55].

Our findings question the high heritability of VO_{2peak}, at least in Chinese individuals with no familial connection. From the 125 SNPs selected for our study, only one (*ACE* rs4295) was associated with VO_{2peak}. Also, the best model obtained through multiple regression analyses could only explain ~2.2% of the interindividual variance in VO_{2peak}. As in the study by Bye et al. [14], we created a polygenic score to determine whether those individuals with a higher number of alleles associated with VO_{2peak} did indeed present with higher values of this parameter. The only differences found between our findings and those of the Bye et al. study were the number of SNPs included in the polygenic score (7 vs 3, respectively) and the use of an intermediate genotype score for heterozygotes, which was not included by Bye et al. Interestingly, in both studies, participants with the theoretically lowest (or 'less favorable') genotype scores had the lowest VO_{2peak} (22–24 mL/kg/min), which was significantly lower than for those with the theoretically highest (or 'most favorable') genotype score (~32 mL/kg/min). These findings suggest that only a small number of SNPs are associated with the

odds of having high VO_{2peak} values in untrained individuals. The variance of the interindividual variability in VO_{2peak} explained with these genotypes is low and the addition of favorable alleles might produce a change of 8–10 mL/kg/min. This genetic influence might be considerable in clinical terms because each 1-MET (or 3.5 mL/kg/min) increase in CRF has been shown to confer a 12% improvement in survival in Caucasian (North-American) men [6]. Moreover, as mentioned above, it is of clinical importance to surpass the 8-MET threshold, and in fact, adults with a CRF clearly above this level (>10 METs) have a remarkably reduced CVD risk [56]. In this regard, the probability of surpassing the 8-MET threshold (equivalent to 28 mL/kg/min) was doubled in those participants that carried the six 'favorable' alleles (Figure 4).

Only three SNPs were included in the final multiple regression model. *ACE* rs4295 has not been previously associated with endurance performance, but it is located in the same linkage disequilibrium block as the widely studied *ACE* insert(I)/deletion(D) polymorphism (rs4340) [57]. The *ACE* gene encodes angiotensin-converting enzyme and the I allele might be associated with lower circulating levels of enzyme, and the II genotype potentially associated with performance in endurance athletes (odds ratio 1.35; 95% confidence interval, 1.17 to 1.55 [58]). However, several studies have found no association between the *ACE* I/D genotype and VO_{2peak} values in trained [59] and untrained [60,61] individuals. With regard to the *ACE* rs4295 variation found in the present study, although its influence on CRF needs to be replicated in other cohorts, our findings bolster the role of angiotensin-converting enzyme and its coding gene as predictors of CRF-related phenotypes. We also found that carriage of the C allele in the *AGTR1* rs275652 polymorphism was negatively associated with VO_{2peak} values. This gene encodes the angiotensin II receptor 1 (AT_1R), and polymorphisms in *AGTR1* have been suggested to be involved in the physiological response to hypoxia [62]. AT_1R is broadly expressed in different tissues and mediates most of the classical actions of angiotensin II, including vasoconstriction and vascular smooth muscle cell proliferation [63]. Thus, under hypoxic conditions, angiotensin II engages AT_1R to modulate the pulmonary vasoconstrictive response [64]. Although speculative, it is possible that the C allele in *AGTR1* rs275652 might exacerbate pulmonary vasoconstriction during exercise owing to a higher activation of AT_1R for a given concentration of angiotensin II [65]. The last SNP included in the model explaining VO_{2peak} was rs7570532, a genetic variation in *GDF8* encoding myostatin. This and other SNPs in *GDF8* have been indirectly associated with a major cardiometabolic condition, obesity [39], but other authors have reported no association of rs7570532 with endurance performance [40]. Myostatin controls the differentiation and proliferation of skeletal muscle throughout embryonic development and regulates muscle growth during adulthood. Mutations in *GDF8* that produce non-functional myostatin result in the increased growth of skeletal muscle, demonstrating the existence of a powerful mechanism to control muscle size in normal individuals through this protein [66]. Based on these findings and given the positive association between muscle mass and VO_{2peak} [66–68], it is possible that *GDF8* rs7570532 confers a small but significant predisposition to higher VO_{2peak} values. Further research on these three SNPs is clearly warranted.

We acknowledge that the current investigation has some limitations. First, our study sample was heterogeneous in terms of age, sex, and anthropometric characteristics (Table 1). Although we used these variables as covariates in linear regression analyses, the high variability of these variables might have partially influenced our results. In fact, when they were included in multiple regression analyses (Figure 2b), the explained variance of VO_{2peak} increased up to 50.1%. Second, our study only included participants of Han Chinese descent and the results might therefore not be applicable to other ethnicities. Of note, the Han Chinese constitute the world's largest ethnic group (constituting ~18% of the global population), but further studies in other large ethnic groups will be needed to confirm/discard the generalizability of these results. Lastly, we only analyzed 125 SNPs and thus it is plausible that other candidate genes might have an influence on VO_{2peak}.

5. Conclusions

The present study shows that in a cohort of untrained Han Chinese individuals, VO_{2peak} is influenced by a very few polymorphic variations in key genes even in isolation of training adaptations. The genetic influence accounted for ~2.2% of the interindividual variance in VO_{2peak}, at least with the 125 SNPs included in this investigation. Although more research is needed, these data suggest that environment, probably more than genetics, is responsible for most of the interindividual variability in VO_{2peak} among healthy Han Chinese adults.

Author Contributions: Conceptualization, Z.H., J.D.C., P.L.V. and A.L.; methodology, Z.H.; formal analysis, Z.H., J.D.C., R.D.-P., and A.L.; assessments G., Z.G., W.G., R.Y.; data curation, Z.H., J.D.C., and A.L.; writing—original draft preparation, Z.H., J.D.C.; writing—review and editing, Z.H., J.D.C., R.D.-P., P.L.V. and A.L.; supervision, A.L.; funding acquisition, Z.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by key research and development projects of the Ministry of Science and Technology (2018YFF0300402, 2018YFC2000600), China Institute of Sport Science (13-13, 14-08), and projects in the National Science & Technology Pillar Program during the twelfth Five-year Plan Period (2012BAK23B01). P.L.V is supported by University of Alcalá (FPI2016). Research by A.L. is funded by and the Spanish Ministry of Science and Innovation (Fondo de Investigaciones Sanitarias and Fondos FEDER [grant number PI18/00139]) and Fondos FEDER.

Acknowledgments: The authors would like to thank all the participants in the study.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Appendix A

Table A1. List of SNPs investigated for association with VO_{2peak} (mL/kg/min) in Han Chinese untrained individuals.

Gene	SNP	MA	MAF
ACE	rs4295	G	38.1
ACE	rs4341	G	34.9
ACE	rs4363	G	38.7
ACE2	rs6632677	C	9.2
ACSL1	rs10022018	G	24.4
ACSL1	rs11732302	C	24.7
ACSL1	rs12503643	G	45.9
ACSL1	rs12644905	T	19.6
ACSL1	rs13126272	T	11.0
ACSL1	rs1803898	A	7.3
ACSL1	rs2292898	C	7.8
ACSL1	rs13120078	A	8.2
ACSL1	rs2280297	C	47.6
ACSL1	rs2292899	A	38.1
ACSL1	rs3749233	A	25.9
ACSL1	rs3792312	G	41.7
ACSL1	rs4069938	G	37.7
ACSL1	rs6552828	G	35.2
ACSL1	rs902177	C	28.2
ACTN3	rs1815739	T	41.7
AGT	rs10864770	T	34.5
AGT	rs11568046	C	12.9
AGT	rs2478523	C	46.0
AGT	rs2478544	C	21.8
AGT	rs2493132	T	37.3
AGT	rs3789671	G	45.5
AGT	rs3789678	T	18.7
AGT	rs3889728	A	49.4

Table A1. *Cont.*

Gene	SNP	MA	MAF
AGT	rs5050	G	14.8
AGT	rs6687360	C	33.1
AGT	rs699	T	19.7
AGT	rs7079	A	16.2
AGT	rs7536290	G	22.0
AGTR1	rs2131127	T	37.4
AGTR1	rs275652	C	13.6
AGTR1	rs3772616	A	17.8
AGTR1	rs385338	G	18.0
AGTR1	rs5182	C	28.1
AGTR1	rs6801836	C	14.2
BDKRB2	rs10130005	C	18.3
BDKRB2	rs10132462	T	28.1
BDKRB2	rs11160322	C	23.0
BDKRB2	rs11627176	G	12.0
BDKRB2	rs11627761	T	15.3
BDKRB2	rs11848502	T	30.1
BDKRB2	rs12433275	T	16.3
BDKRB2	rs12888402	C	16.7
BDKRB2	rs1799722	C	48.0
BDKRB2	rs1959053	T	25.3
BDKRB2	rs2069575	A	20.4
BDKRB2	rs2069578	G	39.1
BDKRB2	rs2069586	A	16.5
BDKRB2	rs2069588	T	18.0
BDKRB2	rs2369521	G	35.6
BDKRB2	rs4144131	A	43.9
BDKRB2	rs4900315	C	46.5
BDKRB2	rs4900318	A	49.7
BDKRB2	rs4905470	A	20.0
BDKRB2	rs4905474	A	37.1
BDKRB2	rs6575577	G	22.5
BDKRB2	rs7155797	T	44.4
BDKRB2	rs7161665	C	47.9
BDKRB2	rs8013400	T	28.5
BDKRB2	rs8016905	A	32.7
BDKRB2	rs885818	T	13.4
BDKRB2	rs945039	T	42.9
FGFR2	rs2071616	T	8.8
FNDC5	rs16835198	T	47.7
FNDC5	rs3480	G	24.8
FST	rs3797296	G	17.3
FST	rs3797297	T	12.8
FTO	rs1421085	C	10.4
FTO	rs1558902	A	10.6
FTO	rs9939609	A	10.4
GDF-8	rs16832288	A	19.9
GDF-8	rs7570532	G	26.0
IL-15	rs1057972	A	49.9
IL-6	rs1524107	C	29.3
IL-6	rs2069840	G	7.3
IL-6	rs2069830	G	27.2
IL-6	rs2069837	G	20.1
IL-6	rs2069852	G	37.0
ITLN1	rs2274906	A	36.5

Table A1. *Cont.*

Gene	SNP	MA	MAF
ITLN1	rs2274910	T	29.6
ITLN1	rs2297560	T	13.9
ITLN1	rs6427552	C	24.7
PGC-1α	rs12374310	C	43.9
PGC-1α	rs12650562	C	49.5
PGC-1α	rs251468	T	19.8
PGC-1α	rs4452416	G	13.4
PGC-1α	rs4697425	G	30.8
PGC-1α	rs6821591	C	29.9
PRDM16	rs12409277	C	42.0
PRDM16	rs2236518	A	44.5
PYY	rs10853114	C	37.8
PYY	rs12953033	A	6.9
PYY	rs162430	G	35.3
PYY	rs1859223	G	27.2
REN	rs11571078	T	12.7
REN	rs1464816	T	24.4
REN	rs2368564	T	20.3
REN	rs4951313	G	29.1
REN	rs5707	G	40.3
RETN	rs3745367	A	35.5

Abbreviations: MA, minor allele; MAF, minor allele frequency; SNP, single nucleotide polymorphism; VO_{2peak}, peak oxygen uptake. Abbreviations for gene names: *ACE*, angiotensin-converting enzyme; *ACE2*, angiotensin-converting enzyme 2; *ACSL1*, acyl-CoA synthase long-chain member 1; *ACTN3*, alpha-actinin-3; *AGT*, angiotensinogen; *AGTR1*, angiotensin II receptor type 1; *AGTR2*, angiotensin II receptor type 2; *BDKRB2*, bradykinin receptor B2; *FGF21*, Fibroblast growth factor 21; *FGFR2*, fibroblast growth factor receptor 2; *FNDC5*, fibronectin type III domain-containing protein 5; *FST*, follistatin; FTO, fat mass and obesity-associated protein (also known as 'alpha-ketoglutarate-dependent dioxygenase'; *GDF8*, growth differentiation factor 8 (also known as 'myostatin'); *IL-6*, interleukin 6; *IL-15*, interleukin 15; *ITLN1*, intelectin 1; PGC-1α, peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1alpha; *PRDM16*, PR domain containing 16; *PYY*, peptide YY; *REN*, renin; *RETN*, resistin.

Appendix B

Table A2. List of SNPs discarded for analyses because did not meet the Hardy–Weinberg equilibrium.

Gene	SNP	MA	MAF
ACE2	rs2074192	T	42.6
ACE2	rs6632677	C	9.2
AGTR1	rs12721241	A	12.0
AGTR1	rs2675511	G	13.9
AGTR2	rs5193	T	15.9
AGTR2	rs12840631	G	18.1
AGTR2	rs6608590	T	41.6
BDKRB2	rs4900313	A	16.2
PGC1β	rs17110586	G	14.8
PRC	rs17114388	G	19.7

Abbreviations: see Table A1.

Table A3. List of SNPs discarded for analyses because the frequency of the minor allele was inferior to 5%.

Gene	SNP	MA	MAF
FGF21	rs838133	A	1.1
FGF21	rs838145	G	1.3
FNDC5	rs726344	A	0.2

Table A3. *Cont.*

Gene	SNP	MA	MAF
FST	rs12152850	T	1.6
GDF-8	rs1805086	C	0.2
GDF-8	rs3791784	G	2.3
IL-6	rs1800795	C	0.7
IL-15	rs1589241	T	0.8
IL-6	rs1554606	T	1.8
ITLN1	rs11265509	T	4.7

Abbreviations: see Table A1.

Table A4. List of SNPs discarded for analyses because all individuals of the sample had the same genotype.

Gene	SNP	Genotype
AGTR1	rs12721276	CC
PYY	rs432747	GG

Abbreviations: see Table A1.

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