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Original Article

Functional DNA variations associated with Saudi female with low VO_{2max}: a pilot microarray study

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Abstract: The study aims to explore the genetic predispositions and molecular pathways of low cardiorespiratory fitness (VO_{2max}) in young Saudi females (n = 70). Young females were grouped based on the level of VO_{2max} according to the specification of the physical fitness specialist certification as low VO_{2max} (< 28.9; n = 19) and high VO_{2max} (> 33; n = 14) and genotyped for 243345 putative functional exonic variants. The *CYFIP2&FNDC9*-rs10037485T, *C1R*-rs75380747G and *TOP2A*-rs13695C SNPs on chromosome 5, 12 and 17, respectively were found to be the most significant among young Saudi females with low VO_{2max} ($P < 8.01 \times 10^{-05}$). Linkage disequilibrium (LD) analysis among the significant SNPs ($P < 0.001$) have revealed risk and protective haplotypes with high degree (p-value < 1.0×10^{-4}) of LD. The most significant risk haplotypes with the low VO_{2max} in young Saudi females are: Chromosome 1: *LOC112268276*-rs10800201G; *LOC112268276*-rs4657537A; rs4657583T (p-value = 2.00×10^{-04}); Chromosome 3: rs978979G; *CCDC66*-rs7637449A; *CCDC66*-rs111934125T; *FAM208A*-rs9835332G (p-value = 5.00×10^{-04}) and Chromosome 17: *STX2*-rs13696C; *TNS4*-rs1901187C (p-value = 1.00×10^{-04}). Functional Enrichment Analysis revealed that the genes with SNPs $P < 0.001$ have significantly involved in the heart rate (P = 0.00442), body weight (P = 0.00629), breath tests (P = 0.0147), proteolysis (P = 0.00623) and cardiac muscle fiber development (P = 0.0263). In conclusion we could say that the identified genetic predispositions and gene-annotation enrichment in low VO_{2max} in young Saudi females revealed that they are at high risk for developing cardiovascular complications.

Keywords: Cardiorespiratory fitness, genetic predispositions, VO_{2max}, haplotyping, SNP genotyping, risk alleles, cardiovascular disease, predictor genes

Introduction

Cardiorespiratory fitness (VO_{2max}) is a predictor of the symptoms of chronic diseases and risk of premature mortality [1]. The low VO_{2max} is reported to be associated with development of various chronic diseases: higher risk of developing defined obesity, abdominal obesity, cardiovascular disease, hyperinsulinemia and premature mortality [1-8]. Regular exercise is always recommended to reduce the worldwide prevalence of low cardiorespiratory fitness associated diseases such as diabetes, cardiovascular disease, and stroke through various mechanism [https://www.who.int/nmh/about/chp/en/]. Studies have reported the impact of the genetic and environmental factors on the cardiorespiratory fitness [7, 9, 10]. Studies have identified ~100 possible genes associated with the VO_{2max} trainability in various populations [1]. However, there were no studies on the impact of the cardiorespiratory fitness in Arab population. The current study aims to explore the possible genetic predispositions and functional molecular mechanism of low VO_{2max} among young females from Eastern Province of Saudi Arabia.

Materials and methods

Subjects and methods

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board (IRB) at Imam Abdulrahman Bin Faisal University (IRB approv-

al number: IRB-PGS-2017-01-219). This is a cross-sectional study conducted during the period from March 2017 to March 2018. Saudi female college students (n = 70) within the age range 19-25 years were randomly selected from different health colleges (College of Medicine, Dentistry, Nursing, Applied Medical Science and Clinical Pharmacy) of Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia. Simple random selection was performed using a Microsoft Excel sheet. Each student in the campus was given a random value then values were later sorted in an ascending order. Thereafter, the required sample was chosen from the top of the list. The sample size was calculated using G*power 3.1 statistical power analysis software [11] based on the calculation of the effect size to give the study a power of 95%. The effect size: (0.39) was calculated using the mean VO_{2max} value of the null hypothesis H_0: (33.7), the standard deviation SD: (10.97) (mean VO_{2max} and SD values were taken from earlier study) [12]. Whereas the mean VO_{2max} value of the alternative hypothesis was H_1: (40). Criteria for inclusion in the study were apparently healthy females, with normal body mass index (BMI) of 18.50-24.99 ($Kg.m^{-2}$) [https://www.who.int/nutrition/databases/bmi/en/], who were sedentary according to the definition of the International Physical Activity Questionnaire (IPAQ) [https://sites.google.com/site/theipaq/home]. Participants with any contraindication of exercise stress testing [13], who has known chronic illness, who underwent major surgery or are taking any medication apart from nonsteroidal anti-inflammatory drugs were excluded from the study. Additionally, pregnancy, breastfeeding, smoking and using of ergogenic aids were also considered as exclusion criteria. All participants signed an informed written consent in English and a translated Arabic form and a form according to Standing Committee for Research Ethics on Living Creatures. All performed procedures were in agreement with the university ethical committee guidelines.

Exercise testing and direct measurement of VO_{2max}

The exercise was carried out in the Physiotherapy Research Center of the College of Applied Medical Science at the Exercise Physiology Laboratory. All tests were performed during daytime from 10:00 am to 1:00 pm using COSMED system for cardiopulmonary exercise testing (Quark CPET, COSMED, Rome, Italy). COSMED system is composed of: Gas analyzer (breath by breath analyzer) for continuous gas exchange measurements made by an open-circuit spirometry; Electronically braked cycle ergometry (Ergoline, COSMED, Bitz, Germany); Electrocardiogram (ECG) monitor (Wireless 12-lead stress PC ECG, COSMED, Rome, Italy); Arterial blood pressure cuff; Pulse oximeter (Pulse Oximetry, COSMED, Rome, Italy); and computer, online analysis software for final reports.

System calibration

The system was calibrated in accordance with the manufacturer's instructions. Prior to calibration, system was warmed-up for 20 minutes. Analyzers calibration (fast response paramagnetic O_2 analyzer and nondispersive infrared CO_2 analyzer) were done every day and before each test. The appropriate analyzer response was verified by passing known gas mixture concentrations over the analyzers. Inspired gas values were verified using ambient air assumed to contain 20.93% O_2 and 0.03% CO_2. Appropriate expired gas values were verified using certified gas cylinders from the British Oxygen Corporation containing 16% O_2, 5% CO_2 and N_2 for balance. Bidirectional digital turbine was calibrated every week using a 3 liter syringe.

Cardiopulmonary exercise testing

All subjects performed a maximal exercise test on a stationary bicycle ergometer using a one-minute incremental test. The selection of the work rate increase needed for the students was calculated by the formula previously published for cycle ergometry and sedentary females [14].

Work rate increase formula

It is calculated by the following formula:

Work rate increment per minute in watts = (peak VO_2 in milliliters per minute - VO_2 unloaded in milliliters per minute)/100.

Where VO_2 unloaded in milliliters per minute is calculated as: VO_2 unloaded (ml/min) = 150 + (6 × weight in Kilograms) and peak VO_2 in milliliters per minute is calculated by: Peak VO_{2max}

(ml/min) = (height in centimeters - age in years) × 20 for sedentary men and × 14 for sedentary women.

Age predicted maximal heart rate was obtained from the formula (220-age) [14]. Subjects had measurement of resting arterial blood pressure (BP) taken in sitting position from the right arm manually and digitally by a mercury sphygmomanometer (diplomat-presameter®Sphygmomanometer, Riester, Jungingen, Germany) and by a vital signs monitoring device (Spot Vital Signs® Device, Welch Allyn, Skaneateles Falls, New York, USA). The height of the seat was adjusted to keep the subject's legs at near full extension in each pedal revolution [13]. The appropriate size of an ergonomic multi-use silicone face masks (available in 3 sizes) was chosen for each subject to have a good seal about the face and to ensure an accurate test. The duration of the exercise was 6 to 12 minutes as suggested previously. This duration is considered the optimum time to give the highest maximal VO_2 consumption in healthy subjects because tests that are too long will be too boring and the subjects may terminate the test prematurely while too short tests would not give sufficient informative data [14].

After a period of rest, the incremental protocol allowed the subject initially to cycle for 3 minutes of unloaded pedaling as a warm-up period. Then, the power output started at 20 Watts and was increasingly incremented by 15 Watts every minute by computer control until the subject was limited by debilitating symptoms despite verbal encouragement. The cycling frequency was maintained at 50 revolutions per minute (rpm) throughout the exercise with the assistance of the digital display on the bike to maintain the required cycling pace. Finally, the participants were asked to continue cycling for 3 minutes without resistance in the recovery period. Time till exhaustion was recorded and participants were asked about the reason for stopping the exercise test. Heart rate (HR), oxygen saturation (SpO_2%), arterial blood pressure and ECG were monitored at the resting baseline and during the exercise test. At the end of each stage, HR and ratings of perceived exertion (RPE) score of each participant were recorded. Specifically, The Borg Rating of Perceived Exertion scale was used. It rates the intensity of the exercise from 6 to 20 [14]. HR was monitored with the three bipolar leads of the ECG. Oxygen saturation was obtained from the pulse oximeter. The arterial blood pressure was recorded automatically by a pressure cuff attached to the bike every 2 minutes. Maximum HR, blood pressure and RPE of the last achieved stage were determined. To monitor the safety, the bipolar leads were recorded using a wireless ECG belt with a special ECG electrode (Ambu® Blue Sensor T, Ambu, Penang, Malaysia).

Based on breath by breath gas analysis system, Oxygen consumption (VO_{2max}) in ($ml.min^{-1}$) and ($ml.kg^{-1}.min^{-1}$); Carbon dioxide production (VCO_2) in ($ml.min^{-1}$); Minute ventilation (VE); ($l.min^{-1}$); Respiratory rate (RR); ($breaths.min^{-1}$); Tidal volume (TV); (l); Respiratory exchange ratio (RER) and Ventilatory anaerobic threshold (AT) in $ml.min^{-1}$ and $ml.kg^{-1}.min^{-1}$ were measured. In the post-exercise time at the 3-minute recovery period, HR, manual and digital blood pressure were recorded by the same devices mentioned previously.

Maximum VO_2 assessment (VO_{2max}) and statistical analysis

VO_2 values were considered maximal when two out of the four criteria (Plateau of VO_2 despite an increase in workload; Respiratory exchange ratio 1:1 or higher; Heart rate within 15 beats of the age predicted maximum heart rate; An RPE ≥ 17) were achieved [14]. ACSM's Guidelines of indications for terminating exercise testing were followed [14].

Statistical Package for the Social Sciences software version 22 (IBM SPSS Statistics) was used to perform the statistical analyses. All data were expressed as mean ± SD. Pearson correlation was applied for all the ventilator and hematological parameters. All the tested subjects were divided according to the level of VO_{2max} into two groups, and then independent t-test was used to compare the hematological parameters. A p-value of < 0.05 was considered as significant.

DNA extraction and genotyping

QIAamp DNA Blood Mini Kit (Qiagen, Germany) was used to perform DNA extraction on blood samples collected from the study subjects with low VO_{2max} (< 28.9 $n = 19$) and high VO_{2max} (> 33; $n = 14$). For microarray, human Exome bead

chip kit v1.0 and v1.1 Illumina (San Diego, USA), which consists of 243345 putative functional exonic markers, was utilized using Illumina iScan. All DNA samples were processed according the manufacturer's protocol and iScan control software (Illumina, San Diego, USA) was used to acquire data. The DNA extraction, microarray genotyping, and analysis were performed at the Genetic research laboratory of the Institute for Research and Medical Consultation (IRMC), Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia. Genotyping was carried in this lab from 2016 to 2018, using the similar chip and procedures.

Statistical and functional analysis

GenomeStudio 2.0 Data Analysis Software - Illumina was used for initial quality check for the call rate, total of 0 subject were excluded from the analysis due to call rate < 0.98% and re-clustered. Hardy-Weinberg equilibrium (HWE) was tested separately among the case and control groups with 1 degree of freedom genotypic chi-squared test. Differences in clinical characteristics between cases and controls were calculated by the two-sample t-test or the χ^2 test if appropriate using IBM SPSS Statistics version 23 (IBM Co., Armonk, NY, USA). Kaviar [15] and SNP-Nexus [16] were used to confirm variants reported at a base pair position at the respective chromosome as per Genome Reference Consortium Human Build 37 (GRCh37.p13). The case-control association analyses, odds ratios and 95% confidence intervals were calculated to evaluate the effects of different alleles and haplotypes using Haploview version 4.2 [17] and gPLINK version 2.050 [18] Bonferroni corrections or false discovery rate corrections were applied to correct the *p* values of 243345 SNPs (corrected $\alpha = 0.05/243345 = 2.05 \times 10^{-07}$), to control inflation of the Type I error rate. The *p*-value less than 0.05 were considered as significant. The highly significant ($P < 1 \times 10^{-05}$) genes were annotated for functional implications through DAVID 6.7 [19], STRING 10.5 [20], SNPnexus [16], Expression Atlas [21] and Reactome [22].

Results

Saudi female subjects were grouped on the level of VO_{2max} according to the specification of the physical fitness specialist certification as low VO_{2max} (n = 19) and high VO_{2max} (n = 14) (**Table 1**) [23]. Ventilation (L/min), tidal volume (L), VO_{2max} (ml/min), VO_{2max} (ml/min.Kg), CO_2 production (VCO_2) (ml/min), Respiratory exchange ratio VCO_2/VO_2, anaerobic threshold (ml/min), and anaerobic threshold (ml/min.Kg) were significantly different among the selected study group (**Table 1**). The distinct two groups of the Saudi young females were subjected for the genotyping analysis of 243345 putative functional exonic markers.

A total of 106 SNPs was found to be significant with the low VO_{2max} at the *p*-value below 0.001 among the 24,3345 variants (Supplementary Table 1). Three *CYFIP2* and *FNDC9*-rs10037485, and *C1R*-rs75380747 and *TOP2A*-rs13695 SNPs on chromosome 5, 12 and 17, respectively were found to be associated significantly in young Saudi females with low VO_{2max} ($P < 8.01 \times 10^{-05}$) among the 24,3345 variants (**Figure 1**). The most significant ($P < 0.000979$) SNPs that are associated with low VO_{2max} in young Saudi females from the Arab-ancestry are summarized (**Table 2**). All these SNPs obeyed the hardy-Weinberg equilibrium.

All association tests were screened using *p*-value of Hardy-Weinberg equilibrium, and frequency of minor allele in controls to conclude the strongest genetic predisposition ($P < 0.001$) and imputed for linkage disequilibrium in HapMap, separately for regions of chromosomes with more than one SNP (**Figure 2**). Linkage disequilibrium analysis among SNPs with significance level $P < 0.001$ identified risk (lowering VO_{2max}) and protective (keeping VO_{2max} normal) haplotypes with high degree (p-value < 1.0×10^{-4}) of linkage disequilibrium (**Table 3**). The most significant haplotypes such as Chromosome 1: *LOC112268276*-rs10800201G; *LOC112268276*-rs4657537A; rs4657583T (p-value = 2.00×10^{-04}); Chromosome 3: rs978979G; *CCDC66*-rs7637449A; *CCDC66*-rs111934125T; *FAM208A*-rs9835332G (p-value = 5.00×10^{-04}) and Chromosome 17: *STX2*-rs13696C; *TNS4*-rs1901187C (p-value = 1.00×10^{-04}) were found to be risk haplotypes associated with the low VO_{2max} in young Saudi females (**Table 2**; **Figure 2**). The opposite alleles of the most significant haplotypes such as Chromosome 1: *LOC112268276*-rs10800201A; *LOC112268276*-rs4657537G; rs4657583C (p-value = 0.0077); Chromosome 3: rs978979A; *CCDC66*-rs7637449G; *CCDC66*-rs111934125C; *FAM208A*-rs9835332C (p-value = 0.0014)

Table 1. Ventilatory parameters obtained from the maximum cardiopulmonary exercise testing and hematological indices

Parameter	Low VO_{2max} (n = 19)	High VO_{2max} (n = 14)	p value
RBC count × 10^6	4.28 ± 0.57	4.17 ± 0.87	0.649
Hb g/dL	10.59 ± 1.71	11.05 ± 0.91	0.354
Hct%	34.65 ± 5.40	34.57 ± 4.10	0.962
MCH pg	24.69 ± 2.29	26.37 ± 4.4	0.187
MCHC g/dl	30.57 ± 1.63	33.35 ± 6.03	0.085
RDW%	14.99 ± 3.18	15.28	0.865
MCV fl	80.99 ± 8.10	86.05 ± 18.18	0.327
HbF%	0.46 ± 0.50	0.46 ± 0.55	1.000
HbA_2%	2.68 ± 1.04	2.74 ± 0.28	0.801
Serum ferritin µg/L	21.56 ± 23.17	21.44 ± 22.17	0.986
Serum iron µg/L	65.57 ± 31.18	55.47 ± 30.75	0.303
TIBC µmol/L	304.36 ± 90.03	307.09 ± 60.79	0.912
Ventilation L/min	54.68 ± 9.88	63.93 ± 10.79	0.002**
Tidal volume L	1.20 ± 0.17	1.40 ± 0.19	< 0.001**
Respiratory frequency	45.72 ± 7.63	45.96 ± 7.06	0.911
VO_{2max} ml/min	1290.00 ± 154.66	1740.35 ± 200.80	< 0.001**
VO_{2max} ml/min.Kg)	23.81 ± 1.90	33.87 ± 2.65	< 0.001**
CO_2 production (VCO_2) ml/min	1422.88 ± 224.76	1799.45 ± 331.26	< 0.001**
Respiratory exchange ratio VCO_2/VO_2	1.35 ± 0.10	1.26 ± 0.08	0.001**
Anaerobic threshold ml/min	711.85 ± 298.55	931.40 ± 407.91	0.028*
Anaerobic threshold ml/min.Kg	13.16 ± 5.61	18.20 ± 7.90	< 0.009**
Weight Kg	54.27 ± 5.60	51.41 ± 4.95	0.067
Body Mass Index BMI	21.53 ± 1.70	21.10 ± 1.82	0.393
Resting heart rate (beats/min)	99.12 ± 13.57	91.80 ± 13.10	0.059
Resting systolic blood pressure (mmHg)	100.45 ± 11.00	98.25 ± 10.63	0.477
Resting diastolic blood pressure (mmHg)	60.03 ± 11.25	57.10 ± 8.40	0.319
Maximum heart rate (beats/min)	183.61 ± 6.10	178.96 ± 7.08	0.023*
Systolic blood pressure (mmHg) at VO_{2Max}	146.33 ± 11.79	140.65 ± 18.26	0.174
Diastolic blood pressure (mmHg) at VO_{2Max}	82.06 ± 17.75	70.80 ± 16.00	0.024*
Post exercise heart rate at 3 minutes recovery (beats/min)	142.36 ± 11.78	138.74 ± 12.24	0.297
Post exercise diastolic blood pressure (mmHg)	110.94 ± 13.06	111.85 ± 13.69	0.811
Post exercise systolic blood pressure (mmHg)	58.97 ± 10.12	53.70 ± 8.44	0.058

*Significant; **Highly significant.

and Chromosome 17: *STX2*-rs13696T; *TNS4*-rs1901187T (p-value = 1.00 × 10^{-04}) were found to be protective haplotypes associated with the low VO_{2max} in young Saudi females (**Tables 2**, **3**; **Figure 2**). Haplotypes *MMP26* and *OR51F1*-rs1030726C; rs1030723G; rs11033800C; rs11033801A (Risk haplotype; p-value = 0.0011), *MMP26* and *OR51F1*-rs1030726A; rs1030723A; rs11033800A; rs11033801G (Protective haplotype; p-value = 0.0024), *OR11H6*-rs12891553T; rs34693535C; rs17211285G; rs17277221T; rs17277228T (Risk haplotype; p-value = 0.002) and *OR11H6*-rs12891553C; rs34693535T; rs17211285T; rs17277221C; rs17277228C (Protective haplotype; p-value = 0.002) were identified in single gene (**Table 2**; **Figure 2**).

Functional Enrichment Analysis revealed that the genes with SNPs P < 0.001 have significantly involved in the heart rate (6 Genes; P = 0.00442; *HS3ST4, CYFIP2, CNTNAP2, MYH6, ROS1, FNDC9*), body weight (7 Genes; P = 0.00629; *PTPRD, MYO18B, MCTP2, C1ORF220, ZMIZ1, CYFIP2, CNTNAP2*), breath tests (4 Genes; P = 0.0147; *PTPRD, ZMIZ1, CNTNAP2, LDLRAD3*), proteolysis (7 Genes; P = 0.00623; CPA5, MMP26, C1R, KLK1, CPA1, CAPN3, CT-

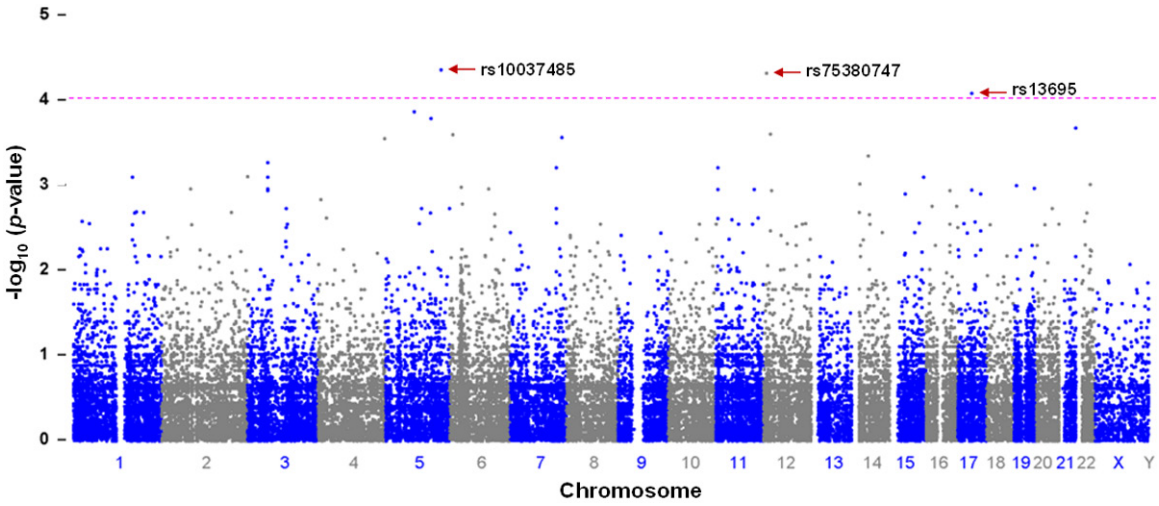

Figure 1. Manhattan plot of putative functional exonic (n = 243,345) variants in the molecular genetics of low VO_{2max} from association study. The-log_{10} (p-values) from the association are plotted according to variant position on each chromosome. Positions of candidate SNP biomarkers of functional exonic variants at *CYFIP2 and FNDC9* (rs10037485), and *C1R* (rs75380747) and *TOP2A* (rs13695) on chromosome 5, 12 and 17, respectively for low VO_{2max} are indicated by arrows. The horizontal dotted line indicates the suggestive threshold of p 1.00 × 10^{-4}.

RL) and cardiac muscle fiber development (2 Genes; P = 0.0263; *MYO18B, MYH6*).

Discussion

The rapid development of socioeconomic status in the gulf regions impose a harmful sedentary lifestyle which burden society with health concerns. According to the WHO, physical inactivity considered one of the leading causes to cardiovascular diseases (CVD) that account for 54% of deaths from non-communicable disease in the Eastern Mediterranean regions [https://www.who.int/nmh/about/chp/en/]. Studies on the sedentary lifestyle with obesity and Physical activity (PA) in Saudi population revealed that physical inactivity level is 66.6% [24] and account for 71% in youth and 52% in children [25]. Different factors such as sex, geographical location and age appears to impact the PA [26]. Moreover, males generally are physically more active than females [27] and this tend to decrease with age [26]. Therefore, many studies were focusing on females as a high-risk group for CVD as per social and environmental factors may contributed to poor PA among Saudi females [28]. The PA and cardiovascular risk factors have been investigated heavily among adolescents and adults of both gender in different regions of Saudi Arabia [29-31]. However, less towards cardiorespiratory fitness which linked to CVD. Cardiorespiratory fitness has been known as a predictor of mortality caused by CVD, and further used to evaluate the cardiovascular health by measuring maximum oxygen consumption (VO_{2max}) during sustained exercise [32]. Different correlation studies of cardiorespiratory fitness have been conducted within Saudi population tackling different aspects. Some indicated a positive correlation between 25(OH) D/body weight and VO_{2max} (as VO_2 peak) in young Saudi females [28]. However, less stringent association was conveyed in other study, improve VO_{2max} after vitamin D supplementation was reported though [33]. Saudi females showed inverse correlation of VO_{2max} and body fat composition with BMI [28, 34]. Researches were aiming to characterize the aerobic fitness of Saudi athletes and soccer players [35]. The promotion towards physical activities have been initiated [25]. Recent findings noticed an association of a high VO_{2max} with decrease incidence of CVD and improve the quality of life [36]. Studies are scanty on the investigation of VO_{2max} in association with type 2 diabetes mellitus, dementia and environment, metabolic and genetic factors to ensure a better understanding of the cardiovascular health and quality life of Saudis. The present study is a pilot model for the genetic association and functional molecular mechanism of low VO_{2max} in young females in Saudi Arabia. The genes (*CYFIP2, FNDC9, C1R, TOP-2A, LOC112268276, LOC112268276, CCDC-*

Table 2. List of the most significant SNPs associated with low VO_{2max}

S. No	RS id	CHR	BP	MA	MAF	CHISQ	P	OR (L95-U95)	Gene	AA	CCF	HWpval
1	rs10037485	5	156770133	A	0.6053	16.75	4.26E-05	12.78 (3.27-49.92)	*CYFIP2 and FNDC9*	T	0.605, 0.107	1
2	rs75380747	12	7188562	A	0.1053	16.59	4.65E-05	0.09 (0.02-0.32)	*C1R*	G	0.895, 0.429	0.365
3	rs13695	17	38545193	A	0.05263	15.56	8.01E-05	0.06 (0.01-0.32)	*TOP2A*	C	0.947, 0.536	1
4	rs7726099	5	79521704	G	0.2105	14.61	0.0001323	0.13 (0.04-0.38)	*SERINC5*	A	0.789, 0.321	1
5	rs45074	5	126993249	C	0.6842	14.25	0.0001598	7.94 (2.56-24.66)	*CTXN3 and CTC-548H10.2*	C	0.684, 0.214	0.002
6	rs743346	21	47851636	A	0.02778	13.78	0.0002056	0.04 (0.01-0.37)	*PCNT*	G	0.972, 0.607	0.003
7	rs4959505	6	8869571	A	0.6316	13.43	0.0002475	7.89 (2.45-25.42)		A	0.632, 0.179	1
8	rs2462603	7	145813854	A	0.1944	13.3	0.0002648	0.13 (0.04-0.42)	*CNTNAP2*	G	0.806, 0.357	0.859
9	rs11726022	4	188170467	A	0.2632	13.23	0.0002751	0.14 (0.05-0.43)		G	0.737, 0.286	1
10	rs9835332	3	56667682	C	0.6053	12.02	0.0005274	7.05 (2.2-22.62)	*FAM208A*	G	0.605, 0.179	1
11	rs11033800	11	4790948	A	0.1111	11.78	0.0005977	0.13 (0.03-0.45)	*CPA5*	C	0.889, 0.500	0.345
12	rs17854248	7	130007388	A	0.1111	11.78	0.0005977	0.13 (0.03-0.45)	*MMP26 and OR51F1*	G	0.889, 0.500	0.345
13	rs4675801	2	242493511	G	0.6316	11.32	0.0007657	6.29 (2.06-19.22)	*BOK-AS1*	C	0.632, 0.214	0.98
14	rs7178698	15	94945719	C	0.02941	11.3	0.0007748	0.05 (0.01-0.46)	*MCTP2*	T	0.971, 0.643	0.321
15	rs978979	3	56533016	A	0.2632	11.29	0.0007776	0.17 (0.06-0.49)		G	0.737, 0.321	0.297
16	rs4657537	1	166268048	G	0.2632	11.29	0.0007776	0.17 (0.06-0.49)		A	0.737, 0.321	1
17	rs1263811	14	21993498	G	0.4118	10.95	0.0009359	17.5 (2.12-144.7)	*SALL2*	G	0.412, 0.038	1
18	rs55989856	22	39493294	G	0.02632	10.92	0.0009507	0.06 (0.01-0.48)	*APOBEC3H*	T	0.974, 0.679	1
19	rs7258236	19	6760974	G	0.1053	10.87	0.0009793	0.14 (0.04-0.49)	*SH2D3A*	T	0.895, 0.536	0.126

CHR: Chromosome; SNP ID: Single nucleotide polymorphism ID; BP: Base pair position at the respective chromosome as per GRCh37.p13; MA: Minor allele name; MAF: Frequency of minor allele in controls; ChisQ: Basic allelic test chi-square; P: *p*-value; OR: Odd ratio; SE: Standard error; L95: Lower bound of 95% confidence interval for odds ratio; U95: Upper bound of 95% confidence interval for odds ratio. AA: Associated Allele; CCF: Case, Control Frequencies; HWpval: *p*-value of Hardy-Weinberg equilibrium.

Table 3. Haplotypes of SNPs with the significance < 0.001 in Saudi female with low VO_{2max}

CHR	Block	Haplotype	Case, Control Frequencies	Chi Square	*P* Value	Haplotypes	Risk/Protective
1	Block 1	GAT	0.468, 0.052	13.512	2.00E-04	rs10800201G; rs4657537A; rs4657583T**	Risk
1		GAC	0.236, 0.226	0.009	0.9244	rs10800201G; rs4657537A; rs4657583C	
1		AGT	0.156, 0.271	1.302	0.2538	rs10800201A; rs4657537G; rs4657583T	
1		AGC	0.048, 0.284	7.097	0.0077	rs10800201A; rs4657537G; rs4657583C	Protective
1		GGT	0.056, 0.034	0.173	0.6777	rs10800201G; rs4657537G; rs4657583T	
1		GGC	0.004, 0.091	3.148	0.076	rs10800201G; rs4657537G; rs4657583C	
1		AAC	0.002, 0.043	1.425	0.2326	rs10800201A; rs4657537A; rs4657583C	
1		AAT	0.031, 0.001	0.831	0.3619	rs10800201A; rs4657537A; rs4657583T	

1	Block 2	CG	0.866, 0.497	10.664	0.0011	rs859667C; rs10218696G	Risk
1		TA	0.024, 0.247	7.654	0.0057	rs859667T; rs10218696A	Protective
1		TG	0.055, 0.146	1.577	0.2092	rs859667T; rs10218696G	
1		CA	0.055, 0.110	0.683	0.4085	rs859667C; rs10218696A	
3	Block 1	GATG	0.605, 0.179	12.016	5.00E-04	rs978979G; rs7637449A; rs111934125T; rs9835332G**	Risk
3		AGCC	0.105, 0.449	10.144	0.0014	rs978979A; rs7637449G; rs111934125C; rs9835332C	Protective
3		AGTC	0.158, 0.229	0.538	0.4634	rs978979A; rs7637449G; rs111934125T; rs9835332C	
3		GGTC	0.079, 0.056	0.126	0.7222	rs978979G; rs7637449G; rs111934125T; rs9835332C	
3		GGCC	0.053, 0.086	0.294	0.5874	rs978979G; rs7637449G; rs111934125C; rs9835332C	
6	Block 1	AC	0.447, 0.821	9.448	0.0021	rs1936797A; rs9491696C	Protective
6		GG	0.447, 0.107	8.836	0.003	rs1936797G; rs9491696G	Risk
6		AG	0.105, 0.071	0.223	0.6365	rs1936797A; rs9491696G	
7	Block 1	TGTC	0.814, 0.428	10.542	0.0012	rs11761888T; rs17854248G; rs17164867T; rs968404C	Risk
7		CTCT	0.131, 0.428	7.432	0.0064	rs11761888C; rs17854248T; rs17164867C; rs968404T	Protective
7		CGTC	0.054, 0.036	0.127	0.7217	rs11761888C; rs17854248G; rs17164867T; rs968404C	
7		CTCC	0.000, 0.036	1.371	0.2416	rs11761888C; rs17854248T; rs17164867C; rs968404C	
7		TGTT	0.000, 0.036	1.371	0.2416	rs11761888T; rs17854248G; rs17164867T; rs968404T	
7		CTTC	0.000, 0.036	1.378	0.2404	rs11761888C; rs17854248T; rs17164867T; rs968404C	
8	Block 1	GT	0.947, 0.679	8.386	0.0038	rs13277113G; rs2618476T	Risk
8		AC	0.053, 0.321	8.386	0.0038	rs13277113A; rs2618476C	Protective
11	Block 1	CGCA	0.868, 0.500	10.674	0.0011	rs1030726C; rs1030723G; rs11033800C; rs11033801A	Risk
11		AGAG	0.105, 0.214	1.49	0.2221	rs1030726A; rs1030723G; rs11033800A; rs11033801G	
11		AAAG	0.026, 0.286	9.211	0.0024	rs1030726A; rs1030723A; rs11033800A; rs11033801G	Protective
14	Block 1	TCGTT	0.737, 0.357	9.515	0.002	rs12891553T; rs34693535C; rs17211285G; rs17277221T; rs17277228T	Risk
14		CTTCC	0.263, 0.643	9.515	0.002	rs12891553C; rs34693535T; rs17211285T; rs17277221C; rs17277228C	Protective
16	Block 1	TG	0.842, 0.500	8.933	0.0028	rs1134760T; rs5923G	Risk
16		CA	0.079, 0.357	7.888	0.005	rs1134760C; rs5923A	Protective
16		CG	0.079, 0.143	0.694	0.4046	rs1134760C; rs5923G	
17	Block 1	CC	0.763, 0.295	14.384	1.00E-04	rs13696C; rs1901187C**	Risk
17		CT	0.184, 0.241	0.314	0.575	rs13696C; rs1901187T	
17		TT	0.000, 0.331	14.608	1.00E-04	rs13696T; rs1901187T*	Protective
17		TC	0.053, 0.134	1.335	0.2479	rs13696T; rs1901187C	
19	Block 1	TA	0.342, 0.750	10.739	0.001	rs4801433T; rs4801200A	Protective
19		CT	0.658, 0.250	10.739	0.001	rs4801433C; rs4801200T	Risk

CHR: Chromosome number; **Risk haplotypes ($P < 1.0 \times 10^{-4}$); *Protective haplotypes ($P < 1.0 \times 10^{-4}$).

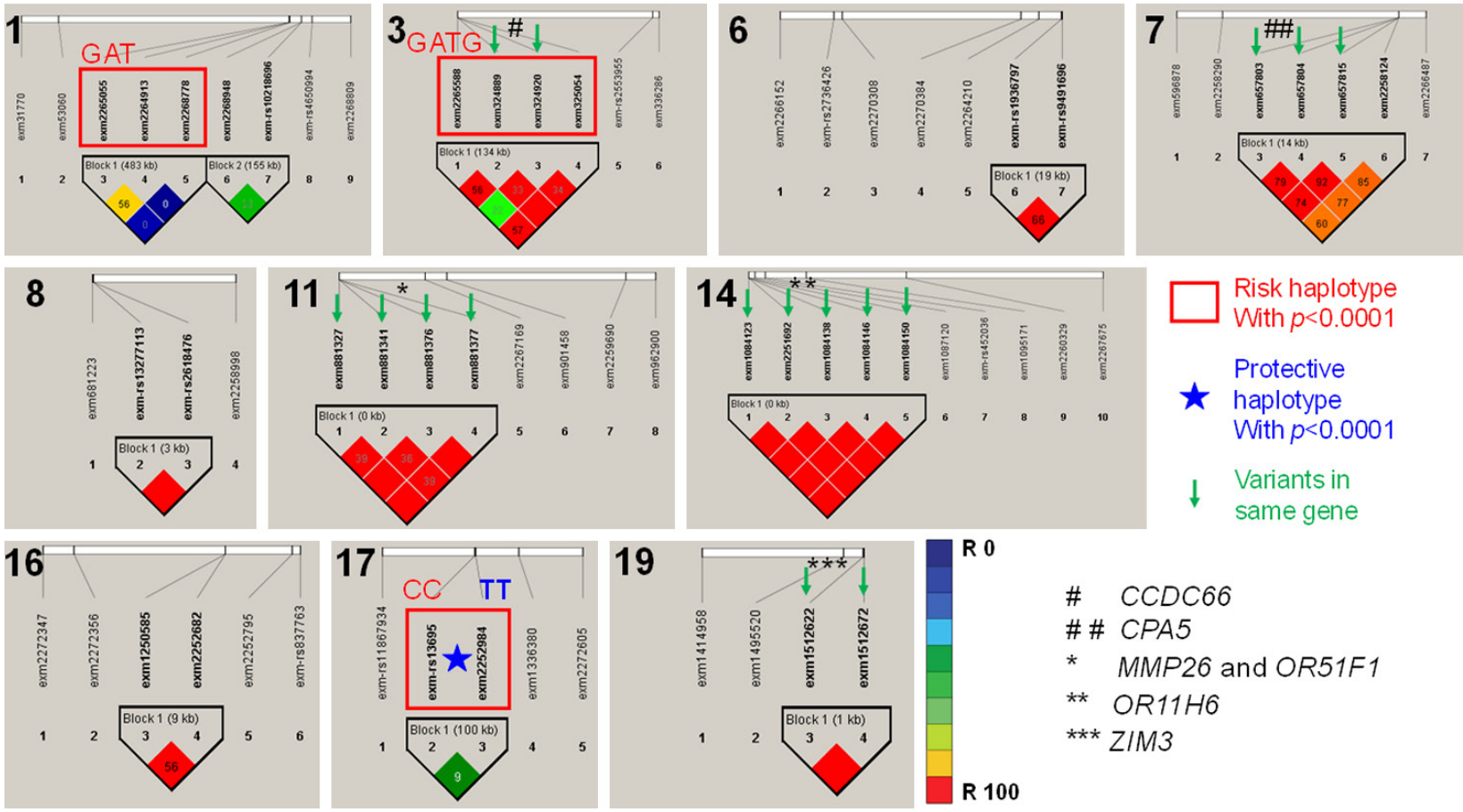

Figure 2. Genetic association and linkage disequilibrium of the significant variants at selected chromosomes in Arab-ancestry females with low VO_{2max} against high VO_{2max}. The numbers at the left top corner of each sector indicates chromosome number. The SNPs indicated with green arrow in a block denotes variants in same gene in the respective chromosome. * and #: Gene names; Red square box: Indicates the most significant risk haplotypes of significant variants; Star indicates the most significant protective haplotype associated with the Arab-ancestry females with low VO_{2max}, the details of the haplotypes and alleles are shown in **Table 3**.

66, FAM208A, STX2, TNS4, MMP26, OR51F1, OR11H6) identified with the most significant SNPs, risk and protective haplotypes and multi SNPs can be considered for the detailed study on the impact of lowering VO_{2max} in young females. Limitation of the study: Number samples are relatively low. None of SNPs were satisfied the Bonferroni corrections (corrected $\alpha = 0.05/243345 = 2.05 \times 10^{-07}$) among the 24,3345 variants. The expression of *TOP-2A* has been shown to be significant response marker to therapy and prognosis in the patients of breast cancer [37], however a study reported insignificant association with breast cancer risk or clinical outcome [38]. A recent study has described the significant association of C/C genotype in rs13695 for lowering the risk of neutropenia in small cell lung cancer patients during chemotherapy [39], the SNP rs13695 has been reported in breast cancer patients [40], hence detailed studies on female with rs13695C are needed to confirm the possibilities to correlate the breast cancer and low VO_{2max}.

Conclusion

The low VO_{2max} in young Saudi females are associated with genetic variants that are significantly involved in the heart rate, body weight, breath tests, proteolysis and cardiac muscle fiber development. Furthermore, the identified genetic predispositions and gene-annotation enrichment in low VO_{2max} in young Saudi females revealed that they are at high risk for developing cardiovascular complications.

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Disclosure of conflict of interest

None.

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Supplementary Table 1. List of the significant SNPs (P < 0.005) that are associated in female with low VO_2 max

S. No	CHR	SNP	BP	A1	F_A	F_U	A2	CHISQ	P	OR	SE	L95	U95
1	5	exm499426	156770133	A	0.6053	0.1071	G	16.75	4.26E-05	12.78	0.6953	3.27	49.92
2	12	exm980015	7188562	A	0.1053	0.5714	C	16.59	4.65E-05	0.08824	0.6521	0.02458	0.3168
3	17	exm-rs13695	38545193	A	0.05263	0.4643	G	15.56	8.01E-05	0.0641	0.8194	0.01287	0.3194
4	5	exm2265965	79521704	G	0.2105	0.6786	A	14.61	0.0001323	0.1263	0.5675	0.04153	0.3842
5	5	exm2256903	126993249	C	0.6842	0.2143	G	14.25	0.0001598	7.944	0.5779	2.56	24.66
6	21	exm1582123	47851636	A	0.02778	0.3929	G	13.78	0.0002056	0.04416	1.085	0.00526	0.3707
7	6	exm2266152	8869571	A	0.6316	0.1786	G	13.43	0.0002475	7.886	0.5971	2.447	25.42
8	7	exm2266487	145813854	A	0.1944	0.6429	G	13.3	0.0002648	0.1341	0.577	0.04328	0.4155
9	4	exm2265900	188170467	A	0.2632	0.7143	G	13.23	0.0002751	0.1429	0.5574	0.04791	0.426
10	3	exm325054	56667682	C	0.6053	0.1786	G	12.02	0.0005274	7.053	0.5947	2.199	22.62
11	7	exm657804	130007388	A	0.1111	0.5	C	11.78	0.0005977	0.125	0.6512	0.03488	0.448
12	11	exm881376	4790948	A	0.1111	0.5	C	11.78	0.0005977	0.125	0.6512	0.03488	0.448
13	2	exm2255224	242493511	G	0.6316	0.2143	A	11.32	0.0007657	6.286	0.5703	2.056	19.22
14	15	exm1191910	94945719	C	0.02941	0.3571	A	11.3	0.0007748	0.05455	1.089	0.006454	0.461
15	1	exm2264913	166268048	G	0.2632	0.6786	A	11.29	0.0007776	0.1692	0.5472	0.05788	0.4945
16	3	exm2265588	56533016	A	0.2632	0.6786	G	11.29	0.0007776	0.1692	0.5472	0.05788	0.4945
17	14	exm1087120	21993498	G	0.4118	0.03846	A	10.95	0.0009359	17.5	1.078	2.117	144.7
18	22	exm2273063	39493294	G	0.02632	0.3214	A	10.92	0.0009507	0.05706	1.091	0.006721	0.4844
19	19	exm1414958	6760974	G	0.1053	0.4643	A	10.87	0.0009793	0.1357	0.6504	0.03794	0.4857
20	6	exm-rs2736426	31745284	G	0.2105	0.6071	A	10.78	0.001028	0.1725	0.555	0.05814	0.5121
21	19	exm1512622	57646570	G	0.6579	0.25	A	10.74	0.001049	5.769	0.5544	1.946	17.1
22	19	exm1512672	57648277	T	0.6579	0.25	A	10.74	0.001049	5.769	0.5544	1.946	17.1
23	3	exm324889	56628031	A	0.5833	0.1786	G	10.7	0.001073	6.44	0.5981	1.994	20.8
24	6	exm2270384	110239704	A	0.5833	0.1786	G	10.7	0.001073	6.44	0.5981	1.994	20.8
25	2	exm2269094	81583557	G	0.5789	0.1786	A	10.69	0.001077	6.325	0.5928	1.979	20.21
26	11	exm881327	4790268	A	0.1316	0.5	C	10.67	0.001087	0.1515	0.6109	0.04576	0.5017
27	11	exm881377	4790951	G	0.1316	0.5	A	10.67	0.001087	0.1515	0.6109	0.04576	0.5017
28	11	exm2259690	108429200	A	0.1316	0.5	C	10.67	0.001087	0.1515	0.6109	0.04576	0.5017
29	17	exm2252984	38646147	A	0.1842	0.5714	G	10.65	0.001103	0.1694	0.5665	0.05579	0.5141
30	3	exm324920	56650054	G	0.1579	0.5357	A	10.61	0.001126	0.1625	0.5844	0.05169	0.5108
31	17	exm2272605	63428940	C	0.08333	0.4286	A	10.46	0.001219	0.1212	0.7138	0.02992	0.491
32	15	exm1153874	42643529	G	0.1053	0.4615	A	10.45	0.001226	0.1373	0.6589	0.03773	0.4993
33	4	exm387823	7780582	A	0.3684	0.03571	G	10.16	0.001434	15.75	1.072	1.925	128.9
34	6	exm2270308	35024695	G	0.4211	0.07143	A	9.935	0.001622	9.455	0.804	1.956	45.71
35	16	exm2272347	16959637	A	0.2895	0.6786	C	9.844	0.001704	0.193	0.5401	0.06696	0.5562

36	16	exm2252795	86817240	A	0.2895	0.6786	C	9.844	0.001704	0.193	0.5401	0.06696	0.5562
37	7	exm657803	130007381	G	0.1667	0.5357	A	9.73	0.001813	0.1733	0.5862	0.05495	0.5468
38	3	exm336348	108754238	A	0.2895	0	T	9.726	0.001816	NA	NA	NA	NA
39	5	exm2261790	101019725	G	0.2895	0	A	9.726	0.001816	NA	NA	NA	NA
40	20	exm1543768	43547677	C	0.2895	0	A	9.726	0.001816	NA	NA	NA	NA
41	5	exm-rs11249661	179277874	A	0.1944	0.5714	G	9.722	0.00182	0.181	0.5685	0.05941	0.5516
42	1	exm-rs4650994	178515312	A	0.6389	0.25	G	9.565	0.001983	5.308	0.5576	1.78	15.83
43	1	exm2268809	198634625	G	0.2632	0.6429	A	9.515	0.002038	0.1984	0.5397	0.06889	0.5714
44	2	exm2261144	197404714	G	0.2632	0.6429	A	9.515	0.002038	0.1984	0.5397	0.06889	0.5714
45	14	exm1084123	20692188	G	0.2632	0.6429	A	9.515	0.002038	0.1984	0.5397	0.06889	0.5714
46	14	exm2251692	20692291	A	0.2632	0.6429	G	9.515	0.002038	0.1984	0.5397	0.06889	0.5714
47	14	exm1084138	20692453	A	0.2632	0.6429	C	9.515	0.002038	0.1984	0.5397	0.06889	0.5714
48	14	exm1084146	20692574	G	0.2632	0.6429	A	9.515	0.002038	0.1984	0.5397	0.06889	0.5714
49	14	exm1084150	20692643	G	0.2632	0.6429	A	9.515	0.002038	0.1984	0.5397	0.06889	0.5714
50	1	exm2268948	172668340	A	0.07895	0.3929	G	9.505	0.002049	0.1325	0.7153	0.0326	0.5382
51	5	exm474346	125802027	A	0.07895	0.3929	G	9.505	0.002049	0.1325	0.7153	0.0326	0.5382
52	22	exm1599671	30857373	A	0.07895	0.3929	C	9.505	0.002049	0.1325	0.7153	0.0326	0.5382
53	6	exm-rs9491696	127452639	G	0.5526	0.1786	C	9.448	0.002114	5.682	0.5915	1.782	18.12
54	14	exm2260329	49904851	A	0.6316	0.25	G	9.424	0.002142	5.143	0.551	1.747	15.14
55	4	exm2269729	23123307	A	0.2368	0.6071	G	9.259	0.002343	0.2008	0.5434	0.06922	0.5826
56	11	exm962900	119216504	A	0.2368	0.6071	G	9.259	0.002343	0.2008	0.5434	0.06922	0.5826
57	11	exm881341	4790471	A	0.02632	0.2857	G	9.211	0.002406	0.06757	1.096	0.00788	0.5794
58	11	exm901458	43876698	A	0.1053	0.4286	G	9.175	0.002453	0.1569	0.6521	0.0437	0.5631
59	1	exm31770	24394811	G	0.2105	0.5714	A	9.074	0.002593	0.2	0.5515	0.06785	0.5895
60	22	exm1593472	24224655	A	0.2105	0.5714	G	9.074	0.002593	0.2	0.5515	0.06785	0.5895
61	17	exm1336380	48603503	G	0.5	0.1429	A	9.057	0.002617	6	0.63	1.745	20.63
62	7	exm657815	130008285	G	0.1316	0.4643	A	8.997	0.002704	0.1748	0.6115	0.05274	0.5795
63	7	exm2258124	130022041	A	0.1316	0.4643	G	8.997	0.002704	0.1748	0.6115	0.05274	0.5795
64	15	exm1183089	82555242	G	0.1316	0.4643	A	8.997	0.002704	0.1748	0.6115	0.05274	0.5795
65	1	exm53060	44290530	G	0.1842	0.5357	A	8.963	0.002754	0.1957	0.5645	0.06472	0.5917
66	5	exm467585	94826655	A	0.1842	0.5357	C	8.963	0.002754	0.1957	0.5645	0.06472	0.5917
67	12	exm-rs10444502	118353871	C	0.1842	0.5357	A	8.963	0.002754	0.1957	0.5645	0.06472	0.5917
68	17	exm-rs11867934	16933404	A	0.1842	0.5357	G	8.963	0.002754	0.1957	0.5645	0.06472	0.5917
69	16	exm1250585	67964203	G	0.1579	0.5	A	8.933	0.0028	0.1875	0.5838	0.05972	0.5887
70	18	exm2253586	9478794	A	0.6579	0.2857	G	8.932	0.002802	4.808	0.5403	1.667	13.86
71	20	exm1529529	23424613	G	0.3421	0.7143	A	8.932	0.002802	0.208	0.5403	0.07214	0.5997
72	1	exm2265055	166169203	A	0.2353	0.625	G	8.929	0.002807	0.1846	0.5842	0.05875	0.5801

73	2	exm2263899	85299783	C	0.5	0.1429	A	8.905	0.002843	6	0.6346	1.73	20.81
74	6	exm-rs1936797	127432657	G	0.4474	0.1071	A	8.836	0.002954	6.746	0.6927	1.736	26.22
75	12	exm2267381	92117210	G	0.4474	0.1071	A	8.836	0.002954	6.746	0.6927	1.736	26.22
76	3	exm336286	108690221	G	0.5789	0.2143	A	8.777	0.003051	5.042	0.5658	1.663	15.28
77	16	exm-rs837763	88853729	G	0.5789	0.2143	A	8.777	0.003051	5.042	0.5658	1.663	15.28
78	12	exm988079	18649057	A	0.1316	0.4615	G	8.616	0.003332	0.1768	0.6205	0.05238	0.5965
79	17	exm1347023	64216815	A	0.2778	0	G	8.611	0.003341	NA	NA	NA	NA
80	7	exm596878	299881	A	0.3158	0.6786	G	8.518	0.003516	0.2186	0.5344	0.07671	0.6231
81	14	exm2267675	86378529	A	0.3158	0.6786	G	8.518	0.003516	0.2186	0.5344	0.07671	0.6231
82	15	exm-rs7176508	70018990	G	0.3158	0.6786	A	8.518	0.003516	0.2186	0.5344	0.07671	0.6231
83	8	exm-rs13277113	11349186	A	0.05263	0.3214	G	8.386	0.00378	0.1173	0.8316	0.02298	0.5985
84	8	exm-rs2618476	11352541	G	0.05263	0.3214	A	8.386	0.00378	0.1173	0.8316	0.02298	0.5985
85	9	exm2264360	8875954	A	0.05263	0.3214	C	8.386	0.00378	0.1173	0.8316	0.02298	0.5985
86	12	exm997329	48142636	A	0.05263	0.3214	G	8.386	0.00378	0.1173	0.8316	0.02298	0.5985
87	8	exm681223	10466482	A	0.6111	0.25	G	8.288	0.003991	4.714	0.5544	1.59	13.97
88	2	exm2261106	16889372	A	0.5263	0.1786	C	8.285	0.003998	5.111	0.5908	1.606	16.27
89	6	exm2264210	117724462	C	0.6053	0.25	A	8.207	0.004174	4.6	0.5483	1.571	13.47
90	10	exm-rs11593576	81015896	A	0.6053	0.25	G	8.207	0.004174	4.6	0.5483	1.571	13.47
91	11	exm2267169	36146156	A	0.6053	0.25	G	8.207	0.004174	4.6	0.5483	1.571	13.47
92	12	exm2267405	131225471	A	0.6053	0.25	G	8.207	0.004174	4.6	0.5483	1.571	13.47
93	1	exm2268778	166652275	G	0.2895	0.6429	A	8.173	0.004252	0.2263	0.5324	0.07971	0.6427
94	14	exm1095171	31355096	G	0.2895	0.6429	C	8.173	0.004252	0.2263	0.5324	0.07971	0.6427
95	3	exm-rs2553955	107050997	G	0.3824	0.07143	A	8.094	0.004442	8.048	0.8143	1.631	39.7
96	8	exm2258998	88032231	C	0.6471	0.2857	A	8.028	0.004606	4.583	0.5512	1.556	13.5
97	12	exm-rs12424086	66364509	G	0.3158	0.03571	A	7.995	0.004691	12.46	1.076	1.511	102.8
98	22	exm1595697	26157068	G	0.4737	0.1429	C	7.94	0.004836	5.4	0.6303	1.57	18.57
99	7	exm2258290	27363392	G	0.2632	0.6071	A	7.891	0.004968	0.2311	0.5343	0.0811	0.6585
100	12	exm2267380	91277503	A	0.2632	0.6071	G	7.891	0.004968	0.2311	0.5343	0.0811	0.6585
101	14	exm-rs452036	23865885	A	0.2632	0.6071	G	7.891	0.004968	0.2311	0.5343	0.0811	0.6585
102	16	exm2272356	25753251	G	0.2632	0.6071	A	7.891	0.004968	0.2311	0.5343	0.0811	0.6585
103	20	exm-rs6051520	351944	C	0.2632	0.6071	A	7.891	0.004968	0.2311	0.5343	0.0811	0.6585
104	1	exm-rs10218696	172823534	A	0.07895	0.3571	G	7.888	0.004976	0.1543	0.7193	0.03767	0.6319
105	16	exm2252682	67973953	A	0.07895	0.3571	G	7.888	0.004976	0.1543	0.7193	0.03767	0.6319
106	19	exm1495520	51323232	G	0.07895	0.3571	A	7.888	0.004976	0.1543	0.7193	0.03767	0.6319

CHR: Chromosome; SNP: Illumina single nucleotide ID; BP: Base pair position at the respective chromosome as per GRCh37.p13; A1: Minor allele name; F_A: Frequency of minor allele in cases; F_U: Frequency of minor allele in controls; A2: Major allele name; CHISQ: Basic allelic test chi-square; P: *p*-value; OR: Odd ratio; SE: Standard error; L95: Lower bound of 95% confidence interval for odds ratio; U95: Upper bound of 95% confidence interval for odds ratio.