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ORIGINAL ARTICLE

Development of a genetic risk score for obesity predisposition evaluation

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Abstract

Obesity is a major public health issue resulting from an interaction between genetic and environmental factors. Genetic risk scores (GRSs) are useful to summarize the effects of many genetic variants on obesity risk. In this study, we aimed to assess the association of previously well-studied genetic variants with obesity and develop a genetic risk score to anticipate the risk of obesity development in the Iranian population. Among 968 participants, 599 (61.88%) were obese, and 369 (38.12%) were considered control samples. After genotyping, an initial screening of 16 variants associated with body mass index (BMI) was performed utilizing a general linear model ($p < 0.25$), and seven genetic variants were selected. The association of these variants with obesity was examined using a multivariate logistic regression model ($p < 0.05$), and finally, five variants were found to be significantly associated with obesity. Two gene score models (weighted and unweighted), including these five loci, were constructed. To compare the discriminative power of the models, the area under the curve was calculated using tenfold internal cross-validation. Among the studied variants, *ADRB3 rs4994, FTO rs9939609*, *ADRB2 rs1042714, IL6 rs1800795,* and *MTHFR rs1801133* polymorphisms were significantly associated with obesity in the Iranian population. Both of the constructed models were significantly associated with BMI ($p < 0.05$) and the area under the mean curve of the weighted GRS and unweighted GRS were 70.22% ± 0.05 and 70.19% ± 0.05, respectively. Both GRSs proved to predict obesity and could potentially be utilized as genetic tools to assess the obesity predisposition in the Iranian population. Also, among the studied variants, *ADRB3 rs4994 and FTO rs9939609* polymorphisms have the highest impacts on the risk of obesity.

Keywords *FTO* · *ADRB3* · BMI · Obesity · GRS

Introduction

Obesity is a complex health problem correlated with an increased risk of hypertension, type 2 diabetes, cardiovascular diseases, cancer, and poor quality of life (Mokdad Ford et al. 2003). In 2014, more than 40 million children and over 600 million adults were classified as obese worldwide. According to the World Health Organization (WHO), the number of obese adults reached 650 million in 2016 (Ren, Xu et al. 2019). It is estimated that over 30% of adults in the US are obese and, in most countries, the prevalence rate of this abnormality has increased over the last decades (Flegal Carroll et al. 2002; Wang, McPherson et al. 2011). Besides, studies have shown that more than 80% of Iranian adults are obese or overweight (Rahmani, Sayehmiri et al. 2015). Various environmental factors play important roles in developing obesity, such as overeating and lack of adequate physical activity (Carlos, Silva-Nunes et al. 2013). In addition to these factors, genetic risk factors could also contribute to

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the increase in the prevalence of obese adults. Genome-wide association studies (GWASs) have successfully identified the impact of various single nucleotide polymorphisms (SNPs) on obesity and being overweight (Rankinen, Zuberi et al. 2006; Lu and Loos 2013; Locke, Kahali et al. 2015). Among the investigated genetic variants, a common variant at the fat mass and obesity-associated (FTO) locus has powerful effect on body fat and mass (Loos and Yeo 2014). Also, several well-defined SNPs are contributing to obesity and metabolic syndrome, especially in Middle Eastern countries. For example, some studies have shown that methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms are related to obesity (Fu, Li et al. 2019), also polymorphisms in the vitamin D receptor (VDR) gene are involved in the pathogenesis of inflammation and obesity (Al-Daghri, Guerini et al. 2014). Moreover, interleukin 6 (IL6) variants on abdominal obesity have been largely studied (Berthier, Paradis et al. 2003). In addition, SNPs of antioxidant enzymes including glutathione peroxidase (GPX1), catalase (CAT), and superoxide dismutase 2 (SOD2) influence the endogenous free radicals' clearance, which plays an important role in excess weight gain (Saravani, Miri et al. 2015; Hernández-Guerrero, Parra-Carriedo et al. 2018). Fatty acid-binding protein (FABP) is another critical gene that its polymorphisms cause an increased triglyceride transport in intestinal cells, which may result in metabolic disorders (Albala, Santos et al. 2004). The polymorphisms of β2 and β3 adrenergic receptors (ADRB2 and ADRB3, respectively) regulate the energy balance and are associated with obesity (Saliba, Reis et al. 2014). Many researchers have confirmed an association between transcription factor 7-like 2 (TCF7L2) variants and higher BMI and type 2 diabetes (T2D) risk (Cauchi, Choquet et al. 2008). The other potential risk locus is on the angiotensin-converting enzyme gene (ACE) and is linked to adiposity and metabolic outcomes (Bordoni, Marchegiani et al. 2017). Other potential candidates include a polymorphism in apolipoprotein C3 (APOC3) that has been associated with both dyslipidemia and insulin resistance (Miller, Rhyne et al. 2007), tumor necrosis factor-alpha (TNF- α) gene polymorphism which is associated with insulin resistance and obesity (Ghareeb, Abdelazem et al. 2021), the variant in caffeine metabolizing enzyme cytochrome P450 1A2 (CYP1A2) (Urry, Jetter et al. 2016) and finally, a polymorphism at the promoter region of the lactase gene (LCT) related to obesity and other anthropometric measurements (Corella, Arregui et al. 2011).

Since each individual allele could only have a limited effect on the risk of obesity, the impact of each variant should be considered in combination with other risk alleles. Thus, information from multiple risk alleles and a carefully constructed genetic risk score (GRS) is vital for obesity risk assessment. These translational genomic tools may be useful in tackling this public health issue (Belsky, Moffitt et al. 2013). Several studies in various ethnicities have suggested new genetic risk tools to predict obesity (Seral-Cortes, Sabroso-Lasa et al. 2021). However, a limited number of studies investigating the effect of these polymorphisms and providing an accurate GRS for Middle Eastern adults. The two main goals of this study were (1) to assess the association of sixteen genetic variants with obesity in the Iranian population and (2) to develop and implement a novel genetic model for this population to anticipate the risk of obesity development, more precisely.

Materials and methods

Subjects

This study was conducted on 968 Iranian subjects (male: 306 (31.6%), female: 662 (68.4%), aged 18–70) residents in Tehran. The proposed cut-off points of body mass index (BMI) for obesity focus on the factors which increase the risk of disease development, especially cardiovascular diseases. According to the WHO classification, a BMI of 25 kg/m^2 is defined as a cut-off point for overweight, and a BMI of 30 kg/m^2 is the cut-off point for obesity (Status 1995). However, Ali Babai et al. defined a new BMI cut-off for Iranians and indicated that Iranian men and women have a higher risk of developing metabolic syndromes with BMI of 25.7 and 27.05 kg/m^2, respectively (Babai, Arasteh et al. 2016). Among 306 men, 239 persons (BMI > 25.7 kg/m^2, 78.1%) were considered as obese cases and the remaining were considered as controls (BMI < = 25.7 kg/m^2, 21.9%). Among 662 women, 360 participants were categorized as obese (BMI > 27.05 kg/m^2, 54.4%), and the remaining as controls (BMI < = 27.05 kg/m^2, 45.6%). All the procedures and the aims of the study were explained to the participants, and just those who signed the informed consent form were enrolled in the study. The ethics committee of Kawsar Human Genetic Research Center (2019) approved this research. All anthropometric parameters were measured by a trained person using standard techniques. Bodyweight and height measurements were done with light clothing and without shoes by Jawon BC 510 bio-electrical impedance analyzer (Jawon, South Korea). Waist circumference was measured at the point of waist narrowing. Hip circumference was determined as the maximum circumference around the hips. BMI was calculated by dividing weight in kilograms by height in meters squared. Moreover, the waist-to-height ratio and waist-to-hip ratio were calculated.

DNA isolation and genotyping

Participants' saliva samples were collected using saliva collection kits (4N6 FLOQSwabs® genetics, Copan Diagnostics Inc.). Genomic DNA from the saliva samples was isolated using the salting-out method. We selected sixteen polymorphisms previously indicated to be associated with obesity, body weight regulation, or lipid metabolism, confirmed in the Middle Eastern population. These variants were within FTO, MTHFR, VDR, IL6, GPX1, FABP2, ADRB2, TCF7L2, ACE, APOC3, SOD2, LCT, TNF, CAT, CYP1A2, and ADRB3 genes. Detailed information about these polymorphisms is reported in Table 2. Genotyping for all the variants was conducted using the ARMS-PCR method. For each polymorphism, one common primer and two allele-specific primers were designed. Each PCR reaction contained 100 ng DNA, 15 µl KBC Alpha PCR Mix (Kawsar Biotech Co., KBC, Tehran, Iran), 1 U KBC Taq DNA polymerase, and 5 pM of each forward and reverse primers. PCR parameters were as follows: an initial denaturation step for 7 min at 95 °C, 1 min at 95 °C, 1 min at 62 °C, 1 min at 72 °C, and final extension for 15 min at 72 °C for 30 cycles.

Statistical analysis

The deviation from Hardy–Weinberg equilibrium (HWE) was assessed using the X^2 test. The test for linkage disequilibrium was performed using the ETDT program. Due to the difference in age and sex in case and control groups, the anthropometric characteristics of the studied individuals were analyzed by ANCOVA adjusted for sex and age. According to previous studies, genotypes of each variant were coded 0, 1, and 2 based on the number of risk alleles. For the first step, a generalized linear model (GLM) was used to establish a primary cut-off point ($p < 0.25$) as an initial filtration to refine the search to seven SNPs. This step was performed to exclude a set of SNPs that did not show a promising behavior in predicting BMI. Then for those seven SNPs that passed the initial filtration, a step-by-step logistic regression algorithm was used to select significant SNPs under the standard *P* value threshold ($p < 0.05$) and integrate them into a multivariate model. The goodness of fit of this model was analyzed by a calibration curve. The independent variables were age, sex, and the selected polymorphisms in both steps, and the dependent variables in the first and second steps were BMI and obesity status, respectively. The unweighted GRS was calculated by summing the number of risk alleles from the five significant SNPs. Also, to construct the weighted GRS, the odds ratio of each significant SNP was used as a measure of their effect sizes. The effect sizes were multiplied by the number of risk alleles at each locus and then the sum of these scores assigned to participants. As logistic regression was used at the final step of building the models, the output ranges of both models were between 0 and 1. A flow chart of this process is illustrated in Fig. 1. To estimate and compare the discriminative power of the constructed GRSs, the area under the receiver operating characteristic (ROC) curves was calculated (Dorfman and Alf Jr 1969). A tenfold cross-validation analysis was performed to validate the models internally. To perform this analysis, our dataset was divided into 10 groups, utilizing nine of them to create and train the models and the other one group to validate the models.

The effect sizes were calculated at each fold using the training data points for the weighted GRS model. Pearson's correlation coefficient was calculated to evaluate the correlation between unweighted and weighted GRS models. Lastly, the associations between BMI and both unweighted and weighted GRSs were analyzed using a simple linear regression model to evaluate the reliability of these models. All statistical analyses were performed using SPSS software (V.28.0). P values less than 0.05 were considered statistically

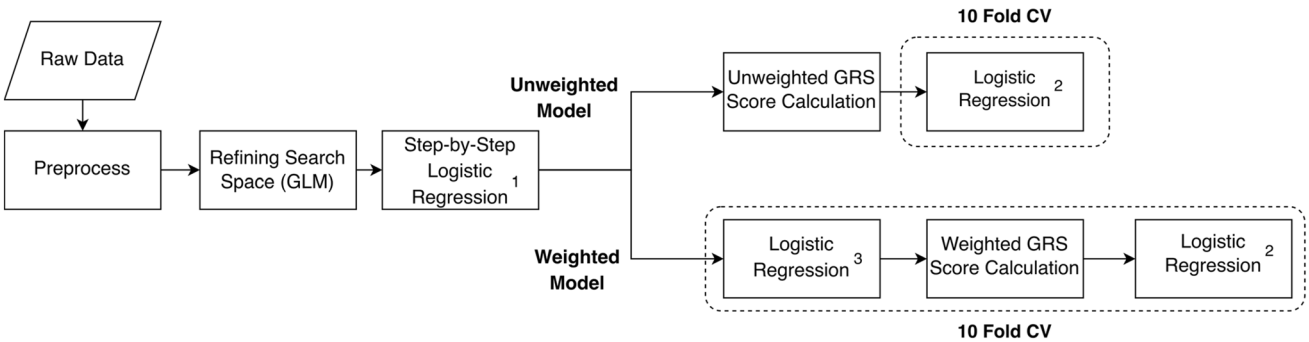

Fig. 1 Analysis and model construction workflow. *CV* cross validation. (1) A step-by-step logistic regression was used to select significant SNPs ($p < 0.05$). At this step, obesity status was the dependent variable. (2) Final logistic regression model using the genetic risk scores. This model was utilized instead of threshold finding for the risk scores. (3) This logistic regression model was used to determine the effect size of each SNP which was used in the construction of the weighted model. At each step of the cross-validation, the effect sizes were calculated solely based on the training dataset

significant. Some plots were drawn using Python (V.3.9.5) with scikit-learn (V.1.0.2) and matplotlib (V.3.4.2) libraries.

Results

The characteristics of obese and control participants are presented in Table 1. According to these results, the differences between the age and sex of obese and control subjects were statistically significant. The obese group had significantly higher BMI, height, weight, waist, and hip circumference values. Initially, 16 SNPs previously indicated to be associated with obesity were selected. Genotype frequencies, HWE P values, and minor allele frequencies (MAF) of the genetic variants analyzed in this study are summarized in Table 2. The genotype frequencies of all the studied polymorphisms except *FABP2 rs1799883 and LCT rs4988235* polymorphisms were in HWE.

Moreover, there was no linkage disequilibrium between the studied SNPs (data not shown). Seven SNPs were determined to be potentially associated with BMI under the $p < 0.25$ cut-off among these genetic variants. The results of the generalized liner model are shown in Table 3. Among the seven variants, *FTO rs9939609*, *IL6 rs1800795*, *ADRB3 rs4994*, *ADRB2 rs1042714*, and *MTHFR rs1801133* polymorphisms were found to be statistically associated with the risk of obesity ($p < 0.05$). The multivariate model´s odds ratios (OR) and confidence intervals (CI) for these five polymorphisms are shown in Table 3. A forest plot of overall effect sizes is displayed in Fig. 2. The effect sizes that calculated at each fold are displayed in Supplementary Table 1.

Two GRSs (weighted and unweighted) including these five SNPs were developed. Both of the constructed models were significantly associated with BMI ($p < 0.05$). The area under the mean ROC curve in the weighted GRS was a little more than the unweighted GRS (Fig. 3). A more detailed analysis of the corresponding AUC, sensitivity, specificity, accuracy, and negative predictive value is shown in Table 4. The scatter plot of unweighted and weighted GRS models is shown in Supplementary Fig. 1. The Pearson's correlation coefficient for these models was 0.9938 with 95% CI of (0.9930, 0.9946).

Table 1 Anthropometric characteristics of studied individuals

Variables	Control ($n=369$, 38.1%)		Obese ($n=599$, 61.9%)		*P* value[b]
Characteristics	Mean	SD	Mean	SD	
Age (years)	37	9.5	39.2	9.7	< 0.001[a]
Gender	*n*	%	*n*	%	
Male	67	21.9	239	78.1	< 0.001[c]
Female	302	45.6	360	54.3	
Height (cm)	166.6	7.9	168.3	9.7	0.012
Weight (kg)	65.6	8.3	90	15.6	< 0.001
Waist circumference (cm)	81.9	9.3	100.6	13.4	< 0.001
Hip circumference (cm)	99.9	9.1	111.9	11.2	< 0.001
Waist to hip ratio	0.82	0.07	0.9	0.12	< 0.001
Waist to height ratio	0.49	0.05	0.59	0.07	< 0.001
BMI (kg/m^2)	23.6	2.4	31.7	4.3	< 0.001

BMI body mass index, *SD* standard deviation

[a]Calculated using *t* test

[b]Adjusted for age and sex in ANCOVA analysis

[c]Calculated by X^2 test

Discussion

This is the first study to investigate the cumulative effect of obesity-associated polymorphisms in a divergent Iranian population. Two GRSs (weighted and unweighted) consisting of five SNPs were developed. Previous studies published on the obesity-specific GRS mostly focused on the European population. In a recent cohort of 1069 European adolescents, Seral Cortes et al. constructed 2 weighted and unweighted GRSs using 21 BMI-related SNPs, which both were significantly associated with BMI ($p < 0.001$) (Seral-Cortes, Sabroso-Lasa et al. 2021). Belsky et al. developed a 32-locus GRS which was a predictor of obesity among white Americans (AUC = 0.57) (Belsky, Moffitt et al. 2013). For European ancestry, Song et al. constructed a GRS which was associated with BMI, and the associations were stronger in women (Song, Zheng et al. 2018). Besides, other GRS development studies in adults, adolescents, and preadolescents have been conducted in different European, Chinese, white American, black American, and African populations (Domingue, Belsky et al. 2014; Liang, Sun et al. 2016; Viljakainen, Dahlström et al. 2019), but none in the Iranian population of any age.

In this study, 16 genetic variants previously indicated to be associated with obesity were assessed in the Iranian population. Among the studied polymorphisms, *ADRB3 rs4994, FTO rs9939609*, *ADRB2 rs1042714, IL6 rs1800795,* and *MTHFR rs1801133* polymorphisms were significantly associated with obesity. Among these SNPs, *ADRB3 rs4994* is the locus that explains the largest association with BMI. The *ADRB3* and *ADRB2* genes are part of the adrenergic system, a major contributor to energy expenditure and body weight regulation. These receptors are expressed in adipose tissue and skeletal muscles, and stimulate lipolysis in fat cells (Rankinen, Zuberi et al. 2006). This explains why polymorphisms in these genes are among the most studied candidates for genetic obesity predisposition. The *ADRB3* is one of the only 6 genes which showed reproducible association with

Table 2 Genotype frequencies and Hardy–Weinberg equilibrium *P* values of the SNPs analyzed in this study

Gene, SNP ID[a] (Allele)	Chromosome[b], function	Minor allele homozygote	Heterozygote	Major allele homozygote	MAF in this study	MAF[c]	HWE *P* value *
VDR, rs1544410 (C/T)	chr12:47846052, Intron	155 (16%)	472 (48.8%)	341 (35.2%)	0.40 (T)	0.30 (T)	0.695
MTHFR, rs1801133 (G/A)	chr1:11796321, Missense	66 (6.8%)	394 (40.7%)	508 (52.5%)	0.27 (A)	0.25 (A)	0.375
IL6, rs1800795 (G/C)	chr7:22727026, Intron	45 (4.6%)	339 (35%)	584 (60.4%)	0.22 (C)	0.14 (C)	0.637
GPX1, rs1050450 (G/A)	chr3:49357401, Missense	125 (13%)	438 (45.2%)	405 (41.8%)	0.36 (A)	0.22 (A)	0.699
FTO, rs9939609 (T/A)	chr16:53786615, Intron	155 (16%)	429 (44.3%)	384 (39.7%)	0.38 (A)	0.34 (A)	0.057
FABP2, rs1799883 (G/A)	chr4:119320747, Missense	171 (17.7%)	384 (39.7%)	413 (42.7%)	0.37 (A)	0.25 (A)	< 0.001*
ADRB2, rs1042714 (C/G)	chr5:148826910, stop gained	74 (7.6%)	398 (41.1%)	496 (51.3%)	0.28 (G)	0.20 (G)	0.634
TCF7L2, rs7903146 (C/T)	chr10:112998590, Intron	150 (15.5%)	425 (43.9%)	393 (40.6%)	0.37 (T)	0.23 (T)	0.050
ACE, rs4646994 (D/I)	chr17:63477061–63498373, Intron	156 (16.1%)	474 (49.0%)	338 (34.9%)	0.41 (I)	-	0.635
APOC3, rs5128 (C/G)	chr11:116832924, 3 prime UTR	66 (6.8%)	345 (35.6%)	557 (57.6%)	0.25 (G)	0.23 (G)	0.210
SOD2, rs4880 (G/A)	chr6:159692840, Missense	222 (22.9%)	452 (46.7%)	294 (30.4%)	0.46 (G)	0.41 (G)	0.058
LCT, rs4988235 (G/A)	chr2:135851076, Intron	16 (1.6%)	78 (8.1%)	874 (90.3%)	0.06 (A)	0.16 (A)	< 0.001*
TNF, rs1800629 (G/A)	chr6:31575254, 2 KB Upstream	8 (0.8%)	155 (16%)	805 (83.2%)	0.09(A)	0.09 (A)	0.858
CAT, rs1001179 (C/T)	chr11:34438684, 2 KB Upstream	43 (4.4%)	326 (33.7%)	599 (61.9%)	0.21 (T)	0.13 (T)	0.872
ADRB3, rs4994 (A/G)	chr8:37966280, Missense	11 (1.1%)	138 (14.3%)	819 (84.6%)	0.08 (G)	0.12 (G)	0.062
CYP1A2, rs762551 (C/A)	chr15:74749576, Intron	132 (13.7%)	485 (50.1%)	351 (36.3%)	0.39 (C)	0.37 (C)	0.080

MAF minor allele frequency

*HWE *P* values were calculated using the chi-square test. ** *P* vaues < 0.05 were considered as not consistence with HWE

[a]According to the dbSNP database

[b]The SNP Chromosome positions (GRCh38.p12) and functions are based on the NCBI human genome

[c]MAF in the 1000 Genomes Phase 3 dataset

obesity during 15 years of research (Loos and Yeo 2021). Xie and colleagues performed a meta-analysis on 12,497 case and controls of East Asia's population, and indicated that the *ADRB3 rs4994* polymorphism significantly increases the risk of childhood and adolescent overweight/obesity (Xie, Hua et al. 2020). Moreover, the molecular alteration at the codon 64 of the *ADRB3* is related to weight gain, insulin resistance, and type 2 diabetes. A large meta-analysis consisting of 4864 T2D patients and 8779 controls demonstrated a significant association between *rs4994* and T2D in Asians (Ryuk, Zhang et al. 2017). Another recent study proposes that *rs4994* alters the body's metabolic function into lower lipolytic activity and resistance to weight loss; hence an appropriate weight loss program for carries is necessary (González-Soltero, de Valderrama et al. 2021). The *rs1042714* variant which occurs at codon 27 alters *ADRB2* function. Although there are conflicting results about the relationship between rs1042714 and obesity, two meta-analysis studies involving this polymorphism and obesity have provided consistent results. First, Jalba, et al., were able to clarify their association in three genetic models (heterozygote, homozygote, and dominant) -in Asians, Pacific Islanders, and American Indians (Jalba, Rhoads et al. 2008). In the most recent meta-analysis which involved 17 studies, authors showed that *rs1042714 (Gln27Glu)* was significantly associated with increased obesity risk in the heterozygote and dominant model in all studied populations (Zhang, Wu et al. 2014). The findings from the current study also suggests an obvious role of the *ADRB3* and *ADRB2* alleles in the development of obesity (O.R. 1.878 and 1.464, respectively).

The well-known variant within the *FTO* gene had the second place for effect size among the five significant polymorphisms, which indicates its high impact in predicting

Table 3 The results of GLM and multivariate logistic regression model

Gene	SNP ID	GLM	Logistic model	
		P value[a]	*P* value[b]	O.R. (95% CI)
VDR	rs1544410	0.446	–	-
MTHFR	rs1801133	0.207	0.031	1.284 (1.023–1.611)
IL6	rs1800795	0.015	0.011	1.368 (1.073–1.743)
GPX1	rs1050450	0.356	–	–
FTO	rs9939609	0.001 >	0.001 >	1.524 (1.247–1.861)
FABP2	rs1799883	0.599	–	–
ADRB2	rs1042714	0.163	0.001	1.464 (1.170–1.831)
TCF7L2	rs7903146	0.869	–	–
ACE	rs4646994	0.336	–	–
APOC3	rs5128	0.403	–	–
SOD2	rs4880	0.795	–	–
LCT	rs4988235	0.173	0.588	–
TNF	rs1800629	0.04	0.140	–
CAT	rs1001179	0.378	–	–
ADRB3	rs4994	0.001	0.001	1.878 (1.277–2.762)
CYP1A2	rs762551	0.593	–	–

[a]Calculated using GLM model. Adjusted for age and sex. *P* values less than 0.25 were considered as significant

[b]Calculated using logistic model. Adjusted for age and sex. *P* values less than 0.05 were considered as significant

obesity in our models. This genetic variant has been associated with the risk of obesity among distinct populations and ages, including European, African-American and Mexicans (Villalobos-Comparán, Flores-Dorantes et al. 2008; Liu, Zhu et al. 2010). Moreover, some studies indicate that this SNP can be considered a prognostic marker of obesity risk among Iranians (Koochakpour, Esfandiar et al. 2019; Mozafarizadeh, Mohammadi et al. 2019; Ostadsharif, Ebrahimi et al. 2019). The A Allele of *rs9939609* has also been shown to associate with several metabolic syndrome components such as diabetes mellitus, coronary artery disease, and triglyceride and total cholesterol measurement in European and Asian populations. (Zhou, Liu et al. 2012), and thereby could be considered an important preventive marker. According to what Smemo et al. indicated, this gene is responsible for regulating *IRX3* expression which is related to body composition (Smemo, Tena et al. 2014). This gene is highly expressed in the hypothalamus, adrenal and pituitary glands, and is involved in controlling food intake, which suggests a probable role in the hypothalamic–pituitary–adrenal axis (Todendi, Klinger et al. 2019). In a meta-analysis including 27 observational studies in different populations, Gholami et al., also concluded that *IL6 rs1800795* increases the risk of obesity based on different genetic models (Gholami, Sharifi et al. 2019). *IL6* (174G/C) gene polymorphism can affect obesity by playing an important role in *IL6* expression (Ibrahim, Gabre et al. 2017). A higher concentration of *IL6* is related to activation of adipose tissue inflammation, leading to lipid accumulation in adipocytes and obesity (Todendi, Klinger et al. 2015). It is well-established that this cytokine has a crucial role in physiological conditions such as immune function, metabolism regulation, and oncogenesis, thus the association between *rs1800795* and hypertension, insulin resistance, type 2 diabetes mellitus and cancer progression is no surprise (Mauer, Denson et al. 2015). Barati et al. demonstrated an association between presence of *IL6* variant and metabolic syndrome components in Iranian obese patients (Barati, Ghazizadeh et al. 2019). In line with these findings, our results confirmed that *rs1800795* is a potential risk marker for obesity in our population (O.R. 1.368).

In addition, the fifth SNP included in our GRS is a functional variant located on the *MTHFR* gene, and substantially decreases the activity of the *MTHFR* enzyme (nearly 50%) and inhibits the efficient deoxynucleoside synthesis and intracellular methylation reactions (Cho, Amin et al. 2017). *MTHFR rs1801133* polymorphism is responsible for the increased risk of obesity and also coronary artery diseases, but the exact mechanism of its influence on obesity is unclear. (Luo, Lu et al. 2018; Fu, Li et al. 2019). A meta-analysis by Meng et al. provided significant evidence

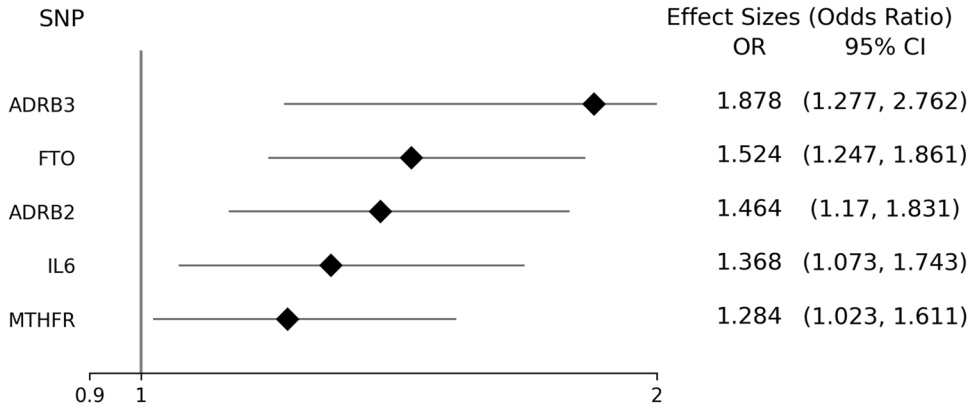

Fig. 2 Forest plot of genetic variants significantly associated with the risk of developing obesity

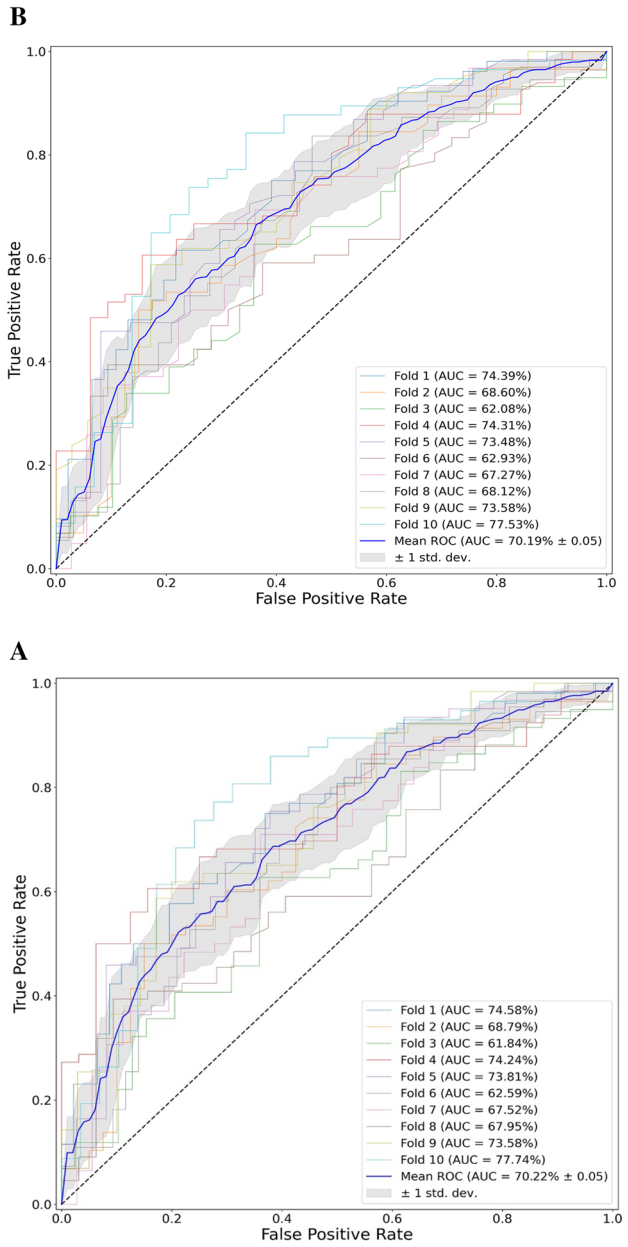

Fig. 3 Receiver operating characteristics (ROC) curves of the weighted GRS (**A**) and unweighted GRS (**B**) with tenfold cross validation

for this MTHFR mutation role in T2DM susceptibility in Asians (Meng, Liu et al. 2019). Other studies have reported the same association in the Iranian population (Raza, Abbas et al. 2017; Poodineh, Saravani et al. 2019). MTHFR is responsible for maintaining methionine and homocysteine balance, through its role in the one-carbon cycle. Decreased activity of this enzyme leads to a reduction in the conversion of 5,10-methylenetetrahydrofolate (5,10-MeTHF) to 5-methyltetrahydrofolate (5-MTHF), resulting in an elevated homocysteine level. Hence, improving the homocysteine levels could be helpful in the prevention and treatment of obesity and other related diseases (Fu, Zhang et al. 2018).

Both unweighted and weighted GRSs were statistically associated with the risk of obesity ($P < 0.05$). The area under the mean ROC in the unweighted GRS was 70.19% and in the weighted GRS increased to 70.22%. Comparing the difference between AUCs for these two models, no significant difference in discriminatory ability to estimate obesity was observed between the two models. And the Pearson's correlation coefficient for these models show that they are highly correlated ($r^2 = 0.988$). ROC curve is proposed to evaluate the usefulness of genetic profiling, and the magnitude of the area under ROC (AUC) should be more than 0.8 to indicate an acceptable test for clinical screening of high-risk individuals (Janssens, Aulchenko et al. 2006). Although statistical power was low in the current study, it meets an acceptable discrimination power for a model (AUC equal to or more than 0.7). Our findings may significantly contribute to identifying people with susceptibility to developing greater BMI and obesity-related problems. Although the BMI is a highly heritable trait (between 40 and 70%) (Maes, Neale et al. 1997), almost all previous GRS models based only on genetic factors explained just a small fraction of BMI variance and a more holistic approach considering the complexity of the biological system is required (Eichler, Flint et al. 2010). Other studies suggest that risk scores incorporating healthy life style scores and childhood obesity have higher prediction ability. However, identification and replication of obesity-associated variants and GRS construction still provide further insights into weight regulation (Locke, Kahali et al. 2015). As a result, the current GRSs could be useful to minimizing T2D, cardiovascular events and other obesity-related concerns, especially in the Iranian population, which has been poorly studied before. However, our study has several potential limitations, including small sample size, a

Table 4 The area under the mean ROC curve (AUC), sensitivity, specificity, accuracy, negative predictive value (NPV), and positive predictive value (PPV) analysis showed in percentages (%)

Model	AUC	Sensitivity	Specificity	Accuracy	NPV	PPV
Unweighted GRS	70.19 ± 5	82.14	41.57	66.73	59.45	69.56
Weighted GRS	70.22 ± 5	82.44	41.94	67.03	60.07	69.76

lack of the previous GWAS on the Iranian population, small number of investigated risk loci and not incorporating environmental factors before modeling. Even though the GRS models were validated by performing a tenfold cross-validation analysis, it is clear that the optimal validation process would be in an independent cohort. The odds ratios used to construct the weighted model were small which can reveal the influences of other genetic variants and also non-genetic factors such as depression status. This can be an explanation for the similarity of the results produced by our GRS models. Even though the results of our models were not significantly different, the weighted model performed better and is promising for future studies.

In conclusion, our findings suggest that ADRB*3 rs4994, FTO rs9939609*, *ADRB2 rs1042714, IL6 rs1800795,* and *MTHFR rs1801133* SNPs are associated with the risk of obesity. Moreover, our results suggest that the developed GRSs in this study could be considered useful genetic tools for anticipating the risk of obesity development in the Iranian population. More studies with larger sample sizes and considering non-genetic factors are critical to constructing more accurate models. The alarming prevalence of obesity growing each year is a public health problem for many countries including Iran. This kind of study, which provides new insights into the biology and genetics of this abnormality, will assist us in developing and implementing novel therapies in the future.

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Author contributions ND was responsible for designing the study, data collection, interpreting results, model development and writing the report. SBZ and MHSA contributed to sample analysis, data interpretation, writing the report, and created the tables. AS contributed to data analyses, model constructing and writing the report. SZ provided supervision in all the steps and writing the report.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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