Effect of gene polymorphisms in transmembrane protein 18 (TMEM18) and neuronal growth regulator 1 (NEGR1) on body mass index in obese subjects

Müjgan Ozdemir Erdogan, Kamuran Avci, Salilha Handan Yildiz, Evrim Suna Arikan Terzi, Zafer Soylemez, Nuray Varol and Mustafa Solak

Faculty of Medicine, Department of Medical Biology, Afyon Kocatepe University, Afyonkarahisar, Turkey; Faculty of Medicine, Department of Medical Genetics, Afyon Kocatepe University, Afyonkarahisar, Turkey

ABSTRACT

Obesity is a complex disorder with nearly epidemic proportions in many parts of the world. Genome-wide association studies have demonstrated high heritability for obesity and body mass, with associations of certain candidate genes and their variations with respect to race, geographical location/country of origin. However, the functional mechanisms and different ethnic data of these loci are still poorly understood. In this case-control study, we investigated two single nucleotide polymorphisms, rs2815752 in the neuronal growth regulator 1 (NEGR1) gene and rs6548238 in the transmembrane protein 18 (TMEM18) gene, for association in a group of obese residents of Afyonkarahisar province (Turkey). Polymorphisms were genotyped in 172 obese subjects and 77 healthy controls. The results showed no significant differences between the obese subjects and the controls in terms of the allele and genotype frequencies of the NEGR1 gene rs2815752 and the TMEM18 gene rs6548238 polymorphisms. There were no significant associations of the rs2815752 polymorphism in obese subjects and controls with regard to anthropometric measurements and body composition parameters. However, several significant associations were found for the rs6548238 polymorphism with regard to anthropometric measurements and body composition. Consequently, there were no significant differences between the genotype and allele frequencies of NEGR1 gene rs2815752 and TMEM18 gene rs6548238 polymorphisms in the obese group and the controls. There were significant associations for the rs6548238 polymorphism, but not the rs2815752 polymorphism, with the anthropometric measurements and body composition parameters in the group of obese subjects.

Introduction

Obesity is a complex outcome of genetic background and environmental interaction [1]. Despite of all obesity preventive measures, its prevalence has increased far and wide in the last two decades [2]. Its upsurge is likely the result of individual lifestyle and obesogenic conditions [3]. However, existence of healthy people within obesogenic environments has led to investigations of genetic susceptibility variants that direct phenotypical expression favouring obesity [4]. Genetic heritage is regarded as one of the driving forces that determine personal sensitivity and resistance in this process [5]. Genetic heritability varies from 40% to 70% for body mass index (BMI) and/or obesity in twin and family studies [3, 6]. There are many genes that have been associated with obesity [7]. Some gene variants identified in genome-wide association studies (GWAS) play a role in the control of the eating behaviour via the hypothalamus region or of obesity related cells, i.e. adipocyte cells [5, 7–10]. The two major gene variants identified by GWAS are transmembrane protein (TMEM18) and neuronal growth regulator 1 (NEGR1) [6, 11].

NEGR1 is a neurone specific cell adhesion molecule that belongs to the immunoglobulin (Ig) superfamily [12]. NEGR1 expression regulates the body weight in mice via the hypothalamus [13]. Walley et al. [14] showed different NEGR1 expression profiles between normal-weight and obese siblings and a determinative role of NEGR1 in a central ‘hub’. Various NEGR1 variants have also been associated with BMI and obesity [15, 16]. The rs2815752 polymorphism was initially defined by Willer et al. [9] in a GWAS analysis. Subsequently, another GWAS analysis reported it again among significant variants [10]. The rs2815752
polymorphism designates a 43-kb deletion which is also tagged by an obesity related copy number variant (CNV) [9, 17]. Both the CNV and the rs2815752 polymorphism are located upstream of NEGR1 [18]. The risk allele of the rs2815752 polymorphism was associated with low BMI, visceral adipose tissue (kg), nonvisceral adipose tissue (kg), and total adipose tissue (kg) in 332 nondiabetic Caucasian subjects [19]. Bauer et al. [20] associated NEGR1 with weight, BMI and macronutrient intake. Mágì et al. [21] investigated bariatric samples and found strong association of the rs2815752 polymorphism with severe obesity.

TMEM18 was discovered as a novel modulator that regulates neural stem cell migration towards glioma in the rat brain [22, 23]. TMEM18 localises to the nuclear membrane in a dimerised form and binds to DNA in a sequence-specific manner [24]. TMEM18 is expressed in the hypothalamus and other tissues with certain tissue-specific differences [5]. For example, TMEM18 is differentially expressed in subcutaneous and visceral adipose tissue, and obese subjects have lower TMEM expression in their adipose tissue [25]. Gutierrez-Aguilar et al. [26] showed that TMEM18 expression was down-regulated with high-fat diet (HFD) in the liver and soleus muscle of rats. Some studies show that TMEM18 also has epigenetic interactions. In this regard, adipocyte TMEM18 expression was suppressed by insulin and synthetic glucocorticoid dexamethasone [26]. Rohde et al. [27] associated inter-depot variance of TMEM18 mRNA expression with adipose tissue depot specific TMEM18 promoter methylation. Bernhard et al. [28] showed that (si) RNA-mediated knockdown of TMEM18 significantly inhibited adipocyte maturation. Various TMEM18 single nucleotide polymorphisms (SNPs) have been repeatedly associated with obesity and BMI in different populations [22, 29–31]. The rs6548238 polymorphism was primarily associated with increased BMI and body weight by a GWAS analysis [9]. This polymorphism had the second largest effect size among all obesity associated loci identified so far via GWAS [4].

The rs2815752 and rs6548238 polymorphisms were associated with obesity and BMI by Willer et al. [9] at the third wave of GWAS and two independent follow-up studies confirmed their effects [10, 15]. In this study, we conducted a follow-up replication study with NEGR1 rs2815752 and TMEM18 rs65482382 polymorphisms which were evaluated for association with obesity risk and BMI in a cohort of 172 obese and 77 healthy control adult individuals.

### Subjects and methods

#### Subjects

This study was conducted in Afyon Kocatepe University, Faculty of Medicine between June 2015 and August 2016. All participants were comprehensively evaluated and classified according to their BMI categories: normal weight from 18.5 to 24.9 kg/m², overweight from 25 to 29.9 kg/m² and obese 30 kg/m² or above. Exclusion criteria were Cushing’s disease, hypothyroidism, hyperthyroidism, hypertension, type 2 diabetes mellitus, coronary artery disease, pregnancy and history of substance abuse. A total number of 249 individuals were enrolled. There were 172 obese individuals (BMI ≥30 kg/m²) and 77 normal-weight control subjects (BMI <25 kg/m²). The demographic characteristics of the participants are given in Table 1.

Each subject gave their written informed consent. The study was approved by the Afyon Kocatepe University, Medical Ethics Committee.

#### Anthropometric measurements and body composition analysis

Anthropometric parameters were determined by standard techniques. Waist circumference was measured at the lowest rib and the iliac cres. Hip circumference was measured with the maximum value over the buttocks. The waist-to-hip ratio (WHR) was also calculated. Height was measured on bare foot. Body composition parameters [total body fat (TFB), body fat percentage (BFP), lean body mass (LBM), total body water (TBW) and total body water percentage (TBWP)] were determined by bioelectrical impedance analysis. Measurements were performed with light clothes and bare feet by TANITA BF 350 (TANITA, Sindelfingen, Germany). Exclusion criteria were dehydration and

#### Table 1. Phenotypic characteristics of obese patients and healthy controls

| Parameter                  | Control (n = 77) | Obese (n = 172) | P     |
|----------------------------|-----------------|-----------------|-------|
| Age (year)                 | 27.6 ± 9        | 39.3 ± 9.5      | <0.001 |
| Height (cm)                | 165.9 ± 9.4     | 161.7 ± 9.1     | <0.001 |
| Weight (kg)                | 60.4 ± 10       | 95.6 ± 15.2     | <0.001 |
| BMI (kg/m²)                | 21.9 ± 2.2      | 36.7 ± 5.8      | <0.001 |
| WHR                        | 0.8 ± 0.1       | 0.9 ± 0.1       | <0.001 |
| Total body water (kg)      | 34.8 ± 9        | 41.5 ± 7.3      | <0.001 |
| Total body water percentage (%) | 56.3 ± 5.5   | 43.6 ± 5.6      | <0.001 |
| Total body fat (kg)        | 12.9 ± 4.4      | 39.0 ± 12.1     | <0.001 |
| Body fat percentage (%)    | 21.7 ± 7.5      | 40.3 ± 8.6      | <0.001 |
| Lean body mass (kg)        | 47.6 ± 10.1     | 56.8 ± 10.9     | <0.001 |

Note: Data are represented as mean values with standard deviation (±SD).
menstruation. All parametric results are presented as mean values with standard deviation (±SD) for each group.

**Genotyping**

Peripheral blood samples (2 mL) were collected and stored in ethylenediaminetetraacetic acid (EDTA) coated vacutainers. Genomic DNA was extracted from whole blood by using a High Pure Template Preparation kit (Roche Diagnostics, Mannheim, Germany). Each DNA sample was quantified by a Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies, Inc., V3.7, Wilmington, DE, USA). We selected two obesity-associated SNPs according to the following criteria: (i) genome-wide significant level of association ($P < 2 \times 10^{-20}$) for BMI, (ii) genome-wide level of significance ($P < 5 \times 10^{-20}$) reported in a population of European ancestry, (iii) high allele frequency. Two SNPs were genotyped using real-time polymerase chain reaction on a LightCycler$^\text{®}$ 480 real-time polymerase chain reaction system (Roche Diagnostics, Mannheim, Germany), LightSNIP NEGR1 rs2815752 and TMEM18 rs6548238 Reagent Mix (Tib Molbiol, Berlin, Germany). Both SNPs passed the quality control criteria of Hardy–Weinberg equilibrium ($P > 0.01$) for cases and controls.

**Statistical analysis**

Prior to research, statistical sample size calculations were performed with QUANTO software (biostats.usc.edu/Quanto), using the current prevalence of obesity in Turkey and x-level of 0.05 [32]. Statistical analysis was performed using the PASW Statistics for Windows, version 18.0 (SPSS Inc., Chicago, IL, USA). Collations were carried out between obese (BMI $\geq 30$ kg/m$^2$) and normal-weight (BMI $< 25$ kg/m$^2$) subjects. Previously, obesity associated alleles were defined as risk alleles for each SNPs. In the obese subjects and the controls, the genotype and allele frequencies of the NEGR1 gene rs2815752 and the TMEM18 gene rs6548238 polymorphisms were compared using chi-square test. Independent samples $T$-test was used to test polymorphisms for anthropometric and body composition parameters. $P$-values of less than 0.05 were considered significant.

**Results and discussion**

In this study, the genotype and allele frequencies of NEGR1 gene associated rs2815752 and TMEM18 gene associated rs6548238 polymorphisms are analysed in obese subjects and normal-weight controls. The phenotypic characteristics are summarised in Table 1. The examination sample comprised 172 obese subjects (53 males, 119 females; BMI $\geq 30$ kg/m$^2$, mean BMI $36.7 \pm 5.8$ kg/m$^2$, $p < 0.001$) diagnosed with BMI classification and 77 healthy controls (37 males, 40 females; BMI $< 25$ kg/m$^2$, mean BMI $21.9 \pm 2.2$ kg/m$^2$, $p < 0.001$). In the obese subgroup, 47.7% were moderately obese (30 kg/m$^2$ $\leq$ BMI $< 35$ kg/m$^2$), 29.7% were severely obese (35 kg/m$^2$ $\leq$ BMI $< 40$ kg/m$^2$) and 22.6% were morbidly obese (BMI $\geq 40$ kg/m$^2$). The mean ages of the obese and control groups were 39.3 ($\pm 9.5$) years and 27.6 ($\pm 9$) years, respectively.

The distribution of the rs2815752 genotype frequencies in the obese group were 9.3% (16/172) for GG, 37.2% (64/172) for AG and 53.5% (92/172) for AA; and in the control group, the distribution of genotypes were 7.8% (6/77) for GG, 42.9% (33/77) for AG, 49.3% (38/77) for AA. There were no significant differences between the rs2815752 genotype frequencies in the obese group and the controls ($P = 0.688$, $p > 0.05$). The G and A allele frequencies of the rs2815752 polymorphism were 27.9% and 72.1% in the obese group and 29.2% and 70.8% in the control group, respectively. These differences between the two groups were also non-significant ($P = 0.764$, $p > 0.05$). G and A indicate the ancestral allele and the risk allele for the rs2815752 polymorphism, respectively. However, possession of the risk allele (GA + AA) was not significantly associated with either of the two groups ($P = 0.698$, $p > 0.05$) (Table 2). In the same way, no significant association was found for anthropometric

| rs2815752 | Obese | Control | Total | $P$ |
|-----------|-------|---------|-------|-----|
| Genotype frequencies |       |         |       |     |
| AA        | 92 (53.5) | 38 (49.3) | 130 (52.2) |       |
| AG        | 64 (37.2) | 33 (42.9) | 97 (39) | 0.688 |
| GG        | 16 (9.3) | 6 (7.8) | 22 (8.8) |       |
| GG        | 16 (9.3) | 6 (7.8) | 22 (9) |       |
| AA + AG   | 156 (90.7) | 71 (92.2) | 227 (91) | 0.698 |
| Allele frequencies |       |         |       |     |
| A         | 72.1 | 70.8 | 71.7 | 0.764 |
| G         | 27.9 | 29.2 | 28.3 |       |
| rs6548238 |       |         |       |     |
| Genotype frequencies |       |         |       |     |
| TT        | 8 (4.7) | 4 (5.2) | 12 (4.8) |       |
| TC        | 61 (35.4) | 24 (31.2) | 85 (34.2) | 0.801 |
| CC        | 103 (59.9) | 49 (63.6) | 152 (61) |       |
| TT        | 8 (4.7) | 4 (5.2) | 12 (4.8) |       |
| TC + CC   | 164 (95.3) | 73 (94.8) | 237 (95.2) | 0.853 |
| Allele Frequencies |       |         |       |     |
| T         | 22.4 | 20.8 | 21.9 |       |
| C         | 77.6 | 79.2 | 78.1 | 0.689 |

Note: $N$, number.
measurements and body composition parameters within obese subjects (including height (cm), weight (kg), BMI (kg/m²), WHR, TBW (kg), TBWP (%), TBF (kg), BFP (%), LBM (kg)) in the obese group (P > 0.05) (GG and GA + AA) (Table 3).

The distribution of the rs6548238 genotype frequencies in the obese group was as follows: 59.9% (103/172) for CC, 35.4% (61/172) for TC and 4.7% (8/172) for TT; and in the control group: 63.6% (49/172) for CC, 35.4% (61/172) for TC and 4.7% (8/172) for TT. There were no significant differences in the rs6548238 genotype frequencies comparing the obese group and the controls (P = 0.801, p > 0.05). The T and C allele frequencies of the rs6548238 polymorphism were 22.4% and 77.6% in the obese group and 20.8% and 79.2% in the control group, respectively. There were no significant differences in the allele frequencies of rs6548238 comparing the obese group and the controls either (P = 0.689, p > 0.05). The C allele indicates both ancestral and risk allele for the rs6548238 polymorphism. According to risk allele possession (TC + CC), no significant association with obesity was found when comparing the group of obese subjects and the control group (P = 0.853, p > 0.05) (Table 2). However, there were significant associations of the rs6548238 polymorphism with some anthropometric measurements and body composition parameters. These were height (cm) (P = 0.020), TBW (kg) (P = 0.017), TBWP (%) (P = 0.024), BFP (%) (P = 0.006) and LBM (kg) (P = 0.021). On the other hand, no association was found for weight (kg), BMI (kg/m²), WHR or TBF (kg) (P > 0.05) (Table 3).

The main aim of obesity associated risk allele assessment in different populations is to reach a potential use in clinical practice [29]. Therefore, we investigated two SNP variations for genotypic and allelic frequencies in our case-control study. Besides, we also analysed some anthropometric measurements and body composition parameters and sought potential association with carriership of the risk allele. Our results did not fully agree with other published data. These discrepancies might be related to different ethnic groups, limited cohort size, regional differences, life style or age. Additionally, our study did not consider individual factors, such as physical activity, sleep patterns, food intake and family environment. These determinants may be involved as either confounder effects or gene–environment interaction parameters [33]. A limitation of this study is that, our obese and normal-weight groups were not age-matched and there was mean age difference. This needs to be taken into account when interpreting the results from the statistical comparison, as obesity is often age related. On the other hand, we analysed all obese subjects without grouping and obesity subgroups could be differentiated with regard to their SNP associations. Mägi et al. [21] analysed 32 obesity risk GWAS gene variants and found significant association of the rs2815752 polymorphism of the NEGR1 gene with BMI. However, they only replicated their results with their class III obesity subgroup. In the same way, Mimila et al. [31] investigated 26 obesity associated gene variants in a Mexican population and reported significant
association of the rs9939609 polymorphism of *Fat mass and obesity associated gene* (*FTO*) with class III obesity, whereas none for class I/II obesity.

To the best of our knowledge, this study was the first to examine the *NEGR1* gene rs2815752 and *TMEM18* gene rs6548238 polymorphisms in a case-control study of 249 Turkish individuals. Within the limitations of our study, there was no association of the rs2815752/rs6548238 genotype and allele frequencies with the control group or the obese group. However, the obese subjects showed significant associations for anthropometric measurements and body composition parameters according to possession of the risk allele (TC + CC) in the rs6548238 polymorphism. This result could be attributed, at least in part, to the anthropometric effect of *TMEM18* loci with age. For example, Hoed et al. [4] showed that the rs6548238 polymorphism had the strongest effect among 15 variants, and Graf et al. [34] highlighted the importance of this locus for obesity risk over the course of life. Moreover, Mimila et al. [31] reported nominal association for *TMEM18* loci in children, whereas significant association in adults.

The risk allele frequency of the *NEGR1* gene rs2815752 polymorphism in the population studied by us was lower than those reported in Japanese (0.92%) [30] and Chinese Han populations (0.89%) [33] and higher than the ones in Northern Europe (0.58%) [35] and Western Europe (0.66%) [17]. The *NEGR1* gene rs2815752 polymorphism risk allele frequency observed in our study was higher than that in the GWAS reported by Willer et al. [9] and Speliotes et al. [10]. Conversely, we obtained concordant results with Mexican (0.73) [36] and Greek populations (0.79) [29]. The risk allele frequency of the *TMEM18* gene rs6548238 polymorphism determined in our cohort was lower than that in Mexican (0.93%) [31], Philippine (0.92%) [37], Japanese (0.92%) [30], Chinese (0.91%) [33], Swedish (0.87%) [22], Northern Europe (0.84%) [35] and Greek populations (0.82%) [29]. Most similar results were seen with American Indians (0.78%) [38]. Willer et al. [9] observed higher risk allele frequency (0.84%).

Rouskas et al. [29] studied 24 obesity risk-associated SNPs in 510 obese and 469 healthy control subjects in a Greek population. They found no association for the rs2815752 polymorphism with obesity in respect of its genotype distribution and risk allele frequency. They reported the same risk allele frequency of the rs2815752 polymorphism for obese subjects and controls. The risk allele frequency in our study was higher in the obese group and lower in the control group than those reported by Rouskas et al. [29]. However, they reported higher risk allele frequency of the rs6548238 polymorphism in the obese group and found that its genotype distribution was associated with obesity. The risk allele frequency in our obese group was lower than reported by Rouskas et al. [29], and the rs6548238 polymorphism was not associated with obesity. Similarly, Hotta et al. [30] analysed 27 SNP variants in a Japanese population of 1129 obese and 1736 healthy control subjects and reported that the rs2815752 polymorphism was not associated with obesity. The risk allele frequencies in the obese and control groups in their study were higher than the ones determined in our cohort. On the other hand, they reported higher risk allele frequencies of the rs6548238 polymorphism in the obese and control group than our values and found rs6548238 polymorphism association with obesity via allele frequencies. Haupt et al. [19] investigated six obesity risk-associated SNPs, previously identified by GWAS, in a German population of subjects that had BMI values lower than 30 kg/m². They associated the rs2815752 polymorphism with BMI and total fat mass (TFM) parameters according to the genotype distribution, contrary to our results. In the same way, they associated the rs6548238 polymorphism with obesity and found significant associations for BMI and body weight according to genotype distribution. But they also reported significant association for body fat ratio and insignificant association for fat-free mass and TFM parameters of the rs6548238 polymorphism, which were all consistent with our findings. Cuypers et al. [2] investigated obesity-susceptible loci associated with BMI and waist circumference (WC) change from adolescence into young adulthood at an 11-year interval and whether physical activity modifies adiposity-related traits. They reported lower risk allele frequency for rs2815752 and higher risk allele frequency for rs6548238 polymorphisms than our findings. Consistent with our findings, they found no association for rs2815752 and rs6548238 polymorphisms according to genotype distribution with BMI in a Norwegian population. Wang et al. [33] investigated European derived GWAS gene variants in Chinese Han population with regard to obesity-related indices. They reported higher risk allele frequency than our results for both polymorphisms. However, they found no association of the rs2815752 polymorphism, while they associated the rs6548238 polymorphism via genotype distribution and allele frequency. Croteau-Chonka et al. [37] studied obesity risk GWAS gene variants in a Philippine population with 1792 female
subjects who had BMI lower than 30 kg/m². They found higher risk allele frequencies for both polymorphisms than in our obese and control group. Likewise, they found no association for rs2815752 or rs6548238 polymorphisms with obesity through genotype distribution. Renström et al. [35] investigated the association of GWAS gene variants singly and in combination with adipose features in 3885 Swedish individuals with BMI value lower than 30 kg/m². Although they found lower risk allele frequencies than in our study, they associated the rs2815752 polymorphism with BMI in terms of genotype distribution. However, similar to our results, they could not find any association with TFM. On the other hand, they reported higher risk allele frequencies for the rs6548238 polymorphism and no association with BMI and TFM parameters by means of genotype distribution. Rukh et al. [39] studied the genetic susceptibility to obesity and its relation with diet intake levels among 4127 obese, 11850 overweight and 12234 healthy individuals over a 22-year interval. Contrary to our results, they associated the risk allele of the rs2815752 polymorphism with BMI, height, body fat ratio and TFM; and the risk allele of the rs6548238 polymorphism with BMI, weight and TFM. However, similar to our results, they found no association of weight and fat-free mass with the rs2815752 polymorphism or of body fat rate and fat-free mass with the rs6548238 polymorphism. Mägi et al. [21] studied 32 different SNP variants in a European descent case-control cohort. Contrary to our observations, they reported lower risk allele frequency than our results and found an association of rs2815752 with BMI. Shi et al. [40] investigated 18 SNP variants in Chinese Han population with 2,076 female subjects who had BMI values lower than 27 kg/m². They reported higher risk allele frequency than our results and found no association with obesity similarly for the rs2815752 polymorphism. Mejía-Benítez et al. [36] analysed six obesity-related GWAS gene variants in 514 obese and 949 healthy children in Mexico. The risk allele frequencies that they identified in every group correlated very well with our results. But they also associated the rs2815752 polymorphism risk allele frequency with BMI for obesity. Jarick et al. [17] investigated different CNVs and the rs2815752 polymorphism to determine missing heritability factors of obesity in a German population. They replicated findings about the location of the rs2815752 polymorphism adjacent to a CNV region at 1p31.1. Although they found lower risk allele frequencies than those in our case and control groups, they reported an association of body weight with the rs2815752 polymorphism. Almén et al. [22] investigated the functional features of the TMEM18 gene and its obesity association in a Swedish population. They found association of the rs6548238 polymorphism with obesity through allele frequencies and genotype distribution. The risk allele frequency in the obese group was higher than that in the healthy group and all values were higher than our results. However, BMI and body weight were not associated with the genotype distribution of the rs6548238 polymorphism. Moreover, the parameter ‘height’ was not associated with the genotype distribution of the rs6548238 polymorphism. Coenen et al. [41] investigated eight obesity associated genetic variants in 85 Latina women who had BMI values higher than 25 kg/m². They reported a higher risk allele frequency than in our obese and control groups. Similar to our results, rs6548238 was not found to be associated with obesity and BMI according to risk allele frequency. Mimila et al. [31] analysed 17 GWAS gene variants in a Mexican population of 945 adults and 1218 children. They observed higher risk allele frequencies for the adult and children groups than our findings and associated the rs6548238 polymorphism with obesity. However, they only found significant associations of the rs6548238 polymorphism with BMI in the adult group.

Conclusions

In this study, we could not demonstrate any association of the studied NEGR1 or TMEM18 gene polymorphisms with obesity risk in our study groups through genotype distribution and allele frequency. However, the TMEM18 gene polymorphism was associated with some anthropometric measurements and body composition parameters via risk allele-oriented grouping within obese subjects. Our study provided important data about two key SNPs in the Turkish population. However, additional criteria need to be studied such as obesity subgroups, other SNP variations, the functional effects of SNP variations, classification parameters and larger population samples.

Disclosure statement

The authors have reported no conflicts of interest.

Funding

This study was supported by the Afyon Kocatepe University Scientific Research Projects Commission with a project numbered as 15.SAG.11 under grant number 15.SAG.11.
References

[1] Saad MK, Sarah EHC, Mehera A, et al. Establishing a genetic link between FTO and VDR gene polymorphisms and obesity in the Emirati population. BMC Medical Genetics. 2018; [cited 2018 Jun 22]19:11.

[2] Cuypers K, Frans RL, Kirsti K, et al. Obesity-susceptibility loci and their influence on adiposity-related traits in transition from adolescence to adulthood - The HUNT Study. PLoS ONE. 2012; [cited 2018 Jun 22]:7:e46912.

[3] George LW, Benjamin WD, Fred U, et al. Genetic predisposition to obesity and medicare expenditures. Gerontol A Biol Sci Med Sci. 2018;73:66–72.

[4] Hoed M, Ulf E, Søren B, et al. Genetic susceptibility to obesity and related traits in childhood and adolescence. Diabetes. 2010;59:2980–2988.

[5] Carla SD, Monica CV, Claudia IE, et al. Chromosomal microarray analysis in the genetic evaluation of 279 patients with syndromic obesity. Mol Cytogenet. 2018; [cited 2018 Jun 22]11:14.

[6] Zandonà MR, Sangalli CN, Campagnolo PB, et al. Validation of obesity susceptibility loci identified by genome-wide association studies in early childhood in South Brazilian children. Pediatr Obes. 2017;12:85–92.

[7] Valérie T, Yingchang L, Heather MH, et al. Protein-altering variants associated with body mass index implicate pathways that control energy intake and expenditure in obesity. Nat Genet. 2018;50:26–41.

[8] Fall T, Ingelsson E. Genome-wide association studies of obesity and metabolic syndrome. Mol Cell Endocrinol. 2014;382:740–757.

[9] Willer CJ, Speliotes EK, Loos RJF, et al. Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. Nat Genet. 2009;41:25–34.

[10] Speliotes EK, Willer CJ, Berndt SI, et al. Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. Nat Genet. 2010;42:937–948.948.

[11] Loos R. Genetic determinants of common obesity and their value in prediction. Best Pract Res Clin Endocrinol Metab. 2012;26:211–226.226.

[12] Lee A, Hengstler H, Schwald K, et al. Functional inactivation of the genome-wide association study obesity gene neuronal growth regulator 1 in mice causes a body mass phenotype. PLoS One. 2012;7:e41537.

[13] Mccormack S, Grant S. Genetics of obesity and type 2 diabetes in african americans. J Obes. 2013; [cited 2018 Jun 22]2013:1.

[14] Walley AJ, Jacobson P, Falchi M, et al. Differential coexpression analysis of obesity-associated networks in human subcutaneous adipose tissue. Int J Obes. 2012;36:137–147.

[15] Thorleifsson G, Walters GB, Gudbjartsson DF, et al. Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. Nat Genet. 2009;41:18–24. DOI:10.1038/ng.274

[16] Hinney A, Anna V, Jochen A. Genes and the hypothalamic control of metabolism in humans. Best Pract Res Clin Endocrinol Metab. 2014;28:635–647.

[17] Jarick I, Vogel C, Schera C, et al. Novel common copy number variation for early onset extreme obesity on chromosome 11q11 identified by a genome-wide analysis. Hum Mol Genet. 2011;20:840–852.

[18] Boender AJ, Gestel MAV, Garner KM, et al. The obesity-associated gene Negr1 regulates aspects of energy balance in rat hypothalamic areas. Physiol Rep. 2014; 2:e12083.

[19] Haupt A, Claus T, Martin H, F, et al. Novel obesity risk loci do not determine distribution of body fat depots: a whole-body MRI/MRS study. Obesity (Silver Spring). 2010;18:1212–1217.

[20] Bauer F, Elbers CC, Adan RA, et al. Obesity genes identified in genome-wide association studies are associated with adiposity measures and potentially with nutrient-specific food preference 1–3. Am J Clin Nutr. 2009:90:951–959.

[21] Mägi R, Manning S, Youssef A, et al. Contribution of 32 gwas-identified common variants to severe obesity in european adults referred for bariatric surgery. PLoS One. 2013;8:e70735.

[22] Almén MS, Jacobsson JA, Shaik JH, et al. The obesity gene, TMEM18, is of ancient origin, found in majority of neuronal cells in all major brain regions and associated with obesity in severely obese children. BMC Med Genet. 2010;11:58

[23] Jurvansuu J, Zhao Y, Leung D, et al. Transmembrane protein 18 enhances the tropism of neural stem cells for glioma cells. Cancer Res. 2008;68:4614–4622.

[24] Jurvansuu J, Goldman A. Obesity risk gene TMEM18 Encodes a sequence-specific dna-binding protein. PLoS One. 2011;6:e25317.

[25] Schwenk R, Vogel H, Schurmann A. Genetic and epigenetic control of metabolic health. Mol Metab. 2013; 2:337–347.

[26] Gutierrez-Aguilar R, Dong-Hoon K, Stephen W, et al. Expression of new loci associated with obesity in diet-induced obese rats: from genetics to physiology. Obesity (Silver Spring). 2012;20:306–312.

[27] Rohde K, Keller M, Klöß M, et al. Adipose tissue depot specific promoter methylation of TMEM18. J Mol Med. 2014;92:881–888.

[28] Wehner F, Landgraf K, Klöting N, et al. Functional relevance of genes implicated by obesity genome-wide association study signals for human adipocyte biology. Diabetologia. 2013;56:311–322.

[29] Rouskas K, Kouvatsi A, Paletas K, et al. Common Variants in FTO, MC4R, TMEM18, PRL, AIF1, and PCSK1 Show Evidence of Association With Adult Obesity in the Greek Population. Obesity (Silver Spring). 2012;20:389–395.

[30] Hotta K, Nakamura M, Nakamura T, et al. Association between obesity and polymorphisms in SEC16B, TMEM18, GNPDA2, BDNF, FAIM2 and MC4R in a Japanese population. J Hum Genet. 2009;54:727–731.

[31] Mimila P, Villamil H, Villalobos M, et al. Contribution of common genetic variants to obesity and obesity-related traits in mexican children and adults. PLoS One. 2013; [cited 2018 Jun 22]8:e70640.
[32] Erem C. Prevalence of overweight and obesity in Turkey. Metab Syndr Relat Disord. 2003;1:285–290

[33] Wang J, Mei H, Chen W, et al. Study of eight GWAS-identified common variants for association with obesity-related indices in Chinese children at puberty. Int J Obes Relat Metab Disord. 2012;36:542–547.

[34] Graff M, North KE, Richardson AS, et al. BMI loci and longitudinal bmi from adolescence to young adulthood in an ethnically diverse cohort. Int J Obes (Lond). 2017;41:759–768.

[35] Renström F, Payne F, Nordstrom A, et al. Replication and extension of genome-wide association study results for obesity in 4923 adults from northern Sweden. Hum Mol Genet. 2009;18:1489–1496.

[36] Mejía-Benitez A, Klünder-Klünder M, Yengo L, et al. Analysis of the contribution of FTO, NPC1, ENPP1, NEGR1, GNPDA2 and MC4R genes to obesity in Mexican children. BMC Med Genet. 2013;14:21.

[37] Croteau-Chonka D, Amanda M, Ethan L, et al. Genome-wide association study of anthropometric traits and evidence of interactions with age and study year in Filipino women. Obesity (Silver Spring). 2011;19:1019–1027.

[38] Fesinmeyer MD, North K, Ritchie M, et al. Genetic risk factors for body mass index and obesity in an ethnically diverse population: results from the Population Architecture using Genomics and Epidemiology (PAGE) Study. Obesity (Silver Spring). 2013;21:1–21.

[39] Rukh G, Sonestedt E, Melander O, et al. Genetic susceptibility to obesity and diet intakes: association and interaction analyses in the Malmö Diet and Cancer Study. Genes Nutr. 2013;8:535–547.

[40] Shi J, Long J, Gao Y, et al. Evaluation of genetic susceptibility loci for obesity in Chinese women. Am J Epidemiol. 2010;172:244–254.

[41] Coenen K, Sharon K, Sabina G, et al. Genetic risk score does not correlate with body mass index of latina women in a clinical trial. Clin Transl Sci. 2011;4:323–327.