Research Article

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The possible link between endocannabinoid system gene polymorphisms and overweight/obesity susceptibility in individuals living in the Marmara Region

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Abstract

Objectives: Obesity is a global health problem related to reduced life quality and shorter life expectancy. Given the fact that genetic factors play a role in obesity development, studies have suggested that polymorphisms of Cannabinoid Receptor 1 (*CNR1*), Monoacylglycerol lipase (*MGLL*), and Fatty Acid Amide Hydrolase (*FAAH*) genes located in the endocannabinoid system (ECS) are related to overweight/obesity risk. Accordingly, we aimed to assess the genetic susceptibility to overweight/obesity in individuals with Turkish ancestral origin concerning these ECS genes.

Methods: The present study comprised 194 unrelated individuals with Turkish ancestral origin living in the Marmara Region. The study population is categorized into two groups according to their body mass index (BMI) overweight/obese group (BMI≥25) and normal weight group (18.5<BMI<25). Genotyping of the *CNR1 rs1049353*, *MGLL rs60430*, and *FAAH rs324420* polymorphisms was performed using the Real-Time Polymerase Chain Reaction (RT-PCR).

Results: The GG genotype frequency of *CNR1 rs1049353* was significantly higher in overweight/obese individuals than in normal weight individuals (75.5 vs. 61.4 %, respectively; *p=0.034*). No difference was observed in terms of *MGLL rs604300* and *FAAH rs324420* polymorphisms between the groups (*p>0.05*). The genotype distributions of *CNR1*, *MGLL*, *and FAAH* genes were compatible with the Hardy–Weinberg equilibrium (*p>0.05*).

Conclusions: Our findings supported that individuals with AA/AG genotype of *CNR1 rs1049353* have reduced overweight/obesity risk; however, neither *FAAH rs324420* nor *MGLL rs604300* showed any association. A relatively smaller sample size and lack of inclusion of obesity-related measurements could be considered the limitations of this study. Further research is needed to confirm or disprove our results.

Keywords: obesity; overweight; endocannabinoid system; *CNR1*; *FAAH*; *MGLL*

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Introduction

Obesity continues to be a serious health burden worldwide; such that its prevalence has reached epidemic proportions [1]. The World Health Organization (WHO) reported that 43 % of adults over 18 years of age were overweight and 16 % were living with obesity corresponding to one out of eight people in 2022 [2].

The development of obesity is a multifactorial process that has not been fully elucidated yet. Although it is

generally accepted that psychological, behavioral, and biological factors are part of it, even intrauterine life, endocrine-disrupting chemicals, and gut microbiome have an impact [3, 4]. Nevertheless, it is well-known that the direct cause of obesity is overeating, i.e. excessive calorie intake.

The regulation of food intake in the body is controlled by a central and peripheral mediator such as hormones, peptides, transmitters, and endocannabinoids [5, 6]. Central regulation is responsible for nutritional food intake to maintain energy homeostasis, whereas peripheral regulation refers to satiety signals and adiposity [4, 5].

The Endocannabinoid System (ECS) is a complex signaling system that modulates numerous physiological processes such as appetite, food intake, and energy balance [7]. The ECS encompasses cannabinoid receptors (CB1 and CB2), endocannabinoids, and enzymes responsible for the biosynthesis and deactivation of these ligands (FAAH; Fatty acid amide hydrolase, MAGL; Monoacylglycerol lipase). While CB1 is abundant in the central nervous system, CB2 is particularly present in the immune cells, spleen, and some peripheral tissues [8]. It is known that the CB1 receptor activation in the brain stimulates appetite and eating behavior [9]. CB1 receptors, encoded by the *CNR1* gene, are also related to losing control over food intake, resulting in overeating [10]. Likewise, recent studies in animal models suggest that CB2 receptors also modulate food intake [11]. On the other hand, a positive relationship has been found between endocannabinoid levels and obesity [12]. According to experimental studies, *FAAH gene*-deficient animal models tended to gain weight, confirming that FAAH activity suppresses food intake [13, 14]. Lately, convincing evidence has been accumulated that CB1 receptors are isolated from adipose tissue where they directly impact lipid metabolism and adiposity [4]. All this information taken together highlights the ECS involvement in the pathogenesis of obesity at the central and peripheral levels.

Previous studies have constantly emphasized the role of genetics in the obesity pathogenesis [15]. Therefore, it is crucial to identify obesity-related genes and gene polymorphisms to comprehend obesity risk. The single nucleotide polymorphisms (SNP) of endocannabinoid system genes such as the Cannabinoid receptor 1 (*CNR1*), Monoacylglycerol lipase (*MGLL*), and Fatty acid amide hydrolase (*FAAH*) genes are promising candidates in this regard due to the following reasons [16]:

i) Functional polymorphisms of *CNR1* can cause changes in the CB1 receptor activation, which can trigger excessive calorie intake in the body.

ii) Functional polymorphisms of FAAH and MAGL encoding genes (i.e. *FAAH* and *MGLL*, respectively) that change the activity of the degrading enzymes of endocannabinoids can alter the circulating orexigenic endocannabinoid levels, which contributes to the establishment of the obese phenotype.

Although genetic variations in *CNR1* and *FAAH* have previously been shown to be associated with overweight and obesity phenotypes, some have failed to find an association. Recently, a meta-analysis showed that individuals with the mutant genotype of *CNR1* tended to have a lower body mass index (BMI) [17]. In another study, researchers have found that the mutant allele of *FAAH* is associated with higher BMI and anthropogenic measurements [18]. Since no studies were found regarding the *MGLL* polymorphisms and obesity relationship in the literature, its contribution to obesity pathogenesis remains unclear. However, preclinic studies in animal models have indicated that *MGLL* is associated with obesity-related outcomes [14]. In this sense, we aimed to evaluate whether genetic polymorphisms of *CNR1*, *FAAH*, and *MGLL* genes influence overweight or obese phenotype in individuals with Turkish ancestral origin.

Materials and methods

Study population and sampling

This study was approved by the Uskudar University Non-Interventional Research Ethics Board (15/05/2017, No:05/32) and performed with 195 unrelated healthy individuals consisting of 21 females and 174 males with Turkish ancestral origin living in the Marmara Region. The study population was recruited from İstanbul Erenköy Training and Research Hospital and NP İstanbul Neuropsychiatry Hospital. Demographics such as age, gender, educational status, etc. were reached by questionnaire. Informed consent was obtained from all participants after an explanation of confidentiality.

Peripheral blood samples were drawn from each participant by venipuncture using ethylenediamine tetraacetic acid (EDTA) tubes. Body weight (kg) and height (centimeter) of all participants were measured. Body Mass Index (BMI) was calculated according to the following formula:

$$\text{BMI} = \text{Weight in kilogram} / \left(\text{Height in meters}\right)^2$$

Participants were categorized into two groups according to the criteria of WHO as overweight/obese group and the normal weight group (Table 1). Considering the overall study population, 65 individuals (33.33 %) were overweight and 29 individuals (14.87 %) were obese, while 101 individuals

(51.80 %) fell within the healthy weight range (18.5<BMI<25). 15 (51.72 %) of obese individuals have concomitant metabolic disorders, predominantly diabetes.

DNA isolation and genotyping

The genomic DNA was extracted from blood samples using the Exgene™ Blood SV mini DNA isolation kit according to the manufacturer's protocol (GeneAll Biotechnology®, Seoul, Korea). Purified DNA samples were stored at −20 °C until analysis. Genotyping of *CNR1*, *FAAH*, and *MGLL* gene was performed by the Real-Time Polymerase Chain Reaction (RT-PCR) using LightCycler Fast-Start DNA Master HybProbe and Light-SNiP probes which hybridize on the PCR fragments and emit a fluorescent signal on Roche Lightcycler 480 platform (Roche®, Germany). PCR was carried out in 12 µL final volume containing 2 µL DNA for each. Melting curve analysis was carried out and the genotypes were identified with the specific melting points (T_m) of the alleles. PCR steps as follows: denaturation at 95 °C for 10 min, 45 cycles with quantification at 95 °C for 10 s, 60 °C for 10 s and 72 °C for 15 s, melting at 95 °C for 30 s, 40 °C for 2 min and 75 °C for 2 min and cooling at 40 °C for 30 s. A negative control was used for each 96-well plate. The same RT-PCR protocol was applied to all studied SNPs.

Statistical analysis

SPSS version 23 package program was used to analyze the data obtained from this research. The normality of continuous variables was assessed with the Kolmogorov- Smirnov test. The Student's *t*-test was used to compare differences between the mean values of the data with normal distribution, the Mann-Whitney U test was used to compare the groups in case of skewed distribution. Categorical variables were analyzed with Pearson's Chi-square or Fisher's exact test. The data were presented as a number of cases and percentages [n (%)], while descriptive statistics for continuous variables were presented as median (min–max) or mean ± SD, where applicable. The deviation from the Hardy–Weinberg equation was assessed using the Chi-square Goodness of fit test. p-Value less than 0.05 was considered statistically significant.

Table 1: Classification of individuals in this study according to WHO criteria for obesity.

Body-mass index (kg/m^2)	Class	n (%)
≥30 kg/m^2	Obese	29 (14.87)
≥25–29.9 kg/m^2	Overweight	65 (33.33)
[a]<25 kg/m^2	Normal	101 (51.80)

[a]BMI less than 18.5 (underweight) was not included in this study.

Results

We determined the genotype and allele frequencies of *CNR1 rs1049353*, *MGLL rs604300*, *and FAAH rs324420* polymorphisms and their possible link between overweight/obesity risk in Turkish individuals. Descriptive statistics of the study population are presented in Table 2. A statistically significant difference was determined between normal weight and overweight/obese individuals in terms of gender, age, weight, and BMI (*p<0.005*). There was no statistically significant difference in education and smoking status between the groups (*p=0.649* and *p=0.844*, respectively). The groups were similar in terms of mean and median height.

The genotype distributions and allele frequencies of *CNR1*, *FAAH*, and *MGLL* in the normal weight group and overweight/obese group are given in Table 3. Genotype

Table 2: Descriptive statistics of the study population.

	Overall (n=195)	Normal (n=101)	Overweight/ obese (n=94)	*p-Value*
Gender n (%)				
Female	21 (10.77)	17 (16.80)	4 (4.30)	***0.005***
Male	174 (89.23)	84 (83.20)	90 (95.70)	
Age				
Mean ± SD	28.33 ± 8.18	25.68 ± 5.66	31.18 ± 9.44	***<0.05***
Median (min–max)	26 (18–65)	25 (18–44)	29 (18–65)	
Education n (%)				
Elementary	19 (9.70)	8 (7.92)	11 (11.70)	*0.649*
Middle	37 (19.00)	22 (21.78)	15 (15.96)	
High	46 (23.60)	23 (22.77)	23 (24.47)	
Graduate/ postgraduate	93 (47.70)	48 (47.53)	45 (47.87)	
Smoking n (%)				
Yes	94 (48.21)	48 (47.52)	46 (48.94)	*0.844*
No	101 (51.79)	53 (52.48)	48 (51.06)	
Weight, kg				
Mean ± SD	76.38 ± 15.31	65.78 ± 8.01	87.77 ± 12.91	***<0.05***
Median (min–max)	74.0 (46–124)	65 (46–86)	85 (68–124)	
Height, m				
Mean ± SD	1.75 ± 0.08	1.75 ± 0.08	1.75 ± 0.07	*0.953*
Median (min–max)	1.75 (1.56–1.98)	1.75 (1.56–1.97)	1.75 (1.57–1.98)	
BMI, kg/m^2				
Mean ± SD	24.79 ± 4.58	21.35 ± 1.91	28.50 ± 3.60	***<0.05***
Median (min–max)	24 (19–43)	21 (19–24)	28 (25–43)	

distributions were compatible with the Hardy–Weinberg equilibrium for all studied SNPs (*p>0.05*). The study population was dichotomized in homozygous and heterozygous/mutant genotypes of *CNR1*, *FAAH*, and *MGLL* since there were not enough numbers for mutant genotypes in the dataset to compare the two groups.

The A allele (mutant) frequencies of *CNR1 rs1049353* in the normal weight group and overweight/obese group were 0.22 and 0.13, respectively. The wild genotype (GG) frequency of *CNR1 rs1049353* was significantly higher in overweight/obese individuals compared to normal weight individuals (75.5 vs. 61.4 %, respectively; *p=0.034*; OR=1.942; 95 % CI). Similarly, the G allele frequency of *CNR1 rs1049353* was significantly higher in the overweight/obesity group (*p=0.02*8; OR=0.816; 95 % CI).

The variant allele frequency of *FAAH rs324420* in the normal weight group and overweight/obese group was found to be 0.17 and 0.18, respectively. The CC or AA+ AC genotypes of *FAAH rs324420* showed no statistically significant effect on the overweight/obesity risk (p=0.607). Similarly, the C and A alleles of *FAAH rs324420* were not associated with obesity (*p=0.466*; OR=0.826; 95 % CI). For the *MGLL* gene, no significant effect of the variant allele (T) was found on obesity risk (*p=0.838*; OR=1.081; 95 % CI).

Table 3: The comparison of allele frequencies and genotype distributions of *CNR1 rs1049353*, *FAAH rs324420*, and *MGLL rs604300* polymorphisms between normal weight and overweight/obese individuals.

Gene/ polymorphism		Normal n (%)	Overweight/ obese n (%)	*p-Value*	Odds ratio (95 % CI)
CNR1	A	44 (21.8)	25 (13.3)	***0.028***	1.816
(rs1049353)	G	158 (78.2)	163 (86.7)		(1.061–3.108)
	A/A+A/G	39 (38.6)	23 (24.5)	***0.034***	1.942 (1.047–3.601)
	G/G	62 (61.4)	71 (75.5)		
FAAH	A	34 (16.8)	37 (19.7)	*0.466*	0.826
(rs324420)	C	168 (83.2)	151 (80.3)		(0.494–1.382)
	A/A+A/C	33 (32.7)	34 (36.2)	*0.607*	0.856 (0.474–1.547)
	C/C	68 (67.3)	60 (63.8)		
MGLL	C	187 (92.6)	173 (92.0)	*0.838*	1.081
(rs604300)	T	15 (7.4)	15 (8.0)		(0.513–2.277)
	C/C	86 (85.1)	80 (85.1)	*0.993*	1.003
	C/T + T/T	15 (14.9)	14 (14.9)		(0.456–2.209)

Discussion

Obesity is associated with reduced life quality and shorter life expectancy. Given the global increase in obesity rates, it is crucial to understand causal factors that may contribute to obesity phenotype and obesity-related outcomes. It is well-known that ECS is linked with food consumption and hedonic eating. In general, activation of ECS increases the food intake [19, 20].

The scientific research related to ECS genes has remarkably increased in the last decades to understand the molecular genetic basis of obesity. In this study, *rs1049353* SNP in *the CNR1* gene, *rs604300* SNP in *the MGLL* gene, and *rs324420* SNP in *the FAAH* gene were genotyped in 94 overweight/obese and 101 normal weight individuals to assess a possible link between these polymorphisms and overweight/obesity risk. As a result of a detailed literature search, we state that this is the first study that investigates the relationship between endocannabinoid system genes and obesity susceptibility in individuals with Turkish ancestral origin. Our findings supported that individuals with AA/AG genotype of *CNR1 rs1049353* have reduced overweight/obesity risk; however, neither *FAAH rs324420* nor *MGLL rs604300* showed any association. Previous studies assessing the relationship between *CNR1 rs1049353*, *FAAH rs324420*, *MGLL rs604300* polymorphisms, and obesity or obesity-related phenotypes in different populations are summarized in Table 4.

The frequency of the GG genotype (wild type) of *CNR1 rs1049353* was significantly higher in the overweight/obese group than the normal weight group. *CNR1 rs1049353* is a silent variation in codon 435 resulting in the substitution of G to A at the position of 1359 (G1359A). The rare A allele (mutant) frequency of *CNR1* in this study (21.8 %) was similar to that in previous studies conducted in the Caucasian and Brazillian populations (20.9 and 22 %, respectively) [21, 23]. The minor 435A allele was more common in normal weight individuals than overweight/obese individuals. Previous studies reported that A allele carriers have better metabolic profiles, anthropometric measures, reduced BMI, and obesity risk compared to G allele carriers (Table 4). Consistent with the findings of this study, a meta-analysis has elucidated that individuals with the AA/GA genotype had lower BMI compared to those with the GG genotype [17]. Having the A allele of *CNR1 rs1049353* possesses a protective effect on overweight/obesity risk in this study. However, more research is needed to disprove or confirm our results.

The *rs324420* variant of *FAAH* is a missense mutation that leads to a change at position 385 from C to A, resulting in an amino acid substitution from proline to threonine

Table 4: Previously published studies assessing the relationship between *CNR1 rs1049353*, *FAAH rs324420*, *MGLL rs604300* polymorphisms and obesity or obesity-related phenotypes in different populations (in chronological order).

Gene/ polymorphism	Sample size (n)	Ethnicity	Study design	Significance	Finding	Reference
CNR1 rs1049353	451	Caucasian	RCT	-	*CNR1 rs1049353* variant did not significantly affect BMI or metabolic traits.	[21]
	176	Italian	Cross-sectional	+	*CNR1* variant A allele was significantly associated with a lower BMI.	[22]
	756	Caucasian	Case-control	-	No significant association was observed between *CNR1 rs1049353* polymorphism and central obesity.	[23]
	66	NA	Cross-sectional	+	The mutant genotype is related to a better cardiovascular profile (weight, BMI, fat mass, waist circumference, insulin, HOMA, and c reactive protein) than the wild-type.	[24]
	518	Chinese Han	Case-control	+	GG genotype is related to the risk of metabolic syndrome. GA and AA genotypes in subjects with metabolic syndrome had relatively lower levels of BMI, waist circumference, homeostasis model assessment of insulin resistance (HOMA-IR), and serum triglycerides.	[25]
	774	Danish	Cross-sectional	+	GA/GG genotype carriers tended to have higher visceral fat mass and serum triglyceride levels.	[26]
	290	NA	Cross-sectional	+	AA/GA genotypes are related to a better cardiovascular profile (triglyceride, high-density lipoprotein, insulin, homeostasis model assessment levels) than wild-type.	[27]
	917	NA	Cross-sectional	+	AA/GA genotypes are related to a lower prevalence of metabolic syndrome.	[28]
	118	NA	Cross-sectional	+	*CNR1 rs1049353* polymorphism is related to a specific macro-nutrient intake that mediates lower adiposity.	[29]
	258	NA	RCT	+	An allele is related to a lack of improvement in leptin levels.	[30]
	86	NA	RCT	+	A allele carriers showed an improvement in insulin resistance secondary to weight loss after liraglutide treatment. Non-carriers of the A allele showed an improvement in cholesterol levels after weight loss.	[31]
	796	NA	Cross-sectional	+	*CNR1 rs1049353* polymorphism is related to specific macronu-trient intake. The A allele carriers have better lipid profiles (tri-glycerides and HDL cholesterol) than non-carriers.	[32]
	71	NA	Cross-sectional	+	*CNR1 rs1049353* polymorphism is related to physical fitness level (GG genotype had better speeds and accelerations) and could be used as an index for predicting the risk of obesity.	[33]
	189	Mexican	Cross-sectional	+	AA/GA genotypes are related to a lower risk of presenting high scores of restriction in food intake compared with the GG genotype.	[34]
	195	Turkish	Case-control	+	GG genotype is associated with the risk of increased BMI and being overweight/obese.	*This study*
FAAH rs324420	2,667	European-American African-American Asian	Cross-sectional	+	The median BMI was significantly greater in the AA genotype compared to the CA and CC genotypes.	[35]
	451	Caucasian	Cohort	+	A allele carriers have a significantly greater reduction in tri-glycerides and total cholesterol levels after 6 weeks of a low-fat diet.	[21]
	5,801	Danish	Case-control	-	No robust evidence for an association of the *FAAH rs324420* variant with overweight, obesity, and any related quantitative traits.	[36]
	5,109	French	Case-control	+	*FAAH rs324420* polymorphism modestly contributes to class III adult obesity (The C allele was the risk allele).	[37]
	299	Caucasian	Case-control	+	Significant over-representations of the A allele in overweight/ obese subjects.	[38]

Table 4: (continued)

Gene/ polymorphism	Sample size (n)	Ethnicity	Study design	Significance	Finding	Reference
	424	Greek	Case-control	–	No evidence for an association of the *FAAH rs324420* variant with severe obesity and any related quantitative traits.	[39]
	2,415	NA	Cohort	–	The *FAAH rs324420* variant is not associated with measures of adiposity.	[40]
	248	NA	Cohort	+	CA/AA genotypes were associated with a lack of improvement in glucose, insulin, HOMA, and leptin levels after weight loss.	[41]
	163	NA	Case-control	+	CA/AA genotypes and the A allele were found to be associated with antipsychotic-induced weight gain.	[42]
	122	NA	Cohort	+	A allele was associated with larger improvements in glucose, total cholesterol, low-density lipoprotein cholesterol, body mass, and waist circumference after a dietary intervention.	[43]
	192	Chinese Han	Case-control	+	CA/AA genotypes were higher in subjects with metabolic syndrome. CA and AA genotypes of subjects with metabolic syndrome had relatively elevated levels of waist circumference, BMI, homeostasis model assessment of insulin resistance (HOMA-IR), triglycerides, and lower levels of high-density lipoprotein (HDL).	[44]
	453	German	Cohort	–	No evidence for an association of the *FAAH rs324420* variant with weight reduction.	[45]
	200	NA	Case-control	+	The AA genotype was associated with higher levels of anandamide and lower adiponectin levels compared to CC and CA combined.	[46]
	266	NA	Case-control	+	A allele was greater in overweight/obese individuals. A-allele carriers had significantly higher BMI, waist circumference, neck circumference, waist-to-height ratio, and body fat mass. After adjusting, having the CA/AA genotypes increased the probability of obesity risk almost two times.	[47]
	667	African-American Caucasian	Cross-sectional	+	The prevalence of the *FAAH rs324420* variant was higher in obese subjects.	[48]
	189	Mexican	Cross-sectional	+	CC genotype is related to higher body weight and body fat, but not dysfunctional eating patterns.	[34]
	195	Turkish	Case-control	–	*FAAH rs324420* is not related to BMI and overweight/obesity.	*This study*
MGLL rs604300	195	Turkish	Case-control	–	*MGLL rs604300* is not related to BMI and overweight/obesity phenotype.	*This study*

Significant (+) & non-significant (–); RCT, randomized clinical trial; NA, not available.

(P129T). This variation increases the sensitivity of FAAH to degradation and decreases the enzymatic activity similar to its pharmacological blockage, resulting in the overactivation of the CB1 receptors through the increased levels of the endocannabinoids [18]. Most of the studies have demonstrated an association between the *FAAH rs324420* mutant allele (A) and obese phenotype [35, 38, 47, 48], but some have reported opposite findings or no association (Table 4). In the present study, no association existed between *FAAH rs324420* polymorphism and overweight/obesity risk, in parallel with the findings of some other studies [36, 39, 40]. The rare A allele (mutant allele) frequency of *FAAH* in our study (16.8 %) is found to be the same as that in the study of Monteleone et al. (16.8 %) [42]. Differences in the study design and endpoints of published papers render it difficult to draw a definitive conclusion. These conflicting results could be explained by *i)* the multiple phenotypic effects of the ECS, *ii)* the influence of other risky gene loci, and *iii)* gene-environment interactions. Further studies investigating the polygenic associations that control the FAAH activity in specific ethnic groups with larger sample sizes might clarify the possible role of this variant in obesity pathogenesis.

We can not argue the impact of *MGLL rs604300* polymorphism on obesity risk since there are no genetic association studies involving the *MGLL rs604300* polymorphism in the literature. However, preclinic studies in genetically modified animal models have indicated that the *MGLL* gene

is related to weight gain, body fat mass, obesity, and obesity-related outcomes [49]. Moreover, promoter, intron 2, and intron 3 of *MGLL* were found to be associated with BMI, according to a sequence-based association study [50]. Matheson et al. have suggested that MAGL might be a potential drug target in the obesity treatment [14]. However, there is a huge gap in the literature in terms of the relationship between *MGLL* polymorphisms and obesity. More human studies are needed to reveal how *MGLL* variants modulate individual susceptibility to obesity.

We considered that the limitations of our study are relatively smaller sample size and a lack of inclusion of obesity-related anthropogenic and metabolic measures. Another limitation of our study is the unequal number of female and male individuals in the study population and the uneven gender distribution among groups. Different results might have yielded with a different study design which includes obesity endophenotypes. We recommend that future studies investigate the relationship between *CNR1*, *FAAH*, and *MGLL* mutant, heterozygous, and homozygous variants and obesity risk by incorporating anthropometric and metabolic parameters such as body fat mass, waist-to-hip ratio, waist circumference, fasting insulin level, insulin resistance, serum lipid profile in a Turkish population.

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Research ethics: This research related to human use has complied with all relevant national regulations, institutional policies and in accordance with the tenets of Helsinki Declaration and has been approved by the Uskudar University Non-Interventional Research Ethics Board (15/05/2017, No.:05/32).

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