

## ***FTO* gene-lifestyle interactions on serum adiponectin concentrations and central obesity in a Turkish population**

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## ***FTO* gene-lifestyle interactions on serum adiponectin concentrations and central obesity in a Turkish population**

The aim of the study was to investigate whether lifestyle factors modify the association fat mass and obesity-associated (*FTO*) gene single nucleotide polymorphisms (SNPs) and obesity in a Turkish population. The study included 400 unrelated individuals, aged 24-50 years recruited in a hospital setting. Dietary intake and physical activity were assessed using 24-hour dietary recall and self-report questionnaire, respectively. A genetic risk score (GRS) was developed using *FTO* SNPs, rs9939609 and rs10163409. Body mass index and fat mass index were significantly associated with *FTO* SNP rs9939609 ( $P=0.001$  and  $P=0.002$ , respectively) and GRS ( $P=0.002$  and  $P=0.003$ , respectively). The interactions between SNP rs9939609 and physical activity on adiponectin concentrations, and SNP rs10163409 and dietary protein intake on increased waist circumference were statistically significant ( $P_{\text{interaction}}=0.027$  and  $P_{\text{interaction}}=0.044$ , respectively). This study demonstrated that the association between *FTO* SNPs and central obesity might be modified by lifestyle factors in this Turkish population.

Keywords: *FTO* gene variant; obesity; gene-diet interaction; adiponectin; genetic risk score; physical activity

### **Introduction**

Obesity has been recognised as a worldwide public health problem due to its rising prevalence and concomitant health problems. The prevalence of overweight and obesity in Turkey were reported as 64.4% and 28.8%, respectively by WHO (WHO, 2018). Obesity can lead to other chronic diseases including type 2 diabetes (T2D), cardiovascular diseases (CVD), hypertension, cancer and osteoarthritis (Forse et al. 2020). A combination of

interactions between genetic and environmental factors is required for the development of a complex disease such as obesity (Franks and McCarthy 2016; Milagro et al. 2020). Studies have identified approximately 140 genes to be associated with obesity, and the fat mass and obesity associated (*FTO*) gene has been reported to be the strongest susceptibility gene for human obesity (Pigeyre et al. 2016).

The *FTO* gene is located on chromosome 16q12.2 and codes for a protein with 2-oxoglutarate dependent nucleic acid demethylase activity which is involved in DNA repair and the accumulation of fat in the body (Clifton et al. 2006; Chen and Du 2019). *FTO* is highly expressed in the brain, including the hypothalamus, adipocytes, pancreatic islet cells, and adrenal glands (Frayling et al. 2007). *FTO* gene has been suggested to control energy homeostasis and food intake (Abete et al. 2020). Previous studies have shown that, of the various obesity susceptibility genes, single-nucleotide polymorphisms (SNPs) located in the first intron of *FTO* gene has provided the strongest evidence for genetic predisposition to obesity (Frayling et al. 2007; Scuteri et al. 2007; Speliotes et al. 2010; Loos and Yeo 2014; Babenko et al. 2019; Fonseca et al. 2020). The minor allele 'A' of the *FTO* SNP rs9939609 has been consistently associated with higher BMI in various populations (Frayling et al. 2007; Hertel et al. 2011; Peng et al. 2011; Corella et al. 2012; Li et al. 2012; Qi et al. 2014; Wang et al., 2020; Schlauch et al. 2020). Furthermore, a meta-analysis reported that the association between the SNP rs9939609 and BMI was replicated in 13 cohorts with 38,759 participants, where individuals with the 'AA' genotype had 1.67-time higher odds of obesity than those with the 'TT' genotype (Frayling et al. 2007). In the Turkish population, the risk alleles of the *FTO* rs1421085 and rs9939609 polymorphisms were shown to have significant

associations with the risk of obesity in women and metabolic syndrome (MetS) in men (Guclu-Geyik et al. 2016).

Turkish adults are characterized with low levels of total and high-density lipoprotein cholesterol, and high risk of CVD, that distinguish them from Europeans (Onat 2001). They also have increased susceptibility to impaired glucose tolerance and MetS primarily driven by obesity (Onat and Can 2014). Among the non-communicable diseases (NCDs) that accounted for 88.0% of deaths in Turkey, CVD has shown to contribute to 47.73% of overall deaths (WHO, 2018). Targeting modifiable risk factors for NCDs including obesity could prevent many deaths. Therefore, several health promotion campaigns such as “Reducing Portion Sizes” and “Move for Health” have been implemented for the prevention of obesity in Turkey (WHO, 2016; OECD, 2017). However, obesity is a multifactorial disorder, and identifying gene-environment interactions are needed to understand the aetiology and pathophysiology of obesity and also to develop more effective personalised preventative strategies (Castillo et al. 2017; Dahlman and Ryden 2020). To date, several *FTO*-dietary intake interactions on obesity-related outcomes have been examined in different populations (Grau et al. 2009; Sonestedt et al. 2009; Lappalainen et al. 2012; Ortega-Azorin et al. 2012; Phillips et al. 2012; Vimalaswaran et al. 2012; Qi et al. 2014; Merritt et al. 2018; Saber-Ayad et al. 2019) however, there are no such studies to date in a Turkish population. The investigations of the gene-diet interactions in different ethnic groups are crucial to develop personalised nutrition strategies for each ethnic group due to the genetic heterogeneity (Vimalaswaran 2017). The *FTO* SNP rs9939609 has been associated with several dietary components including dietary protein intake (Lappalainen et al. 2012; Qi et al. 2014; Merritt

et al. 2018) and the SNP rs10163409 in *FTO* was among the top associations in a large genome-wide meta-analysis study (GWAS) for total caloric intake (Chu et al. 2013). Therefore, this study aimed to assess whether *FTO* variants, rs9939609 and rs10163409, are associated with obesity in 400 Turkish individuals and to determine whether these SNPs interact with dietary intake and physical activity on obesity outcomes.

## **Materials and Methods**

### ***Study population***

A total of 400 unrelated individuals, aged 24-50 years, were recruited from the outpatient clinic of Department of Endocrinology and Metabolism at the Hacettepe University Hospitals, Ankara, Turkey. This study was conducted as part of the GeNuIne Collaboration that investigates the interactions between genetic and dietary factors on metabolic diseases in different ethnic groups (Vimalaswaran 2017). The study participants were screened based on the following inclusion criteria: 1) routine visits to the outpatient clinic, 2) aged 18-50 years, and 3) having a BMI  $\geq 18.50$  kg/m<sup>2</sup>. The exclusion criteria were: 1) having specific health problems including, liver and kidney diseases, mental and psychological disorders, history of cancer, and serious endocrine disorders (hypothyroidism, hyperthyroidism or hypopituitarism), 2) history of bariatric surgery, 3) being pregnant or lactating, 4) using drugs that affect body weight. Researchers informed and invited the eligible participants for their participation in to the study. The study was approved by the local ethics committee of Hacettepe University (GO 15/612-11), and all the participants provided the signed written consent.

### ***Study design***

A cross-sectional case-control study design was used, where participants were divided into two groups: obese (BMI  $\geq 25.00$  kg/m<sup>2</sup>, n=200) and non-obese (BMI= 18.50-24.99 kg/m<sup>2</sup>, n=200). All participants underwent a physical examination by the research endocrinologists,

followed by clinical, biochemical and lifestyle assessments, and genetic analysis of *FTO* SNPs rs9939609 and rs10163409.

### ***Anthropometrical Measurements***

Body weight and height were measured by standard methods using a calibrated digital scale (Seca 220 Scale, Germany). BMI calculation was based on the body weight (in kilograms) divided by the square of height (in meter) (WHO, 2020). BMI classification of the WHO was used to classify the individuals as non-obese ( $\text{BMI} < 25.00 \text{ kg/m}^2$ ) and obese ( $\text{BMI} \geq 25.00 \text{ kg/m}^2$ ) (WHO, 2005). The waist circumference (WC) was measured by a standard method (WHO, 2011). Increased WC (central obesity) was defined based on cut-points established for Turkish adults ( $\text{WC} \geq 90 \text{ cm}$  for men/  $\geq 80 \text{ cm}$  for women) (Sonmez et al. 2013). Body composition was analysed by bioelectrical impedance using the Tanita MC-980 MA Multi Frequency Segmental Body Composition Analyzer (USA). Fat mass index (FMI) was calculated based on the fat mass (in kilograms) divided by the square of height (in meter) (Peltz et al. 2010). All anthropometrical measurements were taken by the research dieticians.

### ***Biochemical and clinical measures***

Serum adiponectin was analysed by ELISA kits (Ebioscience, Austria) at Hacettepe University Hospitals, Clinical Pathology Laboratory. The physical examination included the measurement of systolic (SBP) and diastolic blood pressure (DBP) using a stethoscope and sphygmomanometer in the right arm of the participants after sitting in a comfortable position in a quiet room for at least 15 min. Both blood pressures were measured twice at 5-minute intervals and recorded on average (Frese et al. 2011).

### ***Dietary assessment***

Dietary intake was assessed using 24-hour dietary recall method that was carried out by trained research dietitians. A photographic atlas of food portion sizes and common household measures were used to facilitate the quantification of the amount of food consumed. Total energy, macro- and micronutrient intakes of participants were analysed from the records using BeBIS software (BeBIS, Nutrition Information System, Version 8).

### ***Other lifestyle factors***

The socio-demographic characteristics, family and medical history, smoking and alcohol consumption were recorded. The physical activity level was assessed using the Turkish version of the International Physical Activity Questionnaire (IPAQ) (Saglam et al. 2010).

### ***SNPs selection and genotyping***

*FTO* gene was selected based on its consistent and strong associations with obesity traits in large-scale GWASs (Frayling et al. 2007). The SNP rs9939609 is the most commonly studied variant and consistently associated with obesity phenotypes across multiple ethnicities (Frayling et al. 2007; Hertel et al. 2011; Peng et al. 2011; Corella et al. 2012; Li et al. 2012; Loos and Yeo 2014; Qi et al. 2014) and SNP rs10163409 has been shown to be associated with dietary energy intake from macronutrients (Chu et al. 2013). Therefore, *FTO* SNPs, rs9939609 and rs10163409, which have been shown to be associated with obesity traits and dietary intake in large GWASs, were genotyped. The genotype frequencies of the *FTO* SNPs, rs9939609 and rs10163409, were in Hardy Weinberg equilibrium ( $p > 0.05$ ).

The genomic DNA was extracted from the whole blood in K2EDTA containing tubes by the salting out method. Genotyping of the SNPs, rs9939609 and rs10163409, were performed using KASP assay (a competitive allele-specific polymerase chain reaction that incorporates a fluorescent resonance energy transfer quencher cassette), and the KASP primers were designed using Kraken software system (LGC, <https://www.lgcgroup.com>). Genotyping assays were carried out according to the manufacturer's instructions with a 7500 Real time PCR System (Applied Biosystems). The following thermal cycling profile were used: 15 min at 94°C; 10 cycles of 20 s at 94°C, 60 s at 61°C with decrement -0.6°C/per cycle and 26 cycles of 20 s at 94°C, 60 s at 55°C; 60 s at 37°C.

### ***Statistical analysis***

SPSS software (version 23.0) was used for statistical analysis. The Hardy-Weinberg equilibrium was assessed using the  $\chi^2$  goodness-of-fit test. Genotype frequencies and distribution in groups were compared using Pearson's chi-squared test. Continuous variables are presented as means and standard deviations (SD), and groups were compared using the independent t-test.

As the number of individuals with rare homozygous genotypes was low, a dominant model was used, where common homozygous genotypes were compared to combined rare homozygous and heterozygous genotypes. A genetic risk score (GRS) was created from both the *FTO* SNPs where the presence of one risk allele of any of the variants was scored as one point. This GRS ranged from 0 (homozygous individuals for non-risk alleles) to 4 points (homozygous individuals for the risk alleles of both *FTO* polymorphisms). The GRS variable

was then categorised into two groups based on the number of points; 1st group: individuals with scores of  $<2$  points; 2nd group: individuals with scores of  $\geq 2$  points.

The independent and joint effects of *FTO* SNPs on the risk of obesity were assessed using the odds ratios (ORs) and 95% confidence intervals (CIs) that were calculated by logistic regression models. Also, the associations between *FTO* polymorphisms (separately and joint) and the continuous outcomes were tested using general linear models. Models were adjusted for age, gender, hypertension, CVD and obesity status wherever appropriate. Furthermore, *FTO* gene-environment interactions on continuous and categorical outcomes were tested using linear and logistic regression models, respectively. Interactions were investigated by including the interaction terms (e.g., carbohydrate\*genotype) in the regression models. Environmental factors that were investigated included dietary intake (carbohydrate, protein, fibre and fat intakes in grams/day) and physical activity. Furthermore, statistically significant interactions were investigated in more depth, where individuals were stratified by the tertiles of the lifestyle factor.

## **Results**

### ***Characteristics of the Participants***

Obese individuals were older, and had higher BMI, WC and FMI and lower adiponectin levels than the controls ( $P < 0.001$ , for each). The cases and controls were not statistically different in terms of their food intake and physical activity levels ( $P > 0.05$ ) (Table 1).

### ***Associations between *FTO* variants and obesity-related traits***

Genotype distributions and minor allele frequencies (MAFs) for both SNPs are shown in Table 2. The MAFs of the SNPs, rs10163409 and rs9939609, were T=0.37 and A=0.39,

respectively. The associations between SNP rs9939609 and BMI ( $P=0.001$ ) and FMI ( $P=0.002$ ) were found significant where the 'A' (AT/AA) allele carriers had significantly higher BMI and FMI than 'TT' homozygotes (Table 3). Furthermore, 'A' allele carriers had significantly higher WC ( $P=0.007$ ) and lower adiponectin levels ( $P=0.031$ ) compared to non-carriers. The *FTO* SNP rs10163409 did not show any significant association with obesity traits (Table 3).

### ***Interactions between FTO variants and dietary intake on obesity-related traits***

#### ***FTO gene-dietary protein intake interactions***

The significant interactions between SNP rs10163409 and protein intake on the risk of increased WC ( $P_{\text{interaction}}=0.044$ ) and WC as a continuous variable ( $P_{\text{interaction}}=0.007$ ) were observed. Stratification of the dietary protein intake into tertiles showed that, in the highest tertile group with a mean  $\pm$  SD of  $138\pm 38$  g/day protein intake, 'T' allele carriers of the SNP rs10163409 had a significantly higher risk of central obesity [OR= 3.3 (95% CI: 1.149-9.478),  $P=0.027$ ] than those with 'AA' genotype (Figure 1).

### ***Interactions between FTO variants and physical activity on obesity-related traits***

The interaction between the SNP rs9939609 and physical activity levels on adiponectin concentrations was statistically significant ( $P_{\text{interaction}}= 0.027$ ), where, among those with lowest levels of physical activity, the adiponectin concentrations were significantly lower in the allele 'A' carriers compared to individuals with 'TT' genotype ( $P=0.006$ ) (Figure 2).

### ***Associations between GRS and obesity-related traits***

The GRS was significantly associated with BMI ( $P=0.002$ ), FMI ( $P=0.003$ ) and increased WC ( $P=0.02$ ) (Figures 3a, 3b and 3c). However, the interactions between GRS and lifestyle factors on obesity traits were not found statistically significant.

## **Discussion**

To our knowledge, this is the first study that investigated the interaction between *FTO* SNPs and dietary intake on obesity traits in a Turkish population. This study has identified the

associations of the *FTO* SNP rs9939609 and GRS with obesity traits, and also showed that the physical activity level can modify the effect of the minor allele 'A' of the *FTO* SNP rs9939609 on adiponectin concentrations, a biomarker of metabolic syndrome (Stojanovic et al. 2015). Furthermore, our study has demonstrated that the higher protein intake was associated with higher risk of central obesity among the 'T' allele carriers of the *FTO* SNP rs10163409 compared to non-carriers. Since Turkish adults have a sedentary lifestyle (WHO, 2018), our findings contribute to the development of effective public health strategies focusing on the prevention and management of central obesity and CVD in Turkish population (IHME, 2017).

This study has shown that the risk allele 'A' of the *FTO* SNP rs9939609 was significantly associated with higher BMI and FMI, in agreement with the findings from other populations (Frayling et al. 2007; Do et al. 2008; Hertel et al. 2011; Peng et al. 2011; Corella et al. 2012; Li et al. 2012; Muc et al. 2015; Merra et al. 2020). A meta-analysis performed on 177,330 individuals from multiple ethnicities have demonstrated an association between *FTO* rs9939609 genotype and BMI, suggesting a higher BMI in 'A' allele carriers (effect per allele=0.30 [0.30, 0.35] kg/m<sup>2</sup>, P=3.6\*10<sup>-107</sup>) (Qi et al. 2014). The reported *FTO*-related genetic associations with BMI have also been confirmed in a study in the Turkish population (Guclu-Geyik et al. 2016), where the *FTO* risk allele, 'C', carriers of the SNP rs1421085, which is in a high linkage disequilibrium (LD) (D'=0.967, r<sup>2</sup>=0.85) with the SNP rs9939609, had significantly increased BMI. Furthermore, parallel to the findings of other studies (Vimalleswaran et al. 2012; De Luis et al. 2016; Saucedo et al. 2017), we have also found that the *FTO* SNP rs9939609 was significantly associated with higher WC and lower adiponectin concentrations. On the contrary, there were no significant association between SNP rs10163409 and obesity. This could be explained by the fact that the SNP rs10163409 is not in LD with other *FTO* variants that have shown significant associations with BMI (Chu et al. 2013).

Our study has provided evidence for gene-diet interaction in the Turkish population. We have demonstrated that, among those in the highest tertile of dietary protein intake, the risk of increased WC/central obesity was higher for the minor allele, 'T', carriers of the *FTO* SNP rs10163409 compared to those with AA genotype. To date, this is the first study analysing gene-diet interactions of the SNP rs10163409, suggesting that high intake of dietary protein might negatively affect WC in genetically susceptible individuals. However, studies investigating other *FTO* SNPs (rs1558902 and rs9939609) have reported conflicting results (Zhang et al. 2012; de Luis et al. 2015; Merritt et al. 2018). It has been suggested that following a high protein diet can modulate the genetic effect of *FTO* variants on obesity traits (Zhang et al. 2012; de Luis et al. 2015; Merritt et al. 2018). According to a 2-year weight loss intervention program, carriers of the risk allele 'A' of the *FTO* rs1558902 had a greater weight loss compared to non-carriers when high protein diets were consumed, whereas a negative genetic effect was found in response to a low-protein intake (Huang et al. 2014). The potential mechanism of *FTO* variants - protein intake interaction is still unclear, however, the regulation of food intake and appetite could be influenced. It has been found that the risk allele 'A' of the SNP rs9939609 was significantly associated with a greater reduction in food cravings and appetite scores among individuals who consumed high-protein diet but not in those in the low-protein diet (Huang et al. 2014). Regarding the SNP rs9939609, there were no significant interactions between the *FTO* variants and any of the dietary components on obesity traits. In agreement with our findings, a study of 11,091 adults from five European countries have found no interactions between the rs9939609 variant and the dietary intake of carbohydrate, glycaemic index, protein or fat on BMI, WC, weight gain and risk of obesity (Vimaleswaran et al. 2012). Furthermore, a meta-analysis of 40 population-based studies reported that the total energy or macronutrient intakes had no effect on the association between the SNP rs9939609 and BMI (Qi et al. 2014). In contrast to our finding, a few large-scale studies demonstrated significant interactions between dietary

macronutrient intakes and *FTO* variants in determining BMI (Grau et al. 2009; Sonestedt et al. 2009; Corella et al. 2011; Lappalainen et al. 2012; Ortega-Azorin et al. 2012; Phillips et al. 2012). A cross-sectional study conducted on 4,839 Swedish participants reported an association between the risk allele of the SNP rs9939609 and higher BMI only in individuals with high fat and low carbohydrate consumption (Sonestedt et al. 2009). A similar interaction between the rs9939609 variant and saturated fatty acids (SFA) intake has been detected in 2,163 individuals from two independent populations of the United States, where individuals homozygous for the risk allele 'AA' had a higher BMI compared to other genotypes, only when the intake of SFA was high (Corella et al. 2011). Furthermore, the *FTO* SNP rs8050136, in LD with rs9939609, significantly interacted with carbohydrate intake on obesity risk among Asian Indian population (Vimalaswaran et al. 2016).

Regarding genetic interactions with physical activity, a previous study conducted among 200 Turkish adults found that BMI was higher in homozygous risk allele 'A' carriers of the SNP rs9939609 than the homozygote the 'T' allele carriers among physically inactive individuals (Kirac et al. 2016). The same interaction but on a biochemical measure of obesity (i.e.: adiponectin level), rather than BMI, was replicated in our study using a larger sample size. We found that, among those with lowest levels of physical activity, the adiponectin concentrations were significantly lower in the carriers of the risk allele 'A' of the *FTO* rs9939609 than 'TT' homozygotes. Adiponectin is a hormone produced and secreted by adipose tissue and commonly known for its antihyperglycemic, anti-inflammatory, antiatherogenic, and cardioprotective effects (Richard et al. 2020; Esmaili et al. 2020; Lee and Shao 2014). Studies have reported a strong correlation between the dysregulation of adipokine production and the onset of several metabolic abnormalities including CVD and cancer (Avogaro and de Kreutzenberg 2005; De Pergola and Silvestris 2013; Xiang et al. 2020). The positive correlation between adiponectin levels and physical activity has been demonstrated in several studies (St-Pierre et al. 2006; Jurimae et al. 2010; Sirico et al. 2018),

where higher levels of physical activity have been shown to reduce adiposity which decreases the production of insulin and leptin, and increases adiponectin production (Nurnazahiah et al. 2016). Indeed, it has been reported that serum concentrations of adiponectin are inversely related to BMI, visceral body fat and blood concentrations of glucose, insulin, and triglycerides (De Rosa et al. 2013; Frithioff-Bojsøe et al. 2020). An intervention study conducted in 400 obese women showed that a weight reduction program resulted in a significant increase in adiponectin levels (Mavri et al. 2011). Given that this is the first study to report an interaction between *FTO* variant and physical activity on adiponectin concentrations, the findings need to be replicated in a larger Turkish cohort.

The main strengths of this study include the use of a biochemical marker of obesity (i.e., adiponectin) and a well-characterised population. Nevertheless, there are some limitations which include the small sample size and the use of self-reported measurements in the assessment of dietary intake and physical activity. However, this study has still confirmed the associations between *FTO* SNP rs9939609 and obesity traits which were also reported in previous studies (Frayling et al. 2007; Hertel et al. 2011; Peng et al. 2011; Corella et al. 2012; Li et al. 2012; Merra et al. 2020; Schlauch et al. 2020). Given that obesity is a multifactorial condition, several genetic factors and lifestyle behaviours provide a predisposition to obesity; even though we have focused on the two important lifestyle factors, diet and physical activity, only two genetic variants were examined. However, to date, the *FTO* gene has been shown to be the strongest susceptibility gene for common obesity (Frayling et al. 2007; Scuteri et al. 2007; Speliotes et al. 2010; Loos and Yeo 2014). Furthermore, the cross-sectional design of this study limits the proof of causality. Even though our analysis was adjusted for several confounders, we cannot rule out the residual confounding caused by unknown factors. Therefore, the observed interactions needed to be confirmed in further studies with larger sample sizes.

## **Conclusion**

In summary, this study has confirmed the associations between the risk allele ‘A’ of the *FTO* rs9939609 and GRS, with obesity related traits including BMI and FMI in this Turkish population. Our study suggests that the impact of the *FTO* polymorphisms, rs10163409 and rs9939609, on obesity among Turkish adults might be affected by dietary protein intake and physical activity levels, respectively, suggesting that increased consumption of protein-rich foods and sedentary lifestyle could possibly increase the genetic risk of central obesity. Our results provide significant public health implications, given that the rising prevalence of central obesity is a major public health problem in Turkey (Pekcan et al. 2017; WHO, 2018). Further studies with large sample size and objective measures of environmental factors are required to provide a better understanding of how these variants interact with lifestyle factors to develop effective prevention and treatment strategies for obesity.

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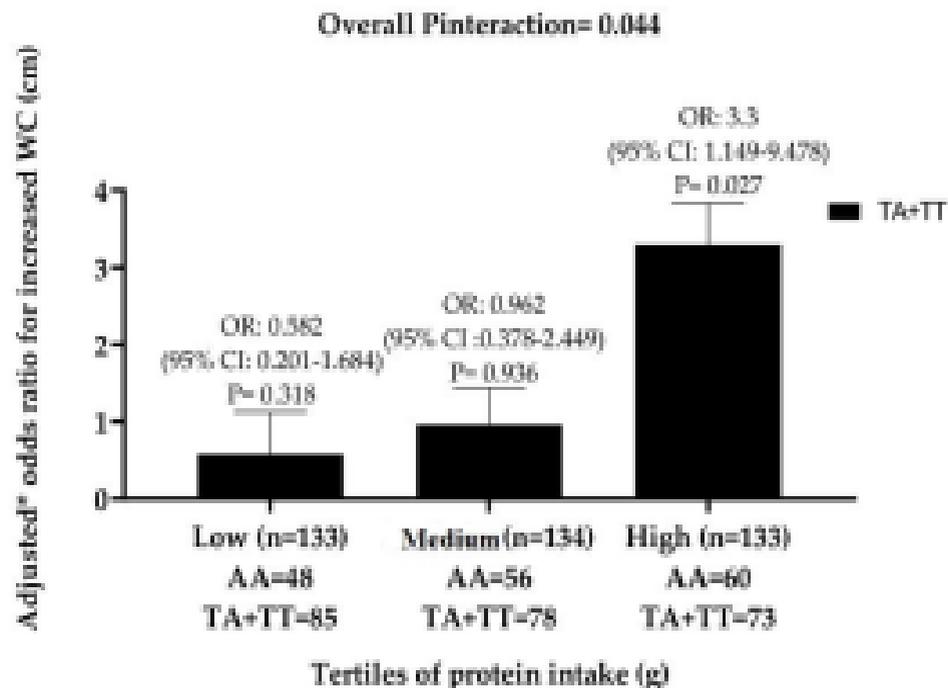
## Figure Captions

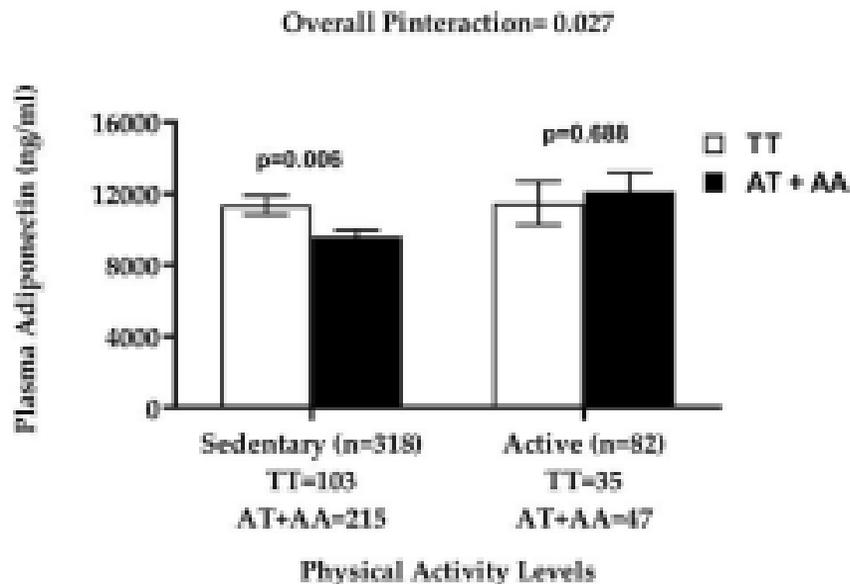
**Figure 1. Interactions of the *FTO* rs10163409 with tertiles of protein intake (g) on increased WC.** WC, Waist Circumference. Black bars implicate the ‘T’ allele carriers (TA+TT). *FTO* SNP rs10163409 showed a significant interaction with protein intake (g) on the risk of increased WC ( $P_{\text{interaction}} = 0.044$ ). Among those in the highest tertile of protein intake (mean  $\pm$  SD: 138 $\pm$ 38 g/day), the minor ‘T’ allele carriers of the SNP rs10163409 had a significantly higher risk of increased WC [OR= 3.3 (95% CI: 1.149-9.478),  $p = 0.027$ ] than those carrying ‘AA’ genotype. \*Odds ratio adjusted for age, gender, hypertension, cardiovascular diseases, total energy intake and obesity status

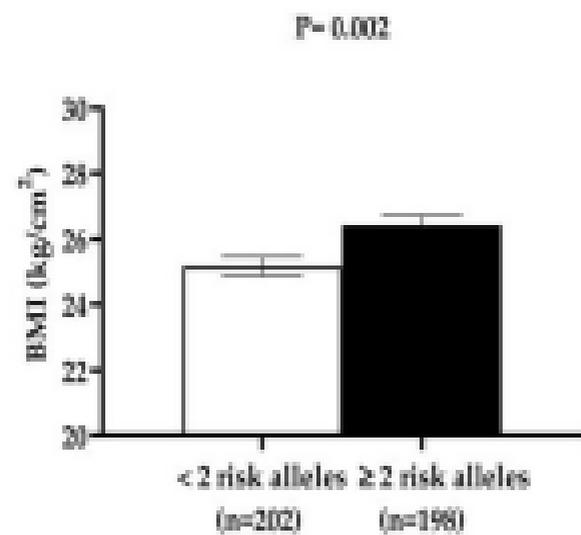
**Figure 2. Interactions between *FTO* rs9939609 variant and physical activity on adiponectin levels.** White bars indicate carriers of ‘TT’ genotype. Black bars implicate the risk allele, ‘A’, carriers (AT+AA). The regression model was adjusted for age, gender hypertension, cardiovascular diseases and obesity status. There was a significant interaction between the *FTO* SNP rs9939609 and physical activity on adiponectin levels ( $P_{\text{interaction}} = 0.027$ ), where, among those with low physical activity levels, carriers of the ‘A’ allele had significantly lower adiponectin levels compared to those with ‘TT’ genotype ( $p=0.006$ ).

**Figure 3. Association between the genetic risk score of the *FTO* SNPs, rs9939609 and rs10163409s and anthropometric measures of obesity.**

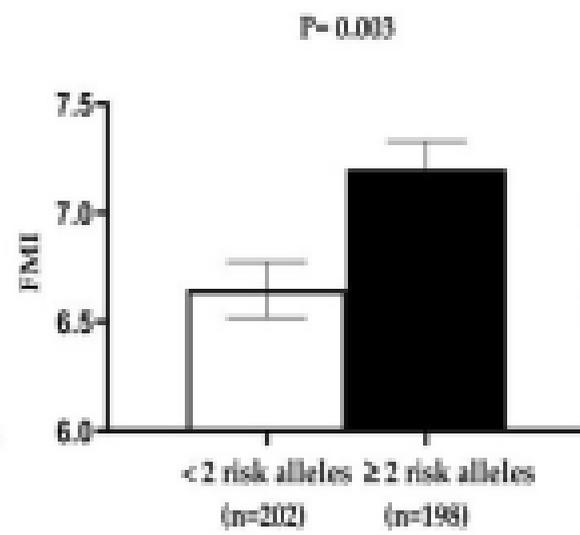
BMI, Body Mass Index; FMI, Fat Mass Index; WC, Waist Circumference. White bars: means of individuals with genetic risk score (GRS) of <2 risk alleles. Black bars: means of individuals with GRS of  $\geq 2$  or more risk alleles. The GRS was significantly associated with BMI (3a), FMI (3b) and WC (3c). Figure 3a; carriers of  $\geq 2$  or more risk alleles of the *FTO* variants (rs9939609 and rs10163409) had higher BMI ( $P=0.002$ ) compared to individuals carrying <2 risk alleles. Figure 3b; carriers of  $\geq 2$  or more risk alleles of the *FTO* variants (rs9939609 and rs10163409) had higher FMI ( $P=0.003$ ) compared to individuals carrying <2 risk alleles. Figure 3c; carriers of  $\geq 2$  or more risk alleles of the *FTO* variants (rs9939609 and rs10163409) had higher WC ( $P=0.020$ ) compared to individuals carrying <2 risk alleles. P values were obtained from linear regression analysis and adjusted for age, gender, hypertension, cardiovascular diseases and obesity status.



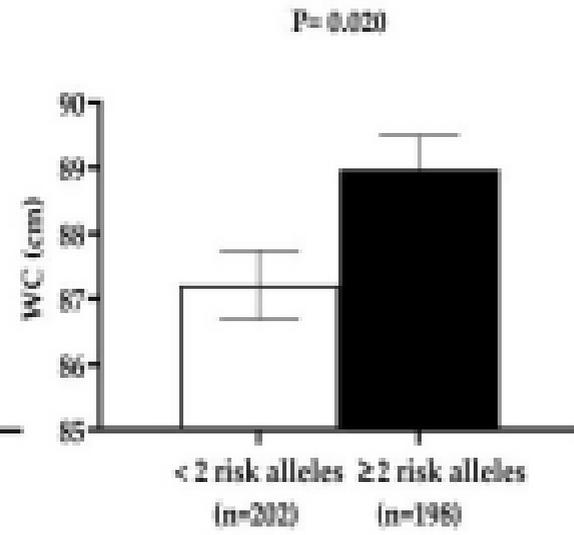




(3a)



(3b)



(3c)

Genetic Risk Score (rs993609 and rs10163409)

**Table 1.** Anthropometric and biochemical characteristic of the study participants

	Non-obese				Obese				P value**
	Total(N=200)	Men(N=100)	Women(N=100)	P value*	Total(N=200)	Men(N=108)	Women(N=92)	P value *	
Age (year)	33.29±6.83	32.64±6.04	33.93±7.51	0.182	36.37±7	36.09±6.78	36.68±7.27	0.552	<0.001
BMI (kg/m <sup>2</sup> )	22.56±1.78	22.83±1.73	22.3±1.79	0.035	29.04±3.38	28.8±3.22	29.33±3.55	0.274	<0.001
WC (cm)	80.68±7.96	85.62±6.52	75.75±5.98	<0.001	95.49±9.84	99.41±8.15	90.89±9.7	<0.001	<0.001
FMI	5.12±1.69	3.97±1.16	6.27±1.31	<0.001	8.72±2.79	7.3±2.16	10.39±2.52	<0.001	<0.001
Adiponectin (ng/ml)	11880.63±6838.36	9095.15±4929.39	14666.11±7350.18	<0.001	9115.13±5766.48	6716.49±3777.58	11930.93±6410.41	<0.001	<0.001
Energy (kcal/day)	2416.44±1064.1	2888.13±1155.72	1944.76±700.65	<0.001	2365.08±1012.1	2728.68±1057.45	1938.23±764.28	<0.001	0.621
Protein (g)	91.34±42.91	106.32±46.98	76.37±32.27	<0.001	87.55±43.81	97.85±50.48	75.45±30.44	<0.001	0.382
Fat (g)	105.31±48	119.48±51.92	91.15±39.13	<0.001	100.8±51.56	113.32±56.41	86.1±40.84	<0.001	0.366
Carbohydrate (g)	270.28±142.83	339.26±157.55	201.29±81.06	<0.001	271.34±128.5	322.74±132.03	211±93.78	<0.001	0.938
Fiber (g)	23.49±10.65	26.71±11.66	20.27±8.43	<0.001	24.03±11.59	26.59±12.84	21.04±9.13	0.001	0.627
SFA (g)	31.13±15.25	33.67±16.56	28.58±13.42	0.018	29.66±16.2	31.87±17.87	27.07±13.64	0.036	0.352
MUFA (g)	35.58±17.78	39.88±19.57	31.27±14.68	0.001	35.12±20.23	39.71±22.78	29.72±15.17	<0.001	0.809
PUFA (g)	28.79±17.35	34.88±19.04	22.7±12.95	<0.001	27.02±16.51	31.57±17.9	21.67±12.88	<0.001	0.296
PAL levels n (%)									
Sedentary	153 (76.5)	78 (78)	75 (75)		165 (82.5)	90 (83.3)	75 (81.5)		
Active	47 (23.5)	22 (22)	25 (25)	0.617	35 (17.5)	18 (16.7)	17 (18.5)	0.737	0.137

\*P values obtained from independent t-test and chi square tests comparing continuous and categorical variables between men and women within obese and non-obese categories\*\* P values obtained from independent t-test and chi square

tests comparing continuous and categorical variables between total obese and total non-obese. Values are mean ± SD and percentages

BMI, Body Mass Index; WC, Waist Circumference; FMI, Fat Mass Index; SFA, Saturated fatty acids; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids; PAL, Physical activity level.

**Table 2.** Genotype frequencies of *FTO* SNPs among cases and controls

<i>FTO rs9939609 SNP</i>				
	<b>Non-obese</b>	<b>Obese</b>	<b>Total</b>	<b>*P value</b>
Additive n (%)				0.217
TT	77 (38.5)	61 (30.5)	138 (34.5)	
AT	99 (49.5)	115 (57.5)	214 (53.5)	
AA	24 (12)	24 (12)	48 (12)	
Dominant n (%)				0.092
TT	77 (38.5)	61 (30.5)	138 (34.5)	
AT+AA	123 (61.5)	139 (69.5)	262 (65.5)	
HWE	0.36	0.007	0.011	
MAF	0.37	0.41	0.39	
<i>FTO rs10163409 SNP</i>				
Additive n (%)				0.772
AA	85 (42.5)	79 (39.5)	164 (41)	
AT	88 (44)	90 (45)	178 (44.5)	
TT	27 (13.5)	31 (15.5)	58 (14.5)	
Dominant n (%)				0.542
AA	85 (42.5)	79 (39.5)	164 (41)	
TA+TT	115 (57.5)	121 (60.5)	236 (59)	
HWE	0.58	0.525	0.392	
MAF	0.36	0.38	0.37	

\*p values obtained from Pearson's chi-squared test comparing genotype frequencies between cases and control

MAF, Minor Allele Frequency; HWE, Hardy Weinberg Equilibrium

**Table 3.** Associations between *FTO* polymorphisms and anthropometric and biochemical parameters of obesity

	<i>FTO</i> rs9939609			<i>FTO</i> rs10163409		
	TT (n=138)	AT+AA (n=262)	*p value	AA (n=164)	TA+TT (n=236)	*P value
BMI (kg/m <sup>2</sup> )	24.81±3.65	26.33±4.41	0.001	25.49±4.03	26.02±4.34	0.212
FMI	6.23±2.61	7.29±3.02	0.002	6.72±2.57	7.06±3.15	0.251
WC (cm)	86.08±10.62	89.14±11.99	0.007	87.26±11.47	88.66±11.7	0.234
Adiponectin (ng/ml)	11306.18±7130.97	10072.14±6059.63	0.031	10865.59±6526.36	10242.35±6427.19	0.377

\*P values obtained from linear regression analysis adjusted for sex, age, hypertension, cardiovascular diseases and obesity status. Values are mean ± SD

BMI, Body Mass Index; WC, Waist Circumference; FMI, Fat Mass Index