

The association of the fat mass and obesity-associated gene (FTO) rs9939609 polymorphism and the severe obesity in a Brazilian population

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Background: Obesity occurs due to the interaction between the genetic background and environmental factors, including an increased food intake and a sedentary lifestyle. Nowadays, it is clear that there is a specific circuit, called leptin-melanocortin pathway, which stimulates and suppresses food intake and energy expenditure. Therefore, the aim of this study was to evaluate the influence of genetic variants related to appetite regulation and energy expenditure on severe obesity susceptibility and metabolic phenotypes in a Brazilian cohort.

Material and methods: A total of 490 participants were selected (298 severely obese subjects and 192 normal-weight individuals). Genomic DNA was extracted and polymorphisms in protein related to agouti (*AGRP*; rs5030980), ghrelin (*GHRL*; rs696217), neuropeptide Y (*NPY*; rs535870237), melanocortin 4 receptor (*MC4R*; rs17782313), brain-derived neurotrophic factor (*BDNF*; rs4074134) and fat mass and obesity-associated (*FTO*; rs9939609) genes were genotyped using TaqMan® probes. Demographic, anthropometric, biochemical and blood pressure parameters were obtained from the participants.

Results: Our results showed that *FTO* rs9939609 was associated with severe obesity susceptibility. This polymorphism was also related to body weight, body mass index (BMI), waist to weight ratio (WWR) and inverted BMI. Individuals carrying the mutant allele (A) showed higher levels of BMI as well as lower values of WWR and inverted BMI.

Conclusion: This study showed that *FTO* rs9939609 polymorphism plays a significant role in predisposing severe obesity in a Brazilian population.

Keywords: leptin-melanocortin pathway, *FTO*, obesity, polymorphisms, hypothalamus

Introduction

Obesity is a serious health and economic problem in the world, affecting low-, middle- and high-income countries.^{1,2} It is defined as an excess in body fat accumulation and can be qualitatively measured using a body mass index (BMI, $\text{BMI} \geq 30 \text{ kg/m}^2$). Recent data estimated that obesity has almost tripled since the 70s and there were more than 650 million obese adults worldwide in 2016.¹ In Brazil, 11.7% of men and 20.6% of women are obese.³ This high prevalence can be explained by environmental changes, resulting in an energy imbalance caused by excessive caloric intake and diminished physical activity. Moreover, obesity is a major risk factor for several comorbidities, including type 2 diabetes, hypertension, osteoarthritis, cardiovascular diseases and

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some cancers.¹ In addition, there is evidence that higher BMI causes a proportional higher risk for these comorbidities and also higher mortality.⁴

Obesity is a complex phenotype influenced by genetic, psychological, behavioral, nutritional and environmental factors.⁵ The most common form of obesity seems not to follow a Mendelian inheritance model and is caused by an interaction of environmental factors with multiple genetic variants with minor effect.⁶ Within the last couple of years, genome-wide association (GWA) and whole exome studies (WES) have identified several variants associated with obesity; however, most of the genetic contribution for this disease remains unknown.^{7–11} Furthermore, the association between the genetic variants and obesity was replicated mainly in European and Asian cohorts, while for Latin American populations data are still scarce.^{10,12–14}

Many of the identified obesity genes are expressed and/or act in the central nervous system (CNS), suggesting a neuronal component in obesity susceptibility.⁸ There, a specific circuit, called leptin-melanocortin pathway, promotes and suppresses appetite and energy expenditure.^{15,16} Ghrelin (GHRH) is abundantly secreted by the stomach when the body is in restriction of food and low energy stock. This peptide binds to the ghrelin receptor and increases the expression of neuropeptide Y (NPY) and agouti-related protein (AgRP) in the orexigenic neurons.^{15,17,18} These neurons project from the arcuate nucleus (ARC) to the paraventricular nucleus (PVN) of the hypothalamus, where AgRP acts as antagonist of the melanocortin-4 receptor (MC4R) and NPY binds to its receptor, increasing appetite and decreasing energy expenditure.^{15,18} Leptin (LEP) is mainly secreted by adipocytes when the body has adiposity and/or the energy was replacement. Within the hypothalamus, LEP binds to orexigenic neurons and inhibits this pathway through AgRP/NPY production. Furthermore, LEP also binds to anorexigenic neurons and stimulates this pathway through the expression of proopiomelanocortin (POMC) and cocaine and amphetamine-related transcript (CART). Both proteins activate MC4R in the PVN, decreasing the appetite and increasing energy expenditure.^{6,18,19}

In addition, other genes expressed in hypothalamic areas also appear to have a neuronal effect on energy balance and appetite, including brain-derived neurotrophic factor (*BDNF*) and fat mass and obesity-associated (*FTO*) genes.^{7,13} *BDNF* has an important role in neuronal survival, maturation and differentiation during hypothalamic development, where it modulates energy metabolism.^{20–23} *FTO*

encodes a demethylation enzyme that plays a central role in the regulation of food intake and energy expenditure in mice and humans. This is supported by studies showing that *FTO* variants have influence on satiety, food choices and energy intake.^{24–27} In rodent models, *Fto* overexpression in hypothalamus led to an increase of food consumption and body fat mass, while a reduced expression of *Fto* mRNA increased the energy expenditure and resulted in a lean phenotype.^{28,29}

Individuals vary in their perceptions of hunger and satiety, and genetic variants associated with appetite regulation may predispose to obesity risk (Table 1). Consequently, it is important to understand these relations in order to design strategies for the prevention of obesity. Therefore, the aim of this study was to investigate whether polymorphisms belonging to genes expressed in the hypothalamic region could influence severe obesity susceptibility. Additionally, we also explored the role of these polymorphisms on obesity-related traits, blood pressure and biochemical parameters.

Materials and methods

Study population

This case–control cross-sectional study enrolled men and women, aged 18 to 70 years from Rio de Janeiro, southeast of Brazil. A total of 527 participants were recruited between July 2012 and December 2017. The selection of this cohort was described previously.¹¹ Briefly, inclusion criteria were participants with normal-weight (controls, $18.5 \leq \text{BMI} \leq 24.9 \text{ kg/m}^2$) and obesity (cases, $\text{BMI} \geq 30 \text{ kg/m}^2$).³⁰ The case group was recruited from a non-governmental organization, called Rescue Group to Self-Esteem and Citizenship of the Obese (in Portuguese, “Grupo de Resgate à Autoestima e Cidadania do Obeso”); and the control group was composed of volunteers from public hospitals in the same city. The exclusion criteria were pregnancy, lactation, and the use of medication to lose or gain weight directly. Pharmacological treatment for mental illness (depression and anxiety) and type 2 diabetes (T2D) was not an exclusion criterion for our study. Clinical and demographic information, anthropometric measurements, blood pressure and peripheral blood collection for biochemical and genetic analyses were obtained from all participants.

From the total of 527 subjects, a subsample was selected. Individuals with obesity with BMI lower than 35 kg/m^2 were excluded from the analyses ($n=31$). Moreover, subjects who self-reported as Asian ($n=5$) and indigenous ($n=1$) were also excluded. Therefore, 490 participants were included in our

Table I Candidate genes and genetic variants included in this study

Gene	Chromosome location	Biological relevance	dbSNP#	SNP location	MAF*	Previous studies
Agouti related protein (AGRP)	16q22	Endogenous antagonist of melanocortin receptors (MC4R and MC3R) that regulates appetite and energy expenditure.	rs5030980	Exon 3	0.015	<ul style="list-style-type: none"> Associated with BMI in European Americans (n=1,982) and body weight in Quebec families (n=874).^{53,54} No association with BMI in Dutch adults (n=582) and Latvian population (n=789).^{12,57}
Melanocortin 4 receptor (MC4R)	18q21.3/18q22	Stimulation of MC4R leads to a reduction in energy intake and an increased basal energy expenditure.	rs17782313	188 kb downstream of MC4R	0.240	<ul style="list-style-type: none"> No association with biochemical or anthropometric variables in individuals with Northern Han Chinese ancestry (n=3,859).⁶⁴ No relation with anthropometric and dietary parameters in a Brazilian childhood cohort followed-up since birth (n=745).¹⁴ Associated with obesity susceptibility in a Japanese (n=1,129), Caucasian (n=1,361) and North Indians cohorts (n=696).^{35,59,65}
Brain-derived neurotrophic factor (BDNF)	11p13	Protein involved in neuronal survival, maturation and differentiation during central nervous system development.	rs4074134	~31 kb of 3'-flanking of BDNF	0.252	<ul style="list-style-type: none"> Associated with BMI, WC, fasting and postprandial glucose, fasting serum insulin and HOMA-IR in subjects with Northern Han Chinese ancestry (n=3,859).⁶⁴ Linked to BMI and body weight in large mixed cohort (n=31,392).⁶⁶ Risk factor for obesity in a Japanese cohort (n=1,129).³⁵
Ghrelin (GHRL)	3p26	Orexigenic peptide that modulates appetite, gastrointestinal motility, gastric acid release and pancreatic secretions.	rs696217	Exon 4	0.083	<ul style="list-style-type: none"> Carriers of the mutant allele (T) showed a significant reduction in BMI after 52 weeks of bariatric surgery (n=100).⁶⁷ Positively associated with BMI and obesity susceptibility in a Japanese cohort (n=5,112).¹⁰ No association with obesity, eating behavior and glucose metabolisms in French families (n=2,334).⁵¹
Neuropeptide Y (NPY)	7q15.1	This peptide regulates energy homeostasis thought binds to its receptor on PVN.	rs535870237	17-bp deletion at 5'UTR	0.00039	<ul style="list-style-type: none"> Observed in one Mexican American family (n=934).⁵⁸

(Continued)

Table 1 (Continued).

Gene	Chromosome location	Biological relevance	dbSNP#	SNP location	MAF*	Previous studies
Fat Mass Obesity-Associated gene (FTO)	16q12.2	Demethylation enzyme involved in controlling of energy homeostasis, DNA methylation and abiogenesis	rs9939609	Intron 1	0.340	<ul style="list-style-type: none"> • No association with obesity phenotype in Egyptian adolescents and children; however, the presence of the risk allele was linked to higher LDL cholesterol (n=200).⁴² • Associated with morbid obesity risk in the Brazilian adults (n=239) and Mexican population (n=1,156).^{13,44} • Associated with satiety sensations (n=70), food intake control (n=97), food choice (n=97) and cardiorespiratory fitness (n=420).^{25,49,68}

Abbreviations: BMI, Body mass index; dbSNP, Single nucleotide polymorphism database; HOMA-IR, Homeostatic model assessment - Insulin resistance; LDL, Low density lipoprotein; MAF, Minor allele frequency; MC3R, Melanocortin 3 receptor; PVN, Paraventricular nucleus; SNP, Single nucleotide polymorphism; UTR, Untranslated region; WC, Waist circumference.

analyses (controls, n=192; cases, n=298). This study was approved by the Ethics Committee of Oswaldo Cruz Foundation and was carried out according to the principles expressed in the Declaration of Helsinki (1964). All individuals accepted to participate and provided a written informed consent.

Demographic variables

Race/skin color was self-reported and categorized according to the criteria of the Demographic Census conducted by the Brazilian Institute of Geography and Statistics (white, brown, black, yellow [Asian] and indigenous). Marital status was stratified as single, married/cohabiting, separated/divorced and widower. Cigarette smoking status was classified as “never smoked” or “already smoked” (current smokers and ex-smokers). Physical activity was classified as “yes”, if the participants have been practicing activities during the last month, or “no”. All information was self-reported and collected using standardized questionnaires by trained interviewers.

Body composition and biochemical analyses

All participants were examined following an overnight fast. Body weight, height, waist and hip circumferences were obtained for each participant. Waist circumference (WC) was measured at the midway between the iliac crest and the last costal arch. Hip circumference (HC) was measured around the greater trochanters. Further, BMI, inverted BMI, waist to weight

ratio (WWR), waist to height ratio and waist to hip ratio (WHR) were calculated in order to estimate the pattern of fat distribution. BMI was calculated as weight (kilograms) divided by the square of height (meters). Body adiposity index (BAI) estimates the percentage of total fat and was calculated by the formula: hip circumference/(height^{1.5})-18.³¹ Blood pressure was recorded in the sitting position using a wrist blood pressure monitor. Hypertension was diagnosed by blood pressure equal or higher than 140×90 mm Hg, or use of antihypertensive drug therapy.³²

Blood samples were collected in order to measure fasting plasma glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c) and triglyceride (TG) using the oxidase-peroxidase method (BioSystems). Low-density lipoprotein cholesterol (LDL-c) was calculated by the Friedewald formula (LDL-c = TC – HDL-c – TG/5). Additionally, C-reactive protein (CRP) was measured by the latex agglutination method and glycated hemoglobin was evaluated using turbidimetric inhibition immunoassay (TINIA). Individuals using medication for these biochemical parameters or blood pressure had their values excluded from the statistical analyses.

Genotyping

Genomic DNA was isolated from peripheral blood leukocytes using the QIAamp Blood Kit (Qiagen, Valencia, CA, USA) according to the manufacturer’s protocol. Genotypes for *AGRP* (rs5030980; C>T), *BDNF* (rs4074134; C>T), *FTO*

(rs9939609; T>A), *GHRL* (rs696217; G>T) and *MC4R* (rs17782313; T>C) polymorphisms were obtained by real-time PCR allelic discrimination using TaqMan® assays (ThermoFisher, Foster City, CA, USA). Amplification was carried out in a StepOne® Plus Real Time PCR System and data analyses were done using StepOne software v2.3.

For the *NPY* gene, a 17 bp deletion (rs535870237) was analyzed by conventional PCR (Supplementary material 1).

Statistical analysis

Normality of continuous variables was analyzed using Kolmogorov–Smirnov and Shapiro–Wilk tests. Continuous parameters were presented as median and interquartile, since they were shown as non-normally distributed data. Categorical variables were presented as number and percentages. Differences between the case and control groups were evaluated using Mann–Whitney (quantitative parameters) or Chi-square tests (qualitative parameters). The effect of potential variables (age, gender, race/skin, marital status, smoking status and physical activity practice) was investigated for severe obesity and was incorporated as covariate in the model when $P \leq 0.05$.

Genotype and allele frequencies were estimated by gene counting. Hardy–Weinberg Equilibrium (HWE) was checked for all polymorphisms using Chi-square test. Association of each polymorphism with obesity status was performed by logistic regression analysis in additive, dominant and recessive models. Odds ratio (OR) and 95% confidence intervals (CI) were also calculated. The same analyses were also performed using allele frequencies. All analyses were adjusted for age and gender (model 1) and further adjusted for age, gender, race/skin color, marital status and exercises habits (model 2). Additionally, genotype score counting was used to calculate the number of risk alleles for each participant as earlier described by Shabana and coworkers.³³ The influence of the combined risk alleles on severe obesity susceptibility was tested by logistic regression and adjusted for the covariates (model 2). The number of risk alleles per group was shown as mean (standard deviation) and the comparison between the groups was carried out by independent sample *t*-tests.

The influence of each polymorphism on the quantitative variables related to obesity was tested using linear regression analysis after a logarithmic transformation of these parameters. Gender, age, race/skin color, marital status and exercises habits were used as possible confounding variables for body weight, BMI, inverted BMI and WWR. Those parameters and BMI

were used as covariates for all other biochemical, blood pressure and anthropometric parameters.

All statistic tests were performed using SPSS software version for Windows (SPSS, Chicago, IL, USA). Since different polymorphisms were studied, a Bonferroni's correction for multiple comparisons was used and a corrected *P*-value of 0.01 was applied as a significance cutoff.

Results

The characteristics of study population are shown in Table S1. The cohort comprised 490 participants, 192 normal-weights (median of BMI =22.8 [21.1; 23.9] kg/m²) and 298 severely obese (median of BMI =46.8 [41.8; 52.8] kg/m²). In terms of clinical and demographic characteristics, a reduced prevalence of cases with white skin and singles were observed, whereas controls were significantly younger and practiced more physical activities. All those variables were included as covariates in the case–control association analyses in order to remove bias from confounding factors. As expected, participants with severe obesity had higher anthropometric, blood pressure and biochemical levels when compared to the normal-weight subjects. The exceptions were in height, inverted BMI, waist to height ratio and HDL-cholesterol, in which the control group had higher values. In addition, the proportion of subjects with hypertension and T2D was 69.8% and 20.2% among severely obese patients, respectively, whereas the values for the controls were 4.7% and 0%, respectively.

Association of the studied polymorphisms with severe obesity

Allele and genotype frequencies were obtained for all polymorphisms (Table 2). *AGRP* rs5030980 polymorphism showed a low frequency in our sample, and we did not find any homozygous for the risk allele (TT). Genetic analyses of *AGRP* rs5030980 were performed using only wild-type and heterozygous genotypes. Regarding *GHRL* rs696217 polymorphism, we identified only one homozygous subject for the risk allele (T) and our analyses were carried out in the dominant model (GG vs GT+TT). Furthermore, no 17-bp deletion (rs535870237) was identified in the *NPY* gene. Therefore, only five polymorphisms were included in our analyses (rs5030980, rs4074134, rs9939609, rs696217 and rs17782313). None of them deviated from HWE for both case and control groups ($P > 0.05$; data not shown).

Results of our case–control analyses are also presented in Table 2. The comparison between genotype frequencies showed that *FTO* rs9939609 AA genotype was associated with severe

Table 2 Association of *AGRP*, *BDNF*, *FTO*, *GHRL* and *MC4R* polymorphisms with obesity susceptibility

Gene (polymorphism)	Control	Case	Model 1		Model 2	
	n=192 (%)	n=298 (%)	OR (95% CI)	P	OR (95% CI)	P
<i>AGRP</i> (rs5030980)^a						
Genotype						
CC	179 (93.2)	278 (93.3)	1.00 (Ref.)	-	1.00 (Ref.)	-
CT	13 (6.8)	20 (6.7)	1.08 (0.50–2.33)	0.841	1.11 (0.47–2.59)	0.811
Allele						
C	371 (96.6)	576 (96.6)	1.00 (Ref.)	-	1.00 (Ref.)	-
T	13 (3.4)	20 (3.4)	1.08 (0.50–2.33)	0.841	1.11 (0.47–2.59)	0.811
<i>BDNF</i> (rs4074134)						
Genotype						
CC	122 (63.6)	204 (68.5)	1.00 (Ref.)	-	1.00 (Ref.)	-
CT	64 (33.3)	82 (27.5)	0.79 (0.51–1.22)	0.293	0.87 (0.54–1.40)	0.571
TT	6 (3.1)	12 (4.0)	1.42 (0.47–4.23)	0.536	2.12 (0.60–7.50)	0.242
Dominant Model						
CC	122 (63.5)	204 (68.5)	1.00 (Ref.)	-	1.00 (Ref.)	-
CT+TT	70 (36.5)	94 (31.5)	0.84 (0.55–1.28)	0.419	0.95 (0.60–1.50)	0.819
Recessive Model						
CC+CT	186 (96.9)	286 (96.0)	1.00 (Ref.)	-	1.00 (Ref.)	-
TT	6 (3.1)	12 (4.0)	1.53 (0.51–4.58)	0.451	2.22 (0.63–7.77)	0.212
Allele						
C	308 (80.2)	490 (82.2)	1.00 (Ref.)	-	1.00 (Ref.)	-
T	76 (19.8)	106 (17.8)	0.92 (0.65–1.32)	0.663	0.92 (0.63–1.34)	0.667
<i>FTO</i> (rs9939609)						
Genotype						
TT	71 (37.0)	73 (24.5)	1.00 (Ref.)	-	1.00 (Ref.)	-
TA	85 (44.3)	146 (49.0)	1.66 (1.05–2.63)	0.031	1.80 (1.09–2.98)	0.021
AA	36 (18.7)	79 (26.5)	2.24 (1.28–3.92)	0.005	2.24 (1.22–4.12)	0.010
Dominant Model						
TT	71 (37.0)	73 (24.5)	1.00 (Ref.)	-	1.00 (Ref.)	-
TA+AA	121 (63.0)	225 (75.5)	1.82 (1.19–2.80)	0.006	1.94 (1.21–3.09)	0.006
Recessive Model						
TT+TA	156 (81.2)	219 (73.5)	1.00 (Ref.)	-	1.00 (Ref.)	-
AA	36 (18.7)	79 (26.5)	1.65 (1.01–2.69)	0.044	1.58 (0.93–2.70)	0.092
Allele						
T	227 (59.1)	292 (49.0)	1.00 (Ref.)	-	1.00 (Ref.)	-
A	157 (40.9)	304 (51.0)	1.53 (1.14–2.00)	0.004	1.53 (1.13–2.07)	0.006
<i>GHRL</i> (rs696217)						
Genotype						
GG	177 (92.2)	277 (93.0)	1.00 (Ref.)	-	1.00 (Ref.)	-
GT+TT	15 (7.8)	21 (7.0)	0.76 (0.35–1.63)	0.475	0.91 (0.38–2.18)	0.838
Allele						
G	369 (96.1)	575 (96.5)	1.00 (Ref.)	-	1.00 (Ref.)	-
T	15 (3.9)	21 (3.5)	0.76 (0.35–1.63)	0.475	0.91 (0.38–2.18)	0.838
<i>MC4R</i> (rs17782313)						
Genotype						
TT	126 (65.6)	172 (57.7)	1.00 (Ref.)	-	1.00 (Ref.)	-
TC	58 (30.2)	113 (37.9)	1.28 (0.84–1.96)	0.254	1.39 (0.87–2.23)	0.170
CC	8 (4.1)	13 (4.4)	1.20 (0.46–3.16)	0.706	1.09 (0.38–3.10)	0.872

(Continued)

Table 2 (Continued).

Gene (polymorphism)	Control	Case	Model 1		Model 2	
	n=192 (%)	n=298 (%)	OR (95% CI)	P	OR (95% CI)	P
<i>Dominant Model</i>						
TT	126 (65.6)	172 (57.7)	1.00 (Ref.)	-	1.00 (Ref.)	-
TC+CC	66 (34.4)	126 (42.3)	1.27 (0.84–1.19)	0.249	1.35 (0.96–2.11)	0.193
<i>Recessive Model</i>						
TT+TC	184 (95.8)	285 (95.6)	1.00 (Ref.)	-	1.00 (Ref.)	-
CC	8 (4.2)	13 (4.4)	1.10 (0.42–2.87)	0.841	0.97 (0.35–2.74)	0.962
<i>Allele</i>						
T	310 (80.7)	457 (76.7)	1.00 (Ref.)	-	1.00 (Ref.)	-
C	74 (19.3)	139 (23.3)	1.20 (0.85–1.69)	0.299	1.23 (0.84–1.80)	0.284

Notes: P-value and OR were calculated using logistic regression and adjusted for gender and age (Model 1). *P*-value and OR were calculated using logistic regression and adjusted for age, gender, race/skin color, marital status and exercises habits (Model 2). ^aNo homozygous mutant was identified.

Abbreviations: AGRP, protein related to aguti; BDNF, brain-derived neurotrophic factor; CI, confidence interval; FTO, fat mass and obesity-associated gene; GHRL, ghrelin; MC4R, melanocortin 4 receptor; OR, Odds ratio; Ref, Reference.

obesity. However, this relation did not remain after adjusting for covariates (model 2) and correcting for multiple testing. This polymorphism was also associated with the dominant model (OR=1.94 [1.21–3.09]; $P=0.006$). Allelic tests showed that individuals carrying the mutant allele (A) were 1.5 times more likely to develop this phenotype when compared to the control group (OR=1.53 [1.13–2.07]; $P=0.006$). Carriers of at least one mutated allele (A) had a 53% higher risk of becoming severely obese. No association of *AGRP* rs5030980, *BDNF* rs4074134, *GHRL* rs696217 and *MC4R* rs17782313 polymorphisms with obesity was observed in our study.

Effect of combined risk alleles on obesity susceptibility

We investigated the influence of the gene score on obesity status. The results showed that 10.1% of severely obese had more than four risk alleles, whereas in normal-weight group only 5.7% of individuals had these number of risk alleles. The average number of risk alleles was higher in cases (1.98±1.13) than controls (1.74±1.10); however, this difference was not statistically significant ($P=0.463$). The risk of becoming obese increase 1.2 times for each risk allele added (OR=1.27 [1.04–1.55]; $P=0.016$) after adjusting for covariates. This association did not remain after correcting for multiple tests.

Association of polymorphisms with metabolic parameters

We also evaluated the potential influence of the studied polymorphisms on anthropometric, blood pressure and biochemical parameters (Table S2). Our results showed that *FTO* rs9939609

was associated with body weight, BMI, WWR and inverted BMI. Individuals with *FTO* rs9939609 AA genotype exhibited higher values of body weight ($\beta=0.030\pm0.010$; $P=0.002$) and BMI ($\beta=0.032\pm0.009$; $P=0.001$) when compared to TA and TT genotypes. These individuals also had lower levels of WWR ($\beta=-0.012\pm0.004$; $P=0.003$) and inverted BMI ($\beta=-0.029\pm0.010$; $P=0.003$). *MC4R* rs17782313 was negatively associated with CRP ($\beta=-0.098\pm0.043$; $P=0.025$); however, this relation did not remain after correcting for multiple testing. None of the other polymorphisms was seen linked to the metabolic parameters.

Discussion

In the present study, we evaluated the contribution of variants in six genes (*AGRP*, *BDNF*, *FTO*, *GHRL*, *MC4R* and *NPY*) in the development of severe obesity in a Brazilian cohort. The products of these genes are expressed or act in the hypothalamic region, and they have been directly or indirectly associated with food intake and basal energy expenditure.^{6,34} Several polymorphisms were identified in these genes; however, their association with obesity varies with the ethnicity of the population.^{12,14,33–35}

Interestingly, *FTO* was locus that presented the most significant association with severe obesity in our cohort. Previous GWA studies have reported that polymorphisms in intron 1 of this gene are associated with obesity susceptibility.^{36–38} Furthermore, the relationship between *FTO* rs9939609 and obesity has been reported in Brazilian and in other populations.^{34,39–41} However, *FTO* rs9939609 does not appear to influence obesity susceptibility in Egyptian and six Oceanic populations.^{42,43}

León-Mimila et al¹³ reported that *FTO* rs9939609 was associated with obesity in a Mexican adult population. Interestingly, the strongest and most significant relation was observed in individuals with morbid obesity (BMI \geq 40 kg/m²). This positive association was also observed in a Brazilian and an Italian cohort.^{44,45} García-Solís reported a similar significant association between *FTO* rs9939609 and obesity in Mexican children.³⁴ However, this finding was not replicated in a different children group from the same population or in an Egyptian cohort.^{13,42}

Regarding the influence of polymorphisms on phenotype variables, we observed an association of *FTO* rs9939609 with several anthropometric parameters (body weight, BMI, WWR and inverted BMI), after excluding possible confounders. However, this polymorphism was not associated with the biochemical parameters or blood pressure. Similarly, Marcadenti et al⁴⁶ reported that this polymorphism has a positive association with BMI and neck circumference in patients with hypertension from the south of Brazil. They did not observe an effect of *FTO* rs9939609 also on blood pressure and other anthropometric variables in their overall population. León-Mimila et al¹³ observed that the Mexican Indigenous adults carrying one risk alleles had an increase of 1.22 kg/m² in BMI. They also reported that this polymorphism was marginally associated with lower triglyceride levels in Mexican-Mestizo adults, but no association was found with BMI, WC, HC, glucose, TC or HDL-c. Sentinelli et al⁴⁵ observed that rs9939609 was associated with BMI and WC. However, no association was found between this polymorphism and biochemical parameters, similar to our results. In Egyptian children and adolescents, *FTO* rs9939609 was only correlated with LDL-cholesterol.⁴² Reasons for the discrepancies might be explained by differences in the genetic background as well as the sample criteria, since we have selected only patients with severe obesity and adults.

FTO is mainly expressed in the hypothalamus and encodes a nucleic acid demethylase which cleaves the methyl groups from nucleotides in the DNA and in the RNA. This nucleic acid demethylation activity may suggest that *FTO* influences the development of obesity at the epigenetic level.⁴⁷ Despite the fact that the function of *FTO* is not totally understood, it is suggested that it may have an important role on satiety, food consumption and energy expenditure.^{25–27} This is supported by studies with murine models that lack *FTO* function and/or *Fto* expression. These mice presented an increase in energy

expenditure and a lean phenotype.^{28,48} In addition, the overexpression of *Fto* resulted in increased food intake, body weight and fat mass.²⁹

Magno et al²⁵ reported that *FTO* rs9939609 may have an important role in the hunger and satiety sensations in a cohort of morbidly obese females from Brazil. Cecil et al⁴⁹ also demonstrated that this polymorphism influenced appetite and food choice. *FTO* rs9939609 polymorphism is located in intron 1 and it seems to exert functional effects on *FTO* by modulation its expression or it is in linkage disequilibrium with another causative genetic variant.^{47,50} More functional studies are required to elucidate the role of *FTO* rs9939609 in the development of obesity.

Furthermore, we have studied different genetic variants in the leptin-melanocortin pathway, but they were not associated with severe obesity susceptibility as well as obesity-related traits in our cohort. As shown in Table 1, the association between these polymorphisms and obesity is controversial in the literature. *GHRL* rs696217 was previously related to adiposity in the Japanese population, in which carriers of one risk allele had an increase of 0.18 kg/m² of BMI.¹⁰ However, this positive association was not observed in a cohort of Italians and French families, similar to our results.^{51,52} The discrepancies may be explained by the different genetic backgrounds between the populations, since the frequency of the risk allele is 20% in Japanese individuals and only 8% in Caucasians.^{10,52}

AGRP rs5030980 polymorphism was associated with anorexia nervosa and also a reduction in BMI, body weight and body fat.^{53–55} In addition, carriers of wild-type genotype were related to total and abdominal adiposity in individuals with late-onset obesity.⁵⁶ Controversially, this polymorphism was not associated with BMI in cohorts from Latvia and the Netherlands.^{12,57} Regarding *NPY* gene, a 17-bp deletion in the 5'UTR was analyzed in our study. We did not find individuals carrying this deletion, suggesting that this genetic variant is rare in our population. Bray et al.⁵⁸ identified this deletion in only one family with morbid obesity. Additionally, the 1000 genomes project also observed a low frequency of this variant in the general population (0.04%).

MC4R rs17782313 polymorphism is located near the gene and was previously associated with obesity and anthropometric variables in Asian and European populations.^{59–61} However, such results were not found in a cohort of Africans and East Asians.^{35,62} In this present study, we observed that the genotype and allele

frequencies were similar between case and control groups. Grant et al.⁶³ found a similar result, in which no association was observed between *MC4R* rs17782313 and obesity in Afro-American individuals. Thus, all these findings suggest that the influence of *MC4R* rs17782313 on the risk for obesity is dependent on the ethnic group.

Finally, few studies have analyzed *BDNF* rs4074134 variation and most of them were in Asian populations.^{35,64} Han et al.⁶⁴ found that this polymorphism was associated with the reduction of BMI, WC, glucose, insulin and the risk for T2D in the Chinese population. However, this genetic variation was positively associated with obesity susceptibility in a sample of Japanese subjects. The discrepancy of these results, and ours, could be explained by differences in the genetic background between the populations and the selection criteria of the samples.

Several limitations should be taken into account before interpreting the results. Despite including race/skin color as covariates, we did not genotype ancestry markers to control for population substructures. We were unable to consider the fluctuation over time of anthropometric, biochemical and blood pressure parameters, since we performed a cross-sectional study. We were not able to exclude patients treated for mental illness and T2D, which may impact the parameters measured. In addition, we do not have information about energy intake, appetite or satiety. Finally, we cannot completely exclude the role of environmental variables, which could impact on the development of severe obesity.

Conclusion

Obesity is caused by the interaction between the individual genetic background and environmental factors, especially due to increased food intake and reduced energy expenditure. It is known that there is a specific circuit in the hypothalamus which can control energy balance. Genetic variants in the genes that act on this system may have an important role in obesity susceptibility. In our study, a significant association between severe obesity and *FTO* rs9939609 was found in our sample. Additionally, the presence of *FTO* risk allele was associated with higher body weight and BMI, and lower inverted BMI and WWR. No association was observed in the other studied polymorphisms. These finds suggest that *FTO* has an important role in severe obesity risk in a Brazilian population.

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Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

PCR procedure for *NPY* polymorphism

The region was amplified using custom designed primers (forward: 5'-GCGGCGAGGAAGCTCCATAA-3'; reverse: 5'-CCTAGACAGACGGGTCGTAG-3'). PCR reactions (25 µl) consisted of 20–50 ng of DNA, 1 unit of Taq DNA polymerase (Biotools B&M Labs. S.A, Madrid, Spain), 0.2

mmol/L of each dNTP, 2 mmol/L MgCl₂ and 0.3 pmoles of each primer. The thermocycling condition started with an initial denaturation at 95 °C for 10 min, followed by 30 cycles of 94 °C for 1 min; 60 °C for 1 min; 72 °C for 1 min; and a final extension step of 72 °C for 10 min. The 159 bp amplified fragment of *NPY* gene was analyzed on a 15% polyacrylamide gel.

Table S1 Phenotypic characteristics of study population

Parameters	n	All	n	Control	n	Case	P*
Age (years)	490	36.0 (27.0; 45.0)	192	29.0 (24.0; 27.0)	298	40.0 (31.0; 48.0)	<0.001
Gender (female/male)							
Female	490	358 (76.1)	192	117 (60.9)	298	241 (80.9)	<0.001
Male		132 (26.9)		75 (39.1)		57 (19.1)	
Race/skin color							
White	489	231 (47.2)	191	128 (67.0)	298	103 (34.6)	<0.001
Brown		169 (34.6)		49 (25.7)		120 (40.3)	
Black		89 (18.2)		14 (7.3)		75 (25.1)	
Marital status							
Single	488	220 (45.1)	192	117 (61.0)	296	103 (34.8)	<0.001
Married/cohabiting		213 (43.6)		64 (33.3)		149 (50.3)	
Separated/divorced		34 (7.0)		4 (2.1)		30 (10.2)	
Widower		21 (4.3)		7 (3.6)		14 (4.7)	
Smoking status							
Already smoked	483	56 (9.4)	191	18 (13.0)	292	38 (11.6)	0.228
Never smoked		427 (90.6)		173 (87.0)		254 (88.4)	
Physical activity practice							
Yes	487	155 (31.8)	192	99 (51.6)	295	56 (19.0)	<0.001
No		332 (68.2)		93 (48.4)		239 (81.0)	
Type 2 Diabetes							
Yes	484	59 (12.2)	192	0 (0.0)	292	59 (20.2)	<0.001
No		425 (87.8)		192 (100)		233 (79.8)	
Hypertension							
Yes	490	217 (44.3)	192	9 (4.7)	298	208 (69.8)	<0.001
No		273 (55.7)		183 (95.3)		90 (30.2)	
Weight (kg)	490	105.1 (67; 132.3)	192	63.0 (57.0; 70.0)	298	126.3 (109.5; 144.2)	<0.001
Height (m)	490	1.65 (1.59; 1.72)	192	1.68 (1.61; 1.74)	298	1.63 (1.58; 1.69)	<0.001
BMI (kg/m²)	490	40.0 (23.4; 48.8)	192	22.8 (21.1; 23.9)	298	46.8 (41.8; 52.8)	<0.001
Waist circumference (cm)	488	120.5 (80.0; 139.9)	192	81.0 (75.0; 85.5)	296	136.0 (125.0; 147.0)	<0.001
Hip circumference (cm)	488	128.0 (99.5; 145.4)	192	98.0 (93.0; 101.4)	296	141.2 (131.1; 152.7)	<0.001
WHR	488	0.91 (0.84; 0.98)	192	0.83 (0.78; 0.89)	296	0.96 (0.90; 1.00)	<0.001
BAI	488	41.8 (28.1; 51.6)	192	26.9 (24.1; 29.6)	296	49.5 (44.0; 56.0)	<0.001
Inverted BMI	490	24.9 (20.4; 42.7)	192	43.8 (41.8; 47.4)	298	21.35 (18.9; 23.9)	<0.001
Waist to height ratio	488	0.70 (0.50; 0.90)	192	0.5 (0.4; 0.5)	296	0.80 (0.80; 0.90)	<0.001
Waist to weight ratio	488	1.10 (1.00; 1.30)	192	1.30 (1.20; 1.40)	296	1.10 (1.00; 1.10)	<0.001
Glucose (mg/dL)	385	93.0 (87.0; 102.0)	176	89.0 (84.0; 95.0)	209	97.0 (90.0; 110.0)	<0.001
Total cholesterol (mg/dL)	423	186.0 (160.0; 216.0)	184	178.5 (156.0; 200.0)	239	193.0 (166.0; 224.0)	<0.001

(Continued)

Table S1 (Continued).

Parameters	n	All	n	Control	n	Case	P*
HDL cholesterol (mg/dL)	423	51.0 (43.0; 61.0)	184	59.0 (48.0; 69.0)	239	47.0 (41.0; 53.0)	<0.001
LDL cholesterol (mg/dL)	416	110.0 (91.0; 135.0)	179	102.0 (86.0; 123.0)	233	117.0 (95.0; 143.0)	<0.001
Triglycerides (mg/dL)	423	102.0 (74.0; 139.0)	184	77.0 (61.0; 101.7)	239	125.0 (96.0; 168.0)	<0.001
Hemoglobin (%)	340	5.3 (4.9; 5.9)	165	5.1 (4.8; 5.4)	175	5.8 (5.1; 6.3)	<0.001
CRP (mg/dL)	336	0.42 (0.12; 1.09)	164	0.13 (0.08; 0.28)	172	1.00 (0.55; 1.59)	<0.001
SBP (mm Hg)	345	121.0 (111.0; 133.0)	178	118.0 (109.7; 125.2)	167	130.0 (117.0; 143.0)	<0.001
DBP (mm Hg)	345	80.0 (70.5; 89.0)	178	76.0 (68.0; 82.0)	167	85.0 (76.0; 92.0)	<0.001

Notes: Data are presented as median values (interquartile range 25–75%) for quantitative traits and n (%) for qualitative traits. Data were analyzed by Mann–Whitney Test (for non-normally distributed continuous variables) or χ^2 test (for categorical variables). P*-value for differences between severely obese and normal-weight subjects.

Abbreviations: BMI, Body Mass Index; WHR, Waist–hip ratio; BAI, Body Adipose Index; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; HDL cholesterol, High-density lipoprotein-cholesterol; LDL cholesterol, Low-density lipoprotein-cholesterol; CRP, C-reactive protein.

Table S2 Association of polymorphisms studied and anthropometric, biochemical and blood pressure variables

Parameters	AgRP (rs5030980)		BDNF (rs4074134)		FTO (rs9939609)		GHRL (rs696217)		MC4R (rs17782313)	
	Genotypes (n)	Values	Genotypes (n)	Values	Genotypes (n)	Values	Genotypes (n)	Values	Genotypes (n)	Values
Weight (kg)	CC (457) CT (33)	104.9 (67.0; 132.1) 111.0 (64.1; 140.8)	CC (326) CT (146) TT (18)	106.1 (66.7; 134.5) 98.5 (64.6; 127.8) 111.9 (73.5; 136.5)	TT (144) TA (231) AA (115)	85.3 (63.0; 126.42) 105.4 (67.5; 131.5) 114.0 (72.0; 140.5)	GG (454) GT+TT (36)	105.4 (66.8; 132.3) 96.9 (67.5; 133.0)	TT (298) TC (171) CC (21)	100.3 (65.1; 132.1) 112.0 (71.2; 134.7) 113.5 (59.4; 128.9)
β		0.005 ± 0.028		-0.003 ± 0.013		0.030 ± 0.010		-0.002±0.027		0.015±0.012
P		0.856		0.808		0.002		0.927		0.229
BMI (kg/m²)	CC (457) CT (33)	40.1 (23.3; 48.9) 39.7 (23.2; 46.9)	CC (326) CT (146) TT (18)	40.4 (23.6; 49.4) 37.8 (22.8; 46.9) 44.3 (24.3; 47.6)	TT (144) TA (231) AA (115)	35.7 (22.5; 46.8) 40.1 (23.6; 47.5) 43.6 (24.5; 52.6)	GG (454) GT+TT (36)	40.1 (23.3; 48.8) 37.2 (24.1; 49.6)	TT (298) TC (171) CC (21)	38.5 (23.1; 48.2) 42.7 (24.0; 49.3) 41.9 (22.2; 47.9)
β		0.001±0.027		-0.004 ± 0.013		0.032±0.009		-0.009±0.026		0.015±0.012
P		0.975		0.757		0.001		0.736		0.198
BAI	CC CT (33)	41.9 (28.1; 51.6) 33.7 (26.3; 48.7)	CC (325) CT (145) TT (18)	42.1 (28.3; 51.7) 37.5 (27.3; 50.4) 44.4 (26.8; 55.3)	TT (143) TA (230) AA (115)	34.2 (26.6; 48.4) 42.1 (29.0; 51.2) 46.6 (31.4; 54.7)	GG (452) GT+TT (36)	42.1 (28.0; 51.6) 38.7 (28.4; 48.1)	TT (297) TC (170) CC (21)	40.9 (27.4; 50.2) 43.6 (29.5; 53.1) 44.5 (27.7; 52.0)
β		-0.016±0.010		0.002±0.005		0.007±0.003		0.005±0.010		0.005±0.004
P		0.101		0.641		0.054		0.631		0.243
Waist to weight ratio	CC (455) CT (33)	1.10 (1.00; 1.30) 1.1 (1.00; 1.25)	CC (325) CT (145) TT (18)	1.10 (1.00; 1.30) 1.20 (1.05; 1.30) 1.10 (1.00; 1.20)	TT (143) TA (230) AA (115)	1.20 (1.00; 1.30) 1.10 (1.00; 1.30) 1.10 (1.00; 1.20)	GG (452) GT+TT (36)	1.10 (1.00; 1.30) 1.10 (1.00; 1.30)	TT (297) TC (170) CC (21)	1.10 (1.00; 1.30) 1.10 (1.00; 1.30) 1.20 (1.10; 1.35)
β		-0.002±0.011		0.002±0.005		-0.012±0.004		-0.007±0.011		0.001±0.005
P		0.831		0.727		0.003		0.489		0.883
Waist to height ratio	CC (455) CT (33)	0.70 (0.50; 0.90) 0.70 (0.50; 0.80)	CC (325) CT (145) TT (18)	0.70 (0.50; 0.90) 0.70 (0.50; 0.80) 0.80 (0.50; 0.82)	TT (143) TA (230) AA (115)	0.60 (0.50; 0.80) 0.70 (0.50; 0.80) 0.80 (0.50; 0.90)	GG (452) GT+TT (36)	0.70 (0.50; 0.90) 0.70 (0.50; 0.80)	TT (297) TC (170) CC (21)	0.70 (0.50; 0.80) 0.80 (0.50; 0.90) 0.70 (0.50; 0.90)
β		0.001±0.008		0.003±0.004		0.001±0.003		-0.011±0.008		0.004±0.003
P		0.937		0.372		0.676		0.153		0.248
Inverted BMI	CC (457) CT (33)	24.9 (20.4; 42.6) 25.2 (21.3; 43.0)	CC (326) CT (146) TT (18)	24.7 (20.2; 42.4) 26.2 (21.3; 43.7) 22.5 (21.0; 41.1)	TT (144) TA (231) AA (115)	27.9 (21.3; 44.3) 24.9 (21.1; 42.4) 22.9 (19.0; 40.8)	GG (454) GT+TT (36)	24.9 (20.5; 42.9) 26.9 (20.1; 41.5)	TT (298) TC (171) CC (21)	25.8 (20.6; 43.4) 23.4 (20.3; 41.7) 23.9 (20.9; 45.0)
β		0.001±0.028		0.001 ± 0.013		-0.029±0.010		0.010±0.027		-0.013±0.012
P		0.976		0.983		0.003		0.712		0.275

(Continued)

Table S2 (Continued).

Parameters	AgRP (rs5030980)		BDNF (rs4074134)		FTO (rs9939609)		GHRL (rs696217)		MC4R (rs17782313)	
	Genotypes (n)	Values	Genotypes (n)	Values	Genotypes (n)	Values	Genotypes (n)	Values	Genotypes (n)	Values
Waist circumference (cm)	CC (455)	120.0 (84.0; 140.0)	CC (325)	121.0 (84.0; 141.7)	TT (143)	101.0 (81.5; 137.0)	GG (452)	121.0 (84.0; 139.9)	TT (297)	116.0 (82.0; 139.0)
	CT (33)	125.5 (81.5; 139.7)	CT (145)	117.0 (92.0; 138.0)	TA (230)	120.5 (84.7; 139.5)	GT+TT (36)	111.5 (82.2; 142.0)	TC (170)	126.2 (86.7; 141.5)
			TT (18)	125.5 (84.4; 137.6)	AA (115)	128.0 (86.0; 146.0)			CC (21)	125.0 (81.7; 143.7)
β		0.003±0.007		0.001 ± 0.013		-0.001±0.002		-0.002±0.007		0.004±0.003
P		0.682		0.778		0.589		0.759		0.156
Hip circumference (cm)	CC (455)	128.0 (100.0; 145.0)	CC (325)	129.5 (100.0; 146.0)	TT (143)	108.0 (98.0; 141.0)	GG (452)	128.0 (99.0; 145.9)	TT (297)	125.0 (99.0; 144.0)
	CT (33)	124.0 (97.5; 145.7)	CT (145)	123.0 (98.5; 142.2)	TA (230)	127.2 (84.7; 139.5)	GT+TT (36)	125.5 (101.2; 144.2)	TC (170)	132.0 (101.9; 147.1)
			TT (18)	136.7 (100.0; 148.6)	AA (115)	135.5 (101.0; 150.0)			CC (21)	131.0 (98.7; 147.5)
β		-0.008±0.005		0.004 ± 0.002		0.002±0.002		0.006±0.005		0.002±0.002
P		0.141		0.128		0.267		0.712		0.378
WHR	CC (455)	0.91 (0.84; 0.98)	CC (325)	0.92 (0.84; 0.98)	TT (143)	0.91 (0.82; 0.98)	GG (452)	0.91 (0.84; 0.98)	TT (297)	0.90 (0.83; 0.97)
	CT (33)	0.93 (0.84; 0.98)	CT (145)	0.90 (0.82; 0.97)	TA (230)	0.91 (0.83; 0.97)	GT+TT (36)	0.90 (0.82; 0.99)	TC (170)	0.93 (0.85; 0.98)
			TT (18)	0.88 (0.84; 0.95)	AA (115)	0.92 (0.85; 0.98)			CC (21)	0.90 (0.84; 0.98)
β		0.011±0.007		-0.003±0.003		-0.004±0.002		-0.007±0.006		0.002±0.003
P		0.108		0.321		0.138		0.260		0.451
Glucose (mg/dL)	CC (354)	92.5 (87.0; 102.0)	CC (260)	94.0 (87.0; 103.0)	TT (110)	91.5 (84.7; 98.2)	GG (357)	92.0 (87.0; 101.5)	TT (239)	92.0 (86.0; 102.0)
	CT (31)	94.0 (86.0; 105.0)	CT (112)	92.0 (86.0; 100.7)	TA (193)	93.0 (87.0; 102.0)	GT+TT (28)	96.0 (90.0; 111.0)	TC (132)	94.0 (87.2; 102.7)
			TT (13)	93.0 (89.5; 102.5)	AA (92)	96.0 (89.0; 108.0)			CC (14)	94.0 (87.0; 104.2)
β		-0.001±0.015		8,139E-5±0.007		0.006±0.006		0.023±0.015		-0.003±0.007
P		0.966		0.991		0.283		0.130		0.643
Total cholesterol (mg/dL)	CC (392)	186.0 (160.0; 216.7)	CC (280)	186.0 (159.2; 218.0)	TT (119)	182.0 (160.0; 212.0)	GG (391)	185.0 (160.0; 215.0)	TT (261)	185.0 (161.0; 220.0)
	CT (31)	182.0 (161.0; 211.0)	CT (129)	189.0 (162.0; 215.5)	TA (210)	187.0 (160.0; 216.2)	GT+TT (32)	192.0 (170.2; 231.5)	TC (146)	186.0 (159.0; 209.5)
			TT (14)	175.0 (153.5; 188.2)	AA (94)	192.0 (161.0; 228.2)			CC (16)	190.0 (160.2; 233.5)
β		-0.005±0.016		-0.003±0.008		0.008±0.006		0.016±0.016		-0.001±0.007
P		0.774		0.694		0.164		0.318		0.920

(Continued)

Table S2 (Continued).

Parameters	AgRP (rs5030980)		BDNF (rs4074134)		FTO (rs939609)		GHRl (rs696217)		MC4R (rs17782313)	
	Genotypes (n)	Values	Genotypes (n)	Values	Genotypes (n)	Values	Genotypes (n)	Values	Genotypes (n)	Values
HDL cholesterol (mg/dL)	CC (392) CT (31)	51.0 (43.0; 61.0) 50.0 (45.0; 57.0)	CC (280) CT (129) TT (14)	49.5 (43.0; 60.0) 53.0 (45.0; 65.0) 50.0 (41.7; 59.5)	TT (119) TA (210) AA (94)	52.0 (45.0; 60.0) 49.0 (43.0; 60.0) 52.5 (43.7; 65.0)	GG (391) GT+TT (32)	51.0 (43.0; 61.0) 49.5 (42.0; 61.2)	TT (261) TC (146) CC (16)	51.0 (43.0; 61.5) 50.0 (42.0; 59.0) 50.5 (46.0; 67.0)
β		0.006±0.017		0.013 ± 0.008		0.010±0.006		-0.006±0.017		0.003±0.008
P		0.739		0.121		0.112		0.717		0.735
LDL cholesterol (mg/dL)	CC (386) CT (30)	110.0 (91.0; 135.0) 105.0 (90.5; 127.7)	CC (275) CT (129) TT (13)	109.0 (92.0; 135.0) 112.5 (90.2; 136.5) 98.0 (77.0; 120.0)	TT (119) TA (207) AA (90)	108.0 (90.0; 132.0) 111.0 (91.0; 133.0) 109.0 (93.0; 139.5)	GG (386) GT+TT (30)	109.0 (90.0; 132.2) 119.0 (99.7; 145.0)	TT (256) TC (144) CC (16)	110.0 (92.0; 137.0) 108.5 (88.0; 126.7) 121.0 (95.7; 158.5)
β		-0.020±0.023		-0.003±0.011		0.004±0.009		0.021±0.023		-0.007±0.011
P		0.384		0.791		0.661		0.359		0.522
Triglycerides (mg/dL)	CC (392) CT (31)	102.5 (74.0; 139.0) 100.0 (70.0; 140.0)	CC (280) CT (129) TT (14)	107.0 (74.2; 142.0) 95.0 (70.5; 131.5) 93.5 (75.5; 133.7)	TT (119) TA (210) AA (94)	96.0 (70.0; 135.0) 104.5 (74.0; 137.2) 109.5 (76.5; 173.2)	GG (391) GT+TT (32)	102.0 (74.0; 139.0) 105.0 (78.0; 141.5)	TT (261) TC (146) CC (16)	100.0 (72.5; 137.5) 113.5 (78.0; 158.0) 95.0 (69.2; 109.2)
β		0.001±0.037		0.015 ± 0.018		0.007±0.014		0.031±0.036		0.008±0.017
P		0.994		0.382		0.612		0.389		0.636
Hemoglobin	CC (318) CT (22)	5.3 (4.9; 5.9) 5.2 (5.0; 6.0)	CC (223) CT (104) TT (13)	5.3 (4.9; 5.8) 5.4 (4.9; 6.0) 5.5 (4.0; 5.9)	TT (99) TA (160) AA (81)	5.2 (4.9; 5.7) 5.4 (4.9; 6.0) 5.4 (4.9; 6.0)	GG (314) GT+TT (26)	5.3 (4.9; 5.9) 5.4 (5.0; 6.1)	TT (211) TC (115) CC (14)	5.3 (4.9; 5.8) 5.4 (4.9; 6.0) 5.2 (4.7; 6.3)
β		0.006±0.020		0.008 ± 0.009		0.003±0.007		-0.002±0.018		-0.007±0.009
P		0.748		0.342		0.627		0.928		0.446
CRP	CC (316) CT (20)	0.43 (0.13; 1.11) 0.16 (0.07; 0.79)	CC (218) CT (105) TT (13)	0.46 (0.13; 1.16) 0.32 (0.10; 1.00) 0.44 (0.09; 0.81)	TT (98) TA (158) AA (80)	0.28 (0.09; 0.90) 0.45 (0.15; 1.14) 0.57 (0.17; 1.14)	GG (310) GT+TT (26)	0.40 (0.11; 1.05) 0.72 (0.17; 1.30)	TT (209) TC (113) CC (14)	0.46 (0.11; 1.14) 0.39 (0.12; 1.00) 0.37 (0.06; 6.3)
β		-0.161±0.105		0.007±0.045		-0.019±0.035		0.104±0.094		-0.098±0.043
P		0.126		0.868		0.578		0.268		0.025
SBP	CC (319) CT (26)	122.0 (110.0; 134.0) 120.0 (113.5; 125.7)	CC (224) CT (105) TT (16)	123.0 (111.0; 133.7) 119.0 (110.0; 130.5) 130.0 (117.0; 156.7)	TT (105) TA (159) AA (81)	121.0 (110.0; 131.0) 120.0 (112.0; 134.0) 124.0 (111.0; 136.5)	GG (322) GT+TT (23)	121.0 (110.0; 133.2) 122.0 (112.0; 130.0)	TT (215) TC (118) CC (12)	120.0 (110.0; 131.0) 126.0 (115.0; 139.2) 118.0 (110.0; 129.2)

(Continued)

Table S2 (Continued).

Parameters	AgRP (rs5030980)		BDNF (rs4074134)		FTO (rs9939609)		GHRl (rs696217)		MC4R (rs17782313)	
	Genotypes (n)	Values	Genotypes (n)	Values	Genotypes (n)	Values	Genotypes (n)	Values	Genotypes (n)	Values
β		-0.016 \pm 0.016		-0.001 \pm 0.007		-0.004 \pm 0.006		0.006 \pm 0.017		0.005 \pm 0.008
P		0.320		0.854		0.456		0.710		0.551
DBP	CC (319)	80.0 (70.0; 90.0)	CC (224)	80.0 (70.0; 90.0)	TT (105)	80.0 (72.5; 90.0)	GG (322)	80.0 (70.7; 89.0)	TT (215)	80.0 (71.0; 89.0)
	CT (26)	77.5 (70.7; 87.5)	CT (105)	80.0 (71.0; 87.0)	TA (159)	80.0 (70.0; 87.0)	GT+TT (23)	78.0 (70.0; 90.0)	TC (118)	80.0 (70.7; 90.0)
			TT (16)	78.5 (69.7; 95.2)	AA (81)	81.0 (71.5; 90.0)			CC (12)	78.0 (62.0; 91.5)
β		-0.005 \pm 0.022		0.012 \pm 0.010		-0.001 \pm 0.008		0.004 \pm 0.023		-0.001 \pm 0.010
P		0.834		0.239		0.889		0.866		0.926

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