The rs9939609 Variant in FTO Increases the Risk of Hypercholesterolemia in Metabolically Healthy Subjects with Excess Weight

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Abstract

\textbf{Introduction:} The fat mass and obesity-associated gene (\textit{FTO}) is largely/primarily expressed in the hypothalamus. It plays a role in energy balance, regulation of food intake, and adipogenesis. According to metabolic phenotypes, studies have associated the \textit{FTO} rs9939609 variant with body mass index (BMI), body fat mass, and dietary intake but not with serum lipids. This study aimed to analyze the association of the \textit{FTO} rs9939609 variant with serum lipids in Mexican adults with different metabolic phenotypes. \textbf{Methods:} We included 306 subjects aged 18–65 years, classified as normal weight or excess weight (EW) according to their BMI. EW included BMI from 25 to 39.9 kg/m\textsuperscript{2}. Participants were classified into two metabolic phenotypes: metabolically healthy/metabolically unhealthy (MH/MUH). We use the homeostatic model assessment of insulin resistance and NCEP-ATP III cutoffs for glucose, triglycerides, high-density lipoprotein, and blood pressure. Subjects with ≥2 altered parameters were classified as MUH. The variant was determined by allelic discrimination with TaqMan\textsuperscript{\textregistered} probes. \textbf{Results:} In subjects with the A allele, significantly higher total cholesterol and low-density-lipoprotein cholesterol were found (\(p < 0.05\)). Furthermore, subjects with EW-MH and the AA or AT genotype had a significantly higher odds ratio for hypercholesterolemia (odds ratio 4.48, 95% confidence interval: 1.48–13.59, \(p = 0.008\)). \textbf{Conclusion:} The \textit{FTO} rs9939609 variant may influence serum lipid concentrations, increasing the risk of hypercholesterolemia.
Introduction

The prevalence of overweight and obesity has increased worldwide during the last decades. In Mexico, it has become one of the leading public health problems [1]. However, not all people with excess weight (EW) have clinical evidence of metabolic disturbances. Since 1982, evidence has demonstrated a group of subjects who do not show metabolic dysregulation despite meeting the body mass index (BMI) criteria for overweight or obesity [2]. These individuals have been considered “metabolically healthy obese” (MHO) [2]. In contrast to the MHO, some patients, despite of being classified with “normal/healthy” BMI (<25 kg/m²), have demonstrated increased metabolic dysregulation or cardiovascular risk [3].

One of the most common metabolic alterations in EW subjects is dyslipidemia [4, 5]. In Mexico, data from the National Health and Nutrition Survey (2020) showed a prevalence of hypercholesterolemia of 26.1% in adults [6]. Moreover, previous local results confirmed the presence of hypertriglyceridemia (26.6%) and hyperalphalipoproteinemia (11.4%) [7]. In addition, the main causes of hypercholesterolemia are a poor-quality diet, sedentary lifestyle, obesity, and genetic factors [8]. Among the genetic factors, it has been reported that the rs9939609 (T>A) variant in FTO might play a relevant role in lipid metabolism; however, controversial data have been found.

The rs9939609 (T>A) variant is in the first intron of FTO and may increase the risk of obesity by 20–30% [9]. This variant has been associated with increased energy intake, which in turn, leads to excess body fat in Scottish children [10] and adult males of mixed European descent [11]. In addition, studies have shown an association between the risk allele A with alterations in the lipid profile. Some studies have linked the FTO variant with lower levels of high-density-lipoprotein cholesterol (HDL-C) [12, 13], higher levels of total cholesterol (TC), and low-density-lipoprotein cholesterol (LDL-C) [14], while in other studies, no associations have been reported [15]. Therefore, this study aimed to assess the association of the FTO rs9939609 variant with hypercholesterolemia risk in Mexican adults with metabolically healthy (MH) or unhealthy (MUH) phenotype.

Materials and Methods

Subjects

This cross-sectional study enrolled 443 unrelated Mexicans; however, only 306 subjects completed the assessment and were included. The study was conducted at the Institute of Translation Nutrigenetics and Nutrigenomics of the University of Guadalajara, Jalisco, Mexico. Subjects aged 18–65 years were included and classified as normal weight (NW), overweight, or obese according to their BMI. The subjects with any medication prescribed for chronic diseases, such as diabetes mellitus type 2, cardiovascular, hepatic, renal, or pancreatic disorders, were excluded. Pregnant or lactating women were excluded due to physiological changes in the lipid profile at these stages of life.

Ethical Considerations

Subjects were informed about the research procedures and, if they agreed, provided a signed written informed consent before enrolling in the study. This study was approved by the Research Ethics Committee of the Universidad de Guadalajara (Registration number: CI/019/2010) and was conducted following the ethical guidelines of the 2013 Declaration of Helsinki [16].

Anthropometric and Clinic Measurements

All parameters were measured after 12 h of fasting. Anthropometric measurements were performed in light clothes and without shoes. A tetrapolar electrical bioimpedance was used to determine body composition, including weight, muscle mass, fat mass, and body fat percentage (InBody 3.0; Biospace Co., Seoul, Korea). Height was determined using a stadiometer with an accuracy of 1 mm (Rochester Clinical Research, Inc., New York, NY, USA). BMI was calculated by dividing kilogram weight by height in meters squared (kg/m²). Subjects were classified according to their BMI as NW (18.5–24.9 kg/m²) or EW if they were overweight or obese (25–40 kg/m²) [17]. Waist circumference was measured at the narrowest diameter between the last rib and the iliac crest and hip circumference at the maximum level of the posterior buttock bulge using a Lufkin Executive® Thinline 2-mm tape measure (Lufkin Executive Thinline, W606PM, MD, USA). All measurements were performed in duplicate, and the mean was reported. Systolic and diastolic blood pressures were assessed with a LifeSource digital sphygmomanometer (LifeSource, Milpitas, CA, USA) after at least a 15-min rest. The subjects were instructed to sit with their back touching the back of the chair and with their arm resting on a horizontal surface without crossing their legs. Two blood pressure measurements were performed, and the mean was recorded.

Definition of the MH and MUH Phenotypes

There is no consensus on the definition of the MUH phenotype. However, in this study and for NW and EW subjects, the following criteria established by Torres-Castillo et al. [18] were considered: blood pressure ≥130/85 mm Hg, triglycerides ≥150 mg/dL, HDL-C <40 mg/dL in men and <50 mg/dL in women, fasting glucose ≥100 mg/dL, and the homeostasis model assessment of insulin resistance (HOMA-IR) >2.5 [19]. If subjects had ≤1 of these altered cutoffs, they were considered as the MH phenotype; otherwise, they were classified as the MUH phenotype (≥2 metabolic alterations). Therefore, individuals were grouped into four groups: NW-MH, NW-MUH, EW-MH, and EW-MUH based on their BMI classification and metabolic conditions.

Dietary Intake

Subjects were given a format and explanations for completing a 3-day food consumption record (including a weekend day) to estimate their usual dietary intake. Food scales and Nasco® models were used to improve the accuracy of portion sizes when a regis-
Biochemical Analysis

Blood samples were collected after 8–10 h of fasting and centrifuged to obtain the serum. Determination of glucose, triglycerides, TC, and HDL-C was performed by dry chemistry using a Vitros 350 Analyzer (Ortho-Clinical Diagnostics, Johnson & Johnson Services Inc., Rochester, NY, USA). LDL-C was calculated using the Friedewald formula when triglyceride levels were <400 mg/dL [20] (LDL-C = TC – [HDL-C + triglycerides/5]). Very-low-density-lipoprotein cholesterol was calculated as follows: (TC – [LDL-C + HDL-C]). The cutoffs used to define alterations in the lipid profile were according to the American College of Cardiology and the American Heart Association, 2018 [21]: hypertriglyceridemia (≥150 mg/dL), hypoalphalipoproteinemia (<50 mg/dL in women and <40 mg/dL in men), and elevated LDL-C (≥100 mg/dL). Hypercholesterolemia was defined as total serum cholesterol ≥200 mg/dL according to the National Cholesterol Education Program (NCEP) III guidelines [22].

Insulin levels were determined using an ELISA assay (Monobind Inc, Lake Forest, CA, USA). Insulin resistance was estimated according to the HOMA-IR [19] calculated as follows: (fasting insulin [µU/ml] × fasting glucose [mg/dL])/405.

DNA Extraction and Genotyping

Genomic DNA was extracted from the peripheral blood using the High Pure PCR Template Preparation kit (Roche Diagnostics, Mannheim, Germany) and then diluted to 20 ng/µL. The FTO rs9939609 variant was determined by allelic discrimination using TaqMan® probes (assay number C__30090620_10; Drug Metabolism Assay, Applied Biosystems, Foster City, CA, USA) in a LightCycler® 96 Real-Time PCR System (Roche Diagnostics, Mannheim, Germany) under the following conditions: 95°C for 10 min and 40 cycles of denaturation at 95°C for 15 s and annealing/extension at 60°C for 1 min. Genotyping was verified using positive controls of the DNA samples corresponding to the three possible genotypes in each 96-well plate. A total of 20% of all samples were analyzed in duplicate.

Statistical Analysis

Statistical power was assessed according to the sample size calculation performed with an estimated margin of error of 5% with a 95% confidence level with an expected prevalence of subjects with MHO of 19% reported in a previous study [19]. The Kolmogorov-Smirnov test was used to determine the distribution of quantitative variables. All variables were log-transformed to improve the normal distribution. To analyze differences between subjects with NW or EW and the MH or MUH phenotype plus FTO genotype, a two-way analysis of covariance was used. Variables were adjusted for sex, age, and kilocalories presented as mean and standard error of the mean. The χ² test was used to compare categorical variables and to calculate the Hardy-Weinberg equilibrium.

The association between metabolic variables and the variant was analyzed by calculating the odds ratio (OR) with a 95% confidence interval. Analyses of association were performed using the χ² test and logistic regression adjusted by age and sex. In addition, multiple linear regression analyses were performed, considering anthropometric, biochemical, and dietary variables as dependent variables and genotype, sex, age, and phenotypes as independent variables. Collinearity was avoided by not including interrelated variables in the same model. All statistical analyses were performed using SPSS v. 28.0 software (IBM Corp., Armonk, NY, USA), and a p value <0.05 was considered statistically significant.

Results

Characteristics of Study Population according to the FTO rs9939609 Variant

The genotype distribution of the FTO rs9939609 variant in the whole sample (n = 306) was TT 54.9%, TA 38.9%, and AA 6.2%. The distribution of the T and A alleles were 74.6% and 25.4%, respectively. Genotypic frequencies were in the Hardy-Weinberg equilibrium (p = 0.73). Moreover, there were no significant differences between genotypes, allele frequency, or dominant genetic model per phenotype (Table 1).

Characteristics of Subjects with the MH or MUH Phenotype

Among all participants, the mean age was 37.3 ± 11.3 years, and 74.5% were women. Based on BMI, 120 subjects (39.2%) were classified as NW and 186 (60.8%) as EW. The MUH phenotype distribution included 30.8% of NW subjects and 67.2% of EW subjects. In addition, sociodemographic, anthropometric, biochemical, and dietary characteristics are presented in Table 2.

The study variables were analyzed according to the genotype and metabolic phenotype in the NW and EW subjects. According to the MH/MUH phenotype, we observed significant differences in all anthropometric variables, very-low-density-lipoprotein cholesterol, TG/HDL ratio, energy, and protein intake. On the other hand, for TC and LDL-C levels, differences by genotypes (TT vs. AT/AA) were statistically significant (p < 0.001, p = 0.006) (Table 2).

Association of the FTO rs9939609 Variant with Hypercholesterolemia

The significant statistical differences of genotypes in TC (p < 0.001) and the trend in the p value of phenotype-genotype interactions (p = 0.058) (Table 2) supported further analyses to assess risk genotypes in the different metabolic phenotypes with hypercholesterolemia. There were associations of the FTO rs9939609 variant with hypercholesterolemia statistically significant unadjusted and adjusted by sex, age, and total energy intake (kcal). These adjusted associations were significant in all sub-
projects (OR 2.17), in those with EW (OR 3.38), and with EW-MH (OR 30.37). Subjects with the AA or AT genotype showed a significantly higher OR for hypercholesterolemia (Table 3).

**Table 1. Genotypes and allelic frequencies of the FTO variant in subjects with MH and MUH phenotype**

| SNP rs9939609 | MUH (n = 138), n (%) | MH (n = 168), n (%) | p value | OR (95% CI) | Total (n = 306), n (%) |
|---------------|----------------------|---------------------|---------|-------------|----------------------|
| **Genotype**  |                      |                     |         |             |                      |
| TT            | 53.6 (90)            | 56.5 (78)           | 0.35    | 1           | 54.9 (168)           |
| AT            | 41.7 (70)            | 35.5 (49)           | 1.24 (0.77–1.99) | 38.9 (119)   |
| AA            | 4.8 (8)              | 8 (11)              | 0.63 (0.24–1.64) | 6.2 (19)     |
| **Allele**    |                      |                     |         |             |                      |
| T             | 74.3 (205)           | 74.4 (250)          | 0.99    | 1           | 74.6 (455)           |
| A             | 25.7 (71)            | 25.6 (86)           | 0.95 (0.69–1.43) | 25.4 (156)   |
| p-HWE         | 0.40                 | 0.22                | 0.73    |             |                      |
| **Dominant genetic model** |                  |                     |         |             |                      |
| TT            | 56.5 (78)            | 53.6 (90)           | 0.60    | 1           | 54.9 (168)           |
| AT + AA       | 43.5 (60)            | 46.4 (78)           | 1.13 (0.72–1.77) | 45.1 (138)   |

*p* corresponds to the χ² test. The dominant model was used based on the total sample size and subclassification of subjects. MH, metabolically healthy; MUH, metabolically unhealthy; OR, odds ratio; CI, confidence interval; p-HWE, p value of the Hardy-Weinberg equilibrium.

***Associations of the FTO rs9939609 Variant with Total Serum Cholesterol***

Multivariable linear regression analysis was performed to analyze the association of TC with the FTO rs9939609 variant (dominant model TT vs. AT/AA). In all subjects, those with EW and with EW-MH models showed a positive and significant association with the presence of the risk allele A after adjusting for sex and age. The EW-MH subjects with the AT or AA genotype had 38.34 mg/dL higher TC compared to those with the TT genotype (*p* = 0.001) (Table 4).

**Discussion**

To our knowledge, this is the first study which analyzes the association between metabolic phenotypes with the FTO rs9939609 variant in the Mexican population. Our analyses indicate that the FTO genotype influences TC and LDL-C levels, even adjusting for intervening variables such as age, sex, and dietary intake. Furthermore, individuals with the EW-MH phenotype and the A allele have an increased risk of hypercholesterolemia.

Other authors have reported the effect of the FTO variant on serum lipids. In a study in 200 Egyptian children and adolescents (100 obese and 100 control) aged 2–17 years, a significant association was observed between the high levels of LDL-C and carriers of the A allele. The authors showed that the carriers of the polymorphic allele had the highest values of LDL-C compared to wild-type homozygotes (TT: 77.3 ± 24.9 mg/dL vs. AA: 103.5 ± 34.3 mg/dL, *p* < 0.05) [23]. Another study was conducted on 788 Mexican adults in which TC was statistically higher in subjects with obesity but without diabetes type 2 and the homozygous risk allele (TT: 200.7 ± 39.9 kcal vs. AA: 221.9 ± 53.7 kcal, *p* = 0.015); however, the authors found no differences in nonobese subjects [24].

Furthermore, another study with 215 Mexican children showed that children with the rs9939609 variant in FTO had higher TC and LDL-C concentrations, although the difference was slight [14]. Both studies on the Mexican population share some similarities with our research. However, they did not classify the subjects by metabolic phenotypes nor explain the possible way in which FTO may modify lipid concentrations.

In addition, a study was conducted in the Iranian population under similar conditions to the present one, comparing the variables by four metabolic phenotypes related to cardiovascular and anthropometric indices and, separately, resembling the frequencies of rs9939609 genotypes by metabolic phenotypes. The authors found the highest OR for the cardiometabolic index in subjects with obesity and MUH phenotypes (OR 32.04, 95% confidence interval: 5.63–182.12, *p* < 0.001). This OR is similar to that found in the present research for hypercholesterolemia. However, these authors did not classify subjects by taking the phenotype and genotype together as we did [25].
Table 2. General characteristics of study subjects according to their metabolic condition and FTO genotypes

|                          | NW-MH (n = 83) | EW-MH (n = 61) | NW-MUH (n = 37) | EW-MUH (n = 125) | p value |
|--------------------------|----------------|----------------|----------------|------------------|---------|
|                          | genotype TT (n = 50) | genotype AT/AA (n = 33) | genotype TT (n = 23) | genotype AT/AA (n = 14) |         |
| Sociodemographic variables |                |                |                |                  |         |
| Women, %                 | 80             | 77.4           | 60.9           | 79.7             |         |
| Age, years               | 33.9±10.7      | 38.8±12        | 38±12.6        | 39±11            |         |
| Anthropometric variables |                |                |                |                  |         |
| Weight, kg               | 58.38±1.73     | 63.11±2.53     | 72.35±2.18     | 86.17±1.52       | <0.001  |
| BMI, kg/m²               | 21.85±0.63     | 23.22±0.92     | 27.65±0.78     | 32.56±0.55       | <0.001  |
| Muscle mass, kg          | 10.99±0.25     | 11.17±0.36     | 11.81±0.30     | 13.33±0.20       | <0.001  |
| Fat mass M, %            | 19.64±1.89     | 18.84±1.96     | 23.11±2.22     | 25.90±1.63       | <0.001  |
| Fat mass W, %            | 26.70±0.86     | 30.87±1.40     | 36.98±1.11     | 41.41±0.76       | <0.001  |
| WC M, cm                 | 84.20±3.19     | 84.67±3.30     | 92.60±3.75     | 100.49±2.75      | <0.001  |
| WC W, cm                 | 73.68±1.63     | 79.21±2.75     | 87.85±1.10     | 95.5±1.45        | <0.001  |
| Biochemical and clinical variables |                |                |                |                  |         |
| TC, mg/dL                | 176.84±4.83    | 182.59±7.09    | 167.3±6.09     | 187.09±4.29      | 0.112   |
| LDL-C, mg/dL             | 10.47±3.12     | 106.69±6.56    | 101.4±5.38     | 114.0±3.7       | 0.678   |
| VLDL-C, mg/dL            | 19.90±1.78     | 31.99±2.61     | 19.32±2.28     | 35.94±1.59       | <0.001  |
| TG/HDL index M           | 2.87±0.87      | 4.14±0.90      | 3.33±1.21      | 7.21±0.81       | <0.001  |
| TG/HDL index W           | 1.84±0.34      | 4.19±0.38      | 1.93±0.49      | 4.79±0.32       | <0.001  |
| Dietary intake           |                |                |                |                  |         |
| Energy, kcal             | 1,820.09±137.97| 1,939.24±212.03| 2,112.79±130.03| 2,105.93±94.47 | <0.001  |
| Carbohydrates, %         | 48.42±1.73     | 47.75±2.63     | 47.91±1.61     | 48.50±1.17       | 0.078   |
| Protein, %               | 15.07±0.79     | 17.11±1.20     | 17.14±0.73     | 16.91±0.53       | 0.497   |
| Fat, %                   | 36.50±1.66     | 35.11±2.52     | 34.94±1.55     | 34.60±1.12       | 0.223   |
| SFA, %                   | 9.71±0.63      | 9.55±0.96      | 10.88±0.67     | 8.81±0.48        | 0.042   |
| MUFA, %                  | 11.82±0.85     | 11.82±1.29     | 12.51±0.89     | 10.64±0.64       | 0.010   |
| PUFA, %                  | 5.12±0.45      | 5.36±0.69      | 5.12±0.48      | 5.13±0.34        | 0.973   |

All data are presented as mean ± SEM and were calculated according to two-way ANCOVA adjusted by sex and age; in the case of the dietary variables, data were also adjusted by total energy. All p values were calculated with variables logarithmically transformed for analysis. Phenotype p values represent whether there were differences between MH and MUH subjects, genotype p values represent whether there were differences between TT and AT/AA participants and interaction p values represent whether there were differences between subjects considering both metabolic phenotype and genotypes.

NW, normal weight; MH, metabolically healthy; EW, excess weight; MUH, metabolically unhealthy; BMI, body mass index; WC, waist circumference; M, men; W, women; WC, waist circumference; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol; TG, triglycerides; HDL-C, high density lipoprotein cholesterol; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Bold numbers represent statistical significance (p < 0.05).
The main advantage of classifying subjects according to the genotype and metabolic phenotype is that analysis of variables according to these interactions may provide the opportunity to reverse or anticipate long-term complications. However, one of the main problems is the lack of standard criteria for classifying subjects and the complexity to compare results with other studies [26].

Moreover, FTO belongs to the superfamily of Fe (II)- and 2-oxoglutarate-dependent dioxygenases. These dioxygenases play a crucial role in the demethylation of nucleic acids [27]. The possibility of regulating the expression of other genes by modifying their methylation-demethylation states has been suggested, due to the property of demethylating N6-methyladenosine, the most abundant RNA modification that stabilizes mRNAs [28].

This mechanism may help to explain the role of FTO in alterations of lipid metabolism, where the increased concentrations of TC and LDL-C may be related with the alteration in the expression of other genes. Reduced N6-methyladenosine levels caused by increased FTO expression have been shown to reduce carnitine palmitoyl transferase 1 (CPT1), hormone-sensitive lipase (LIPE), and adipose triglyceride lipase (ATGL) mRNA expression, leading to reduced fatty acid oxidation and lipolysis. It also causes increased expression of activating transcription factor 4 (ATF4), which stimulates the expression of lipogenic genes, leading to increased de novo lipogenesis in the liver [29].

In addition, recent studies have suggested that some obesity-associated variants in FTO, particularly located in intron 1, are functionally linked to neighboring genes such as Iroquois homeobox 3 (IRX3), Iroquois homeobox 5 (IRX5), and retinitis pigmentosa GTPase-like regulator-interacting protein 1 (RPGRIP1L) and may even influence the transcriptional regulation of these genes related with

### Table 3. Association of the FTO rs9939609 variant with hypercholesterolemia

| Subjects          | Unadjusted | Adjusteda |          | Unadjusted | Adjusteda |          |
|-------------------|------------|-----------|----------|------------|-----------|----------|
|                   | OR (β (95% CI)) | p value   | OR (β (95% CI)) | p value   |           |          |
| All subjects (n = 306) | 1.82 (0.60 (1.13–2.93)) | 0.013 | 2.17 (0.77 (1.12–4.17)) | 0.021 |           |          |
| NW (n = 120)       | 0.93 (−0.07 (0.41–2.10)) | 0.863 | 0.85 (−0.16 (0.26–2.79)) | 0.787 |           |          |
| EW (n = 186)       | 2.50 (0.92 (1.36–4.59)) | 0.003 | 3.38 (1.22 (1.70–7.63)) | 0.003 |           |          |
| NW-MH (n = 83)     | 0.70 (−0.35 (0.23–2.11)) | 0.530 | 0.45 (−0.80 (0.08–2.43)) | 0.354 |           |          |
| NW-MUH (n = 37)    | 1.81 (0.60 (0.46–7.18)) | 0.396 | 1.81 (0.59 (0.23–14.30)) | 0.575 |           |          |
| EW-MH (n = 61)     | 4.48 (1.50 (1.48–13.59)) | 0.008 | 30.37 (3.41 (3.68–250.41)) | 0.002 |           |          |
| EW-MUH (n = 125)   | 1.81 (0.60 (0.88–3.75)) | 0.108 | 1.87 (0.63 (0.73–4.80)) | 0.194 |           |          |

OR, odds ratio; CI, confidence interval; p, value logistic regression; NW, normal weight; EW, excess weight; MH, metabolically healthy; MUH, metabolically unhealthy. aAdjusted for sex, age, and total energy intake (kcal). The dominant model (TT vs. AT/AA) based on total sample size and subclassification of subjects was used.

### Table 4. Association between the FTO rs9939609 variant with TC

| Independent variable | R², % | β (95% CI) | p value |
|----------------------|-------|------------|---------|
| Model 1: All subjects | 7.2   | 13.05 (2.51–23.59) | 0.016   |
| Genotypes AT and AA  |       |            |         |
| Model 2: EW          | 9.9   | 15.38 (2.12–28.63) | 0.023   |
| Genotypes AT and AA  |       |            |         |
| Model 3: EW-MH       | 41.3  | 38.34 (18.04–58.63) | 0.001   |
| Genotypes AT and AA  |       |            |         |

Multiple linear regression models between the FTO variant (dominant model TT vs. AT/AA) as the independent variable and TC as the dependent variable (mg/dL). β, beta-coefficient; CI, confidence interval; SE, standard error; EW, excess weight; MH, metabolically healthy. All models were adjusted for age, sex, and total energy intake (kcal). The dominant model was used according to total sample size and subclassification of subjects.
obesity and fat mass [30]. Regarding the metabolic phenotypes, the fact that subjects with the EW-MH phenotype had a higher risk of developing hypercholesterolemia led us to propose that TC should be a parameter to be included in the classification of metabolic phenotypes; however, further analyses are needed to confirm this.

In contrast, it is not clear why the effect of the variant can no longer be observed in subjects with EW-MUH. Perhaps it could be because they already presented more alterations due to the chronicity of their EW and/or gene-environment interactions that could influence the effect of the variant [31].

Further studies in other populations (considering a larger number of subjects) linking metabolic phenotypes to variants in FTO are strongly recommended to support our results and better understand the associations. Therefore, we should be cautious when comparing various studies of metabolic phenotypes, due to the lack of homogeneity in the classification of subjects across studies.

One of the strengths of this study is the classification of subjects by metabolic phenotypes considering multiple variables to ensure homogeneous groups for comparison. Furthermore, we adjusted our results for potential confounding variables, and we obtained similar results. In addition, this is the first study associating metabolic phenotypes and FTO genotypes, and we expect that this will open the possibility for similar studies in different ethnic groups.

One of the limitations of this study was the small sample size when we analyzed subjects by subgroups, so we suggest replicating these results in a larger sample. Moreover, our results are restricted to the population of Western Mexico, so further studies are needed to compare our findings in different regions. Also, the food records to measure dietary intake have limitations, mainly due to the tendency of participants to report food intakes close to the socially desirable ones and the fact that a high level of motivation is required from participants.

Finally, it was impossible to assess physical activity, smoking habits, or diet quality due to a lack of information. Future studies are needed that include the assessment of dietary parameters more accurately, for example, the type of fatty acid ingested.

Our results suggest that FTO may play an important role in the regulation of lipid metabolism because the risk allele of FTO rs9939609 increases LDL-C and TC concentrations and the risk of hypercholesterolemia in EW-MH subjects. Therefore, we propose the possibility of including hypercholesterolemia as a criterion for classifying MUH subjects.

Acknowledgments
The authors thank the University of Guadalajara and the subjects who participated in the study.

Statement of Ethics
The study was reviewed and approved by the Research Ethical Committee of the University of Guadalajara (Registration number: CI/019/2010). The study was conducted according to the guidelines of the Declaration of Helsinki. Written informed consent was obtained from all subjects involved in the study.

Conflict of Interest Statement
The authors have no conflicts of interest to declare.

Funding Sources
This research was funded in part by the PROINPEP 2018 Universidad de Guadalajara grant for Erika Sierra Ruelas, Barbara Vizmanos, and Erika Martínez López. We also benefit of the support of the Programme for Strengthening Research and Postgraduate Studies 2020 (Programa de Fortalecimiento de la investigación y el posgrado 2020; REC/0342/2020) grant to Erika Martínez López and Barbara Vizmanos and the PRO-SNI 2018 grant to Barbara Vizmanos.

Author Contributions
Erika Sierra Ruelas and Erika Martínez López designed the study; Erika Sierra Ruelas and Nathaly Torres Castillo collected and processed the data; Wendy Campos Pérez, Barbara Vizmanos, and Panblo García Solís analyzed the data; Erika Sierra Ruelas, Wendy Campos Pérez, Nahtaly Torres Castillo, and Erika Martínez López participated in data interpretation and manuscript preparation; Barbara Vizmanos and Erika Martínez López contributed to funding acquisition. All the authors read and approved the final manuscript.

Data Availability Statement
Data cannot be shared for confidentiality reasons. All data generated or analyzed during this study are included in this article. Additional inquiries may be directed to the corresponding author.
