Interaction between FTO rs9939609 and the Native American-origin ABCA1 rs9282541 affects BMI in the admixed Mexican population

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Abstract

Background: The aim of this study was to explore whether interactions between FTO rs9939609 and ABCA1 rs9282541 affect BMI and waist circumference (WC), and could explain previously reported population differences in FTO-obesity and FTO-BMI associations in the Mexican and European populations.

Methods: A total of 3938 adults and 636 school-aged children from Central Mexico were genotyped for both polymorphisms. Subcutaneous and visceral adipose tissue biopsies from 22 class III obesity patients were analyzed for FTO and ABCA1 mRNA expression. Generalized linear models were used to test for associations and gene-gene interactions affecting BMI, WC and FTO expression.

Results: FTO and ABCA1 risk alleles were not individually associated with higher BMI or WC. However, in the absence of the ABCA1 risk allele, the FTO risk variant was significantly associated with higher BMI (P = 0.043) and marginally associated with higher WC (P = 0.067), as reported in Europeans. The gene-gene interaction affecting BMI and WC was statistically significant only in adults. FTO mRNA expression in subcutaneous abdominal adipose tissue according to ABCA1 genotype was consistent with these findings.

Conclusions: This is the first report showing evidence of FTO and ABCA1 gene variant interactions affecting BMI, which may explain previously reported population differences. Further studies are needed to confirm this interaction.

Keywords: Body mass index, FTO and ABCA1 variants, Interaction

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Background
The *FTO* rs9939609 gene variant has been consistently associated with BMI and obesity, however clear population differences have been identified [1]. Despite the high prevalence of obesity in Mexico, the *FTO* risk allele is considerably less frequent, both in admixed and Native populations as compared to Europeans (0.21, 0.06 and 0.46, respectively). Interestingly, rs9939609 has been associated only with class III obesity, but not with overall obesity or BMI in admixed Mexican adults [2, 3], and rs1421085, in high linkage disequilibrium with rs9939609, was not associated with obesity or BMI in admixed Mexican children [4]. It has been stated that loci that are specific to a single ancestry might contribute to genetic susceptibility across populations [5]. The *ABCA1*-R230C variant (rs9282541) is an ancestry-specific polymorphism private to the Americas and has been strongly associated with low HDL-cholesterol (HDL-C), although its association with BMI and obesity is inconsistent [6–8]. This allele is of particular interest, because it is relatively frequent in the Mexican mestizo population (0.11), is functional and was found to interact with BMI affecting abdominal fat distribution particularly in Mexican premenopausal women [8]. The aim of this study was to analyze possible *FTO* rs9939609 - *ABCA1* rs9282541 interactions affecting BMI, waist circumference (WC) and HDL-C levels in individuals from Central Mexico, which could help explain the differences observed between this and European populations.

Methods

Study population description
The studied population included 3938 DNA samples of unrelated Mexican mestizo adults from 4 different cohorts and 636 DNA samples of unrelated school-aged children. All cohorts include samples from Central Mexico and have been previously described (Table 1). Protocols for each cohort were approved by their respective Institutional Ethics Committee. Fully informed written consent for participation was attained from all participants or legal guardians.

Genotyping
The *FTO* rs9939609 and *ABCA1* rs9282541 variants were genotyped in 3938 DNA samples using TaqMan assays (ABI Prism 7900HT Sequence Detection System, Applied Biosystems). In addition, because the Mexican-Mestizo population is admixed, individual ancestry estimates were analyzed for 2354 individuals to test whether the results could be confounded by population stratification. Different panels of ancestry informative markers were used for each cohort (Additional file 1: Table S1).

Expression analysis
*FTO* and *ABCA1* mRNA expression was measured in subcutaneous (SAT) and visceral (VAT) adipose tissue biopsies from 22 admixed Mexican patients (16 female and 6 male), aged 25 to 55 years with BMI > =40 kg/m², who underwent bariatric surgery at the Hospital General Rubén Leñero in Mexico City. Total RNA was extracted with RNeasy Lipid Tissue Mini Kit (Qiagen), cDNA was reverse transcribed with TaqMan Reverse Transcription Reagents Kit (Applied Biosystems). Expression was analyzed using GeneChip Human Genome 2.0 ST Array (Affymetrix). *FTO* and *ABCA1* expression were validated by Real-Time PCR (LightCycler 480 II, Roche), using the following primers and probes: ctcggagaattagtttaggatatttca (forward) tctgaccccaagatcttggtct (reverse) and probe #59 for *FTO*, and tgtctcatgtctttgggac (forward), acctctctcgctgct (reverse) and probe #17 for *ABCA1*. Hypoxanthine phosphoribosyl transferase (*HPRT*) expression was measured as reference [2].

Statistical methods
HDL-C measurements were log-transformed for the analysis. Generalized linear regression (GLM) models were used to evaluate the individual effect of each single nucleotide variant and the interaction between *FTO* and *ABCA1* risk variants, adjusting for age, gender, ancestry and BMI as appropriate. GLM models were also used to compare mean values of subcutaneous and visceral *FTO* gene expression, adjusted for age and gender. Thus a model with main effects for risk variants and the

| Study (Reference) | Sample size (% ancestry) | Region (State) | Males (%) | Mean age (years ± SD) | Mean BMI (Kg/m² ± SD) | Mean WC (cm ± SD) | Mean HDL-C (mg/dL ± SD) |
|------------------|--------------------------|----------------|-----------|-----------------------|-----------------------|-----------------|----------------------|
| Romero-Hidalgo et al. [16] | 525 (67.5) | Central Mexico (Mexico City, Hidalgo, Edo. De México, Morelos, Querétaro) | 31.2% | 46.2 ± 13.6 | 27.7 ± 4.5 | 92.0 ± 14.1 | 44.8 ± 12.7 |
| Velázquez-Cruz et al. [17] | 1207 (50.6) | Central Mexico (Morelos) | 30.3% | 50.9 ± 15.3 | 27.0 ± 4.5 | 93.7 ± 10.8 | 44.3 ± 11.4 |
| Villarreal-Molina et al. [8] | 1511 (72.5) | Central Mexico (Mexico City) | 50.9% | 53.1 ± 9.3 | 28.4 ± 4.3 | 94.8 ± 11.6 | 45.9 ± 13.3 |
| Villalobos-Comparán et al. [2] | 695 (67.5) | Central Mexico (Mexico City) | 36.0% | 40.0 ± 13.6 | 27.2 ± 5.2 | 89.1 ± 13.4 | 46.3 ± 12.5 |
| León-Mimila et al. [3] | 636 | Central Mexico (Mexico City) | 48.23% | 9.4 ± 1.85 | 20.0 ± 3.84 | 70.2 ± 11.3 | 46.8 ± 10.9 |

*Proportion of individuals with an ancestry estimation*
adjusted variables plus the interactions between risk variants was fitted. All statistical analyses were performed using SPSS v.15.

**Results**

*FTO* and *ABCA1* risk allele frequencies were 20.3 and 10.0% in the overall population, respectively. In order to avoid potential population stratification, all individuals included in the analysis were from Central Mexico.

In the overall analysis and under additive inheritance models, the *FTO* “A” risk allele was not significantly associated with higher BMI ($\beta = 0.187$, $P = 0.143$) or higher WC ($\beta = 0.409$, $P = 0.208$), nor was the *ABCA1* “T” risk allele associated with BMI or WC ($\beta = 0.247$, $P = 0.154$ and $\beta = 0.369$, $P = 0.403$, respectively). Furthermore, although HDL-C levels were higher in *FTO* “A” homozygous individuals, the association did not reach statistical significance ($\beta = 0.005$, $P = 0.097$). As expected, the *ABCA1* “T” allele was strongly associated with lower HDL-C levels ($\beta = -0.03$, $P = 2.37 \times 10^{-12}$) (Fig. 1).

In order to assess a possible *FTO-ABCA1* gene interaction, we sought associations between the *FTO* risk variant and BMI, WC and HDL-C, stratified by the absence or presence of the *ABCA1* risk allele (“CC” and “CT/TT” genotypes, respectively). Interestingly, in the absence of the *ABCA1* risk “T” allele, the *FTO* risk variant was significantly associated with higher BMI ($\beta = 0.284$, $P = 0.042$, $n = 3191$) and marginally associated with higher WC ($\beta = 0.650$, $P = 0.063$, $n = 3191$). In contrast, in the presence of the *ABCA1* risk allele, the *FTO* risk variant was not associated with BMI ($P = 0.421$, $n = 747$) or WC ($P = 0.376$, $n = 747$). The interaction analyses between *FTO* rs9939609 and *ABCA1* rs9282541 affecting BMI and WC were statistically significant ($P = 0.040$ and $P = 0.045$, respectively). *ABCA1* and *FTO* gene variants showed no significant interaction affecting HDL-C levels ($P = 0.856$) (Fig. 1).

Individual ancestry estimates were available in 60% of the samples. After adjusting for Native American ancestry, the statistical significance of interactions between

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**Fig. 1** Association of *FTO* rs9939609 and *ABCA1* rs9282541 risk variants, independently and stratified, with BMI, waist circumference (WC) and HDL-cholesterol (HDL-C) levels, adjusting for age, gender and BMI as appropriate
**Discussion**

According to WHO, Mexico has one of the highest rates of adulthood and childhood obesity. This higher prevalence of obesity as compared to European populations could be explained by the Native American component as the result of adaptive processes related to energy saving, or could be the result ancestry-specific allele combinations derived from the admixture process. We thus analyzed whether the functional private ABCA1-R230C risk allele might interact with the most replicated obesity risk allele FTO rs9939609.

**FTO and ABCA1 risk allele frequencies** were 20.3% and 10%, respectively, similar to previous reports [2, 3]. Individually, FTO and ABCA1 risk alleles showed no significant association with BMI or WC. However, in the absence of the ABCA1 risk variant, the effect of FTO on BMI and WC became stronger, statistically significant and similar to what has been reported in European populations [9]. Observations from the cohort of children showed a similar trend, although the gene-gene interaction reached statistical significance only in the adult cohort. The lack of significance in children was likely due to the small sample size. Replication studies in independent adult and childhood cohorts are necessary to confirm this interaction. This type of interaction may explain differences in FTO associations with obesity between Mexican and European populations, but they do not explain the higher prevalence of obesity in Mexico.

**FTO mRNA expression** was significantly higher in SAT than in VAT, in accordance with previous studies in other populations [10]. Interestingly, allele-specific FTO expression in SAT differed significantly only in the absence of the ABCA1 risk allele, which is consistent with the interactions described above. It is noteworthy that no significant differences in allele-specific FTO expression have been observed in SAT biopsies from European
individuals [11, 12]. However, a previous study in Mexican patients with morbid obesity, the rs9939609 “TA” genotype was significantly associated with higher FTO expression [2]. Although the latter biopsies were not genotyped for ABCA1-R230C, it is noteworthy that the only independent studies reporting allele-specific differences were performed in Mexican patients.

Although FTO and ABCA1 are both known to play a role in adipose tissue function, there is no previous experimental evidence directly linking the function of both genes [13, 14]. However, previous evidence supports the role of ABCA1 in body fat distribution both in the Mexican population [8], and in a recent multi-ethnic meta-analysis that identified ABCA1 rs10991437 as a variant associated with higher waist-hip ratio adjusted for BMI [15].

**Conclusions**

To our knowledge this is the first report showing evidence of FTO and ABCA1 gene variant interactions affecting BMI, which may explain previously reported population differences. Further studies are needed to understand the possible biological mechanisms underlying this interaction.

**Additional file**

**Additional file 1:** Panels of ancestry informative markers used for each cohort [18]. (DOC 30 kb)

**Abbreviations**

ABCA1: ATP Binding cassette Transporter A-1; BMI: Body mass index; FTO: Fat mass and obesity associated gene; HDL-C: High Density Lipoprotein Cholesterol; WC: Waist circumference; WHO: World Health Organization

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**Availability of data and materials**

All relevant data are available within the manuscript and its supporting information documents.

**Authors’ contributions**

MVC, BAP, SRH, MTVM responsible for the study design, and writing the manuscript. MVC, SRH, DJ participate in data analysis. MTVM, SCQ, RVC, SRH responsible for the cohort studies conception and design. PALM, HVR, OESH, JAGB, MQ, JLMG, MRTB, MERA, CPR, GBA, FCP, JSC responsible of acquisition of samples, data and carried out the experiments. All authors reviewed and approved the submitted version.

**Competing interests**

The authors declare that they have no competing interests.

**Consent for publication**

Not applicable.

**Ethics approval and consent to participate**

Protocols and informed consent forms for each cohort were approved by their respective Institutional Ethics Committee as follows: Ethics Committee of the National Institute of Public Health [16]; IMSS Research Ethics Committee [17]; Biomedical Research in Humans of the Instituto Nacional de Ciencias Médicas y Nutrición “Salvador Zubirán” (INCMNSZ) [2, 3]; Instituto Nacional de Cardiología “Ignacio Chávez” (INICICH) and the Ethics Committee of the Instituto Nacional de Medicina Genómica (INMEGEN) [8]. Fully informed written consent for participation was attained from all participants or legal guardians.

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