Association between FTO gene rs9939609 and adiposity markers in Chilean children

Asociación entre el polimorfismo rs9939609 del gen FTO y marcadores de adiposidad en población infantil chilena

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What do we know about the subject matter of this study?
The rs9939609 variant of the FTO gene has been recognized worldwide for its association with obesity risk in different populations and ages according to the WHO classification, based on BMI or BMI percentile limits. Our group previously reported that the rs9939609 variant of the FTO gene was associated with a higher risk of increased BMI z-score in Chilean children.

What does this study contribute to what is already known?
Obesity is a pathological state characterized by excess body fat, specifically in the adipose tissue. This study shows the association between the presence of the polymorphism rs9939609 of the FTO gene and the increase of the general adiposity (% of fat mass) and central adiposity (waist circumference and waist/height ratio), in the pediatric population of Chile.
Abstract

Obesity is considered a chronic inflammatory disease with an important genetic component. Although several studies have reported an association between the FTO (fat-mass associated gene) and adiposity in children, there is limited evidence in the Chilean population. **Objective:** To determine the association between the polymorphism rs9939609 of the FTO gene and markers of adiposity in Chilean children. **Patients and Method:** Cross-sectional study which included 361 children aged between 6 and 11 years (50% were girls). Between March and June 2008, clinical data and blood sample collection was carried out. The rs9939609 single-nucleotide polymorphism (SNP) of the FTO gene, was determined using the genomic DNA extracted from leukocytes, using the QIAamp DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany). The adiposity markers included body mass index (BMI), waist circumference (WC), body fat, and WC/H index; which were later compared adjusted by sex, age, and Tanner stage. Linear regression analyses were conducted to detect the association between the polymorphism and obesity markers. **Results:** After adjusting the models by age, sex, and Tanner stage, we found a significant association between the polymorphism and markers of adiposity. For each extra copy of the risk allele, we found an increase of 2.47 kg body weight (95% CI: 1.39-3.55); 1.06 kg/m² BMI (95% CI: 0.56-1.54); 2.55 cm WC, (95% CI: 1.26-3.85); and 1.98% body fat (95% CI: 0.78-3.19). When converting adiposity markers to z-score, we found that WC/height index shows the strongest association with the risk allele FTO. **Conclusion:** This study supports the association between the rs9939609 SNP of the FTO gene and overall and central adiposity markers in Chilean children.

Introduction

Obesity is one of the preventable diseases, even though it is currently highly prevalent, affecting more than 600 million people in 2014 and is associated with the genesis and increased prevalence of chronic metabolic diseases. For this reason, stopping the rise in obesity rates represents one of the nine global goals for preventing and controlling chronic non-communicable diseases that the World Health Organization (WHO) has set for 2025. It has been estimated that the number of obese individuals will reach 1.12 billion by 2030.

In the child population, obesity is a serious and urgent public health problem. Its prevalence has increased significantly in children and adolescents, in both developed and developing countries. In Chile, the increase in childhood obesity has been sustained since the late 1980s. Currently, the prevalence of excess body weight presented in the pre-kindergarten, kindergarten, first, and ninth grade corresponds to 49.3%, 50.8%, 51.1%, and 44.5%, respectively. This scenario confirms that childhood obesity represents a serious and urgent public health problem.

Among the main risk factors for the development of obesity are physical inactivity, sedentariness, the amount of energy intake, and the Western-type dietary pattern, all of which can be modified. Potential non-modifiable risk factors include gender, age, ethnicity, as well as genetic polymorphisms.

Since 2007, after it was reported that the polymorphism rs9939609 of the FTO gene presents an association with body mass index (BMI), the identification of single-nucleotide polymorphisms (SNP) genes associated with increased BMI has rapidly risen. By 2015, more than 97 genetic polymorphisms associated with increased BMI had been identified. Recently, a meta-analysis summarized the existence of 738 SNPs associated with several obesity markers (waist circumference, body fat, visceral adipose tissue, etc.).

Out of all the genetic variants studied, the variant rs9939609 of the FTO gene (fat mass and obesity-associated gene) is the one that has been mostly studied due to its recognized effect on increasing BMI and obesity risk, confirming its association in different adult and child populations worldwide.

Previously we reported that the variant rs9939609 of the FTO gene was associated with a higher risk of increased BMI in Chilean children, but its relationship with other adiposity markers was not evaluated. The objective of this study was to investigate the association between the polymorphism rs9939609 of the FTO gene and markers of general and central adiposity in this Chilean child population.

Patients and Method

Cross-sectional study which included 361 children aged between 6 and 11 years from urban areas in the Biobio Region, Chile. Children suffering from any...
chronic pathology were excluded. This study was approved by the Bioethics Committee of the University of Concepción. The parents or guardians of the children signed informed consent before the inclusion of their child in the study.

**Anthropometric variables: obesity markers**

Height was measured without shoes, using a wall-mounted stadiometer with an accuracy of 0.1 cm (Seca, model 208). The body weight was measured with light clothing and without shoes on a Tanita scale (model TBF-300) with an accuracy of 1 g. BMI was through body weight divided by height squared (kg/m²). BMI z-score, based on age and sex, was calculated according to WHO definitions. Children were classified as normal (BMI ≥ 5 and < 85 percentile) or obese (BMI ≥ 95 percentile) according to the international age and gender percentiles defined by the Center for Disease Control and Prevention (CDC)²⁰. The waist circumference (WC) was measured between the lowest rib and the upper edge of the iliac crest, with a non-distensible tape measure (Seca, model 201) with an accuracy of 0.1 cm. Body composition was evaluated using a bioelectrical impedance analyzer (Tanita, TBF-300). All measurements were taken by trained nutritionists. To define the pubertal stage according to Tanner’s criteria²⁰, children were examined by medical professionals.

**Determination of allelic variation of the FTO gene**

Between March and June 2008, the clinical data and blood samples were collected. The SNP polymorphism (rs9939609) of the FTO gene, was determined using genomic DNA extracted from leukocytes, using the QIAamp DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany), according to the manufacturer’s protocol. Polymerase chain reaction (PCR) amplifications were performed in using the Rotor-Gene 6500 real-time PCR cycler (Corbett Research, Sydney, Australia), using splitters previously described by Lopez-Bermejo et al.²¹ (direct: 5’dACTG GCTCTTGAATGAAATAGGATTCAGA 3’ and inverse: 5’ dAGAGTAACAGAGATCCAGTGCATCAC3’), according to a previously standardized protocol²⁰.

Genotype identification was performed by comparison (confidence intervals, 95% CI) of fusion data with standard genotypes identified by sequencing analysis at the Department of Ecology, Faculty of Biological Sciences, Pontifical Catholic University of Chile. To confirm the existence of a single-stranded PCR product, we performed a 3% agarose gel electrophoresis. All sample analyses were performed in duplicates, with a 98% success rate in genotype determination.

**Statistical analysis**

The characteristics of the population studied are presented as mean and standard deviation (SD) for continuous variables, and as percentages for the categorical ones. Differences between genotypes were determined with regression analysis for continuous variables and with the Chi-square test for the categorical ones.

To identify the association between the FTO gene and obesity markers, we performed a linear regression analysis of BMI, waist circumference (WC), waist/height ratio, and % body fat. The SNP genotype rs9939609 of the FTO gene was encoded according to an additive genetic model (0 = TT - homozygous for the protective allele; 1 = AT - heterozygous for the risk allele; 2 = AA - homozygous for the risk allele), and subsequently, through linear regression analysis, we estimated the increase in the adiposity variable for each additional copy of the risk variant (allele A). These results are shown as average or beta coefficient with their respective 95% confidence intervals (95% CI).

To determine which adipose markers had the highest association with the FTO gene, all variables were standardized to z-score and, therefore, the results were presented as a standardized beta coefficient and their respective 95% CI, for each additional copy of the FTO gene risk allele.

All analyses were adjusted for confounding variables using three statistical models, Model 0 - unadjusted; Model 1 - adjusted for age and sex; Model 2 - adjusted by Model 1, but also by Tanner’s stage. The Hardy-Weinberg principle of the FTO gene alleles was estimated through the Chi-square test in the STATA SE v14 software which was also used for all analyses. The significance level was defined as p < 0.05.

**Results**

The studied population comprises 361 Chilean children, in which anthropometric parameters were measured, and the SNP rs9939609 was genotyped in the FTO gene, finding that the respective allele frequency is distributed following the Hardy-Weinberg equilibrium (allele T = 0.649 and allele A = 0.351, $\chi^2 = 0.053$).

Table 1 describes the general characteristics of the population, which has 178 boys and 183 girls, aged between 6 and 11 years (average age: 8.51 ± 1.44). According to Tanner’s stages, 79.8% of the children were in the pre-pubertal development stage. The average nutritional status showed a percentile 87.0 ± 16.6 with 66.8% of boys and 65.0% of girls obese. The risk variant in its heterozygous form (genotype TA) appears
Table 2. Association between genotipe FTO (rs9939609) and adiposity markers

<table>
<thead>
<tr>
<th>Variables</th>
<th>Genotype FTO rs9939609 (Media [IC 95%])</th>
<th>Effect of additive genetic model</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 0</td>
<td>36.6 (35.0 - 38.2)</td>
<td>2.19 (0.76 - 3.61)</td>
<td>0.003</td>
</tr>
<tr>
<td>Model 1</td>
<td>36.2 (35.0 - 37.4)</td>
<td>2.50 (1.41 - 3.57)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Model 2</td>
<td>36.2 (35.0 - 37.4)</td>
<td>2.47 (1.39 - 3.55)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>IMC (kg/m2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 0</td>
<td>20.9 (20.3 - 21.4)</td>
<td>0.99 (0.47 - 1.51)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Model 1</td>
<td>20.8 (20.3 - 21.3)</td>
<td>1.06 (0.57 - 1.54)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Model 2</td>
<td>20.8 (21.2 - 21.3)</td>
<td>1.06 (0.56 - 1.54)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 0</td>
<td>70.8 (69.2 - 72.4)</td>
<td>2.28 (0.81 - 3.75)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Model 1</td>
<td>70.5 (69.0 - 71.9)</td>
<td>2.56 (1.27 - 3.85)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Model 2</td>
<td>70.5 (69.0 - 71.9)</td>
<td>2.55 (1.26 - 3.85)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>% Fat mass</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 0</td>
<td>28.1 (26.7 - 29.4)</td>
<td>1.93 (0.69 - 3.17)</td>
<td>0.002</td>
</tr>
<tr>
<td>Model 1</td>
<td>27.9 (26.6 - 29.2)</td>
<td>1.99 (0.78 - 3.19)</td>
<td>0.001</td>
</tr>
<tr>
<td>Model 2</td>
<td>27.9 (26.6 - 29.3)</td>
<td>1.98 (0.78 - 3.19)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Data is presented as media and its respective confidence interval, IC 95%, by each genotype. The additive genetic model indicates the increment media of adiposity variable for each additional copy of risk allele (A).
Discussion

In this study, the main result is that for each extra copy of the risk allele rs9939609 of the FTO gene, there was a greater probability of increasing the magnitude of the adiposity indicators studied (% body fat, WC, waist/height ratio, and BMI z-score).

These results indicate that the polymorphism rs9939609 of the FTO gene contributes to a higher level of obesity in Chilean children.

Previously, our research group had shown that rs9939609 of the FTO gene is associated with an increased risk of obesity, expressed as an Odds ratio, in this same cohort, comprised of boys and girls from 6 to 11 years of age, who come from an urban area and of social vulnerability state in the Biobío Region, Chile. However, this publication did not study other obesity indicators, nor did it include a statistical analysis to mitigate the influence of non-modifiable confounding factors (age, sex, Tanner’s stage).

Several studies have shown that FTO is a gene of underlying susceptibility for polygenic obesity and that the influence of the FTO gene on BMI changes over life in European populations.

In this study, the association between rs9939609 polymorphism and obesity markers did not change, despite possible confounding factors (age, sex, or Tanner stages). The onset age of the association has been reported to be as early as 7 years or even earlier, which is consistent with our findings in this 6-11-year-old population.
old population. The children in this study were mostly in a pre-pubertal stage (79.8%), while previous studies show variation in wider age ranges, throughout the first and also the second decade of life\textsuperscript{23}. Besides that, the associations of FTO polymorphism with BMI and obesity were stronger in girls than in boys as has been previously reported\textsuperscript{24,25}, which was not observed in this study. These apparently contradictory results may be due to the low statistical power resulting from the small sample size, along with the relative predominance of a single Tanner stage (pre-pubertal). It is worth to mention that differences in the degree of exposure to obesogenic environments between populations in the different studies cannot be excluded either. In this context, it is essential to carry out new studies with a larger sample size, which allows a better exploration of the possible interactions between age, sex, and pubertal development.

In the Chilean adult population, the association between this FTO gene polymorphism and obesity variables has also been identified\textsuperscript{26}, but unlike in our work, there was no association with WC. This could indicate that the distribution of adiposity changes with age in subjects carrying the rs9939609 FTO gene polymorphism.

This fact is in line with a recent study, where researchers found that the association of BMI with different loci of genetic predisposition to obesity, changes throughout the development of the children in the Santiago Longitudinal Study (ELSOC). Specifically, it was observed that the highest association between core BMI-z and most loci occurs at age 10, except for FTO, which can reach its maximum effect up to age 16\textsuperscript{27}.

It is important to mention that several authors have shown that children who are carriers of the FTO risk variable, present low appetite regulation, and higher food intake\textsuperscript{28-31}. In addition, a meta-analysis showed that dietary protein intake can modify the influence of FTO variants on BMI\textsuperscript{32}. These findings provide new insight into the interrelationships between FTO gene variations, food intake, and obesity and may be useful in improving the design of intervention programs for children in the management of eating behaviors and excessive weight gain.

However, some limitations should be considered. Although the sample size provides sufficient statistical power to detect the overall association of the rs9939609 FTO gene polymorphism with obesity indicators, the sample size was not enough to perform stratified analysis by age, sex, or pubertal stage between the FTO gene genotype and adiposity indicators. Also, only the presence of the rs9939609 polymorphism in the first intron of the FTO gene was analyzed, and currently, it is known that the FTO gene is highly polymorphic as a whole, especially in introns 1 and 8\textsuperscript{28}.

Our analyses did not allow us to rule out the presence of other SNPs of the FTO gene in the analyzed population, and no background information on the children’s lifestyles (eating habits, physical exercise, and others) was recorded, which could influence the association result that we report between the FTO gene and the obesity markers.

In conclusion, this study indicates the association of the FTO gene and its polymorphism rs9939609 with markers of general and central adiposity in the Chilean child population and provides evidence that this association is independent of some non-modifiable confounding factors (age, sex, Tanner stage). It would be interesting to carry out future studies to evaluate modifiable risk factors, such as eating behavior, physical exercise, and other lifestyle indicators of Chilean children carriers of this FTO gene polymorphism, and to determine how these behaviors or lifestyles affect the obesity markers in subjects carrying this genetic variant.

**Ethical Responsibilities**

**Human Beings and animals protection:** Disclosure the authors state that the procedures were followed according to the Declaration of Helsinki and the World Medical Association regarding human experimentation developed for the medical community.

**Data confidentiality:** The authors state that they have followed the protocols of their Center and Local regulations on the publication of patient data.

**Rights to privacy and informed consent:** The authors have obtained the informed consent of the patients and/or subjects referred to in the article. This document is in the possession of the correspondence author.

**Conflicts of Interest**

Authors declare no conflict of interest regarding the present study.

**Financial Disclosure**

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References


25. Hakanen M, Raatikari OT, Lehtimäki T, et al. FTO genotype is associated with body mass index after the age of seven years but not with energy intake or leisure-time physical activity. J Clin Endocrinol Metab. 2009;94:1281-7.


