Polymorphisms in Adipokines in Mexican Children with Obesity

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The high prevalence of childhood obesity in Mexico is alarming in the health-science field. We propose to investigate the contribution of adipokines and cytokines polymorphisms and common BMI/obesity-associated loci, revealed in genome-wide association studies in Caucasian adult cohorts, with childhood obesity. This study included 773 Mexican-Mestizo children (5-15 years old) in a case-control study. The polymorphisms included were ADIPOQ (rs6444174), TNF-α (rs1800750), IL-1β (rs154107; rs2069845), NEGR1 (rs34305371), SEC16B-RASAL2 (rs10913469), TMEM18 (rs6548238; rs7561317), GNPDA2 (rs16857402), LEP (rs2167270), MTCH2 (rs10838738), LEPR (rs34057371), TMEM18 (rs6548238), and KCTD15 (rs29942). No significant contribution was found with adipokines and cytokines polymorphisms in this study. Only both TMEM18 (rs6548238; rs7561317) polymorphisms were found associated with obesity (OR=0.5, P=0.008) and were in linkage disequilibrium (r^2 =0.87). The linear regression showed that the rs7561317 polymorphism of TMEM18 is negatively associated with obesity. This report highlights the influence of TMEM18 in Mexican-Mestizo children obesity, while adipokine and cytokine polymorphisms were not associated with it.

1. Introduction

Mexico has the highest prevalence of childhood obesity worldwide [1]. Children obesity is a predictive factor for adult obesity [2] and leads to the development of insulin resistance, diabetes, hypertension, cardiovascular disease, or cancer [3].

Children obesity develops in response to several factors, where nutrition and energy expenditure play a determinant role [4]. Adipose tissue is an endocrine organ that secretes hormones (adipokines) and proinflammatory cytokines [5]. Adiponectin is secreted by adipocytes with antidiabetic, antiatherogenic, and anti-inflammatory functions [6].

During obesity adiponectin decreases and serum levels are negatively correlated with body mass index (BMI) in prepubertal children [7]. An increase in proinflammatory cytokines like Tumor Necrosis Factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6) suppresses adiponectin expression [8], which is regulated by the ADIPOQ gene, and single nucleotide polymorphisms (SNPs) in this gene have been associated with obesity-related traits [9].

Also, genetic factors also have a significant contribution to obesity as evidenced by the large disparities observed between ethnic groups. Results of large cohort studies showed that American Indian, Mexican, and Hispanic children have
Table 1: Single nucleotide polymorphisms evaluated in this study.

| SNP              | Gene                  | Chromosome |
|------------------|-----------------------|------------|
| rs34305371       | NEGR1                 | 1          |
| rs10913469       | SEC16B-RASAL2         | 1          |
| rs6548238        | TMEM18                | 2          |
| rs7561317        | TMEM18                | 2          |
| rs1143643        | IL-1beta              | 2          |
| rs6444174        | ADIPOQ                | 3          |
| rs16857402       | GNPDA2                | 4          |
| rs1800750        | TNF-alpha             | 5          |
| rs1524107        | IL-6                  | 7          |
| rs2069845        | IL-6                  | 7          |
| rs2167270        | LEP                   | 7          |
| rs10838738       | MTCH2                 | 11         |
| rs925946         | LGRA-LIN7C-BDNF       | 11         |
| rs7138803        | BCDIN3D-FAIM2         | 12         |
| rs6203340        | FTO                   | 16         |
| rs11872992       | MC4R                  | 18         |
| rs17782313       | MC4R                  | 18         |
| rs29942          | KCTD15                | 19         |

a higher risk to develop obesity than Africans children [10]. It has been estimated that obesity heritability goes from 6% to 85% among several populations. Genome Wide Association Studies (GWAS) have revealed several loci highly associated with body mass index (BMI), waist circumference, and body fat percentage (BF%) among populations [11–13]. Fat mass and obesity-associated gene (FTO) has been widely linked to obesity among populations, and posterior replications have shown an additional signal in the melanocortin 4 receptor (MC4R) locus. From the Genetic Investigation of Anthropometric Traits (GIANT) consortium, additional loci were associated with obesity, like transmembrane protein 18 (TMEM18), potassium channel tetramerization domain containing 15 (KCTD15), glucosamine-6-phosphate deaminase 2 (GNPDA2), Src homology 2B (SH2B) family member 1 (SH2B1), mitochondrial carrier 2 (MTCH2), the melanocortin 4 receptor (MC4R), and the neuronal growth regulator 1 (NEGR1) genes [14]. Thus, the aim of this study was to evaluate the association between polymorphisms previously associated in GWAS in Caucasian adult population, adipokines, and proinflammatory cytokines with obesity in Mexican-Mestizo children.

2. Methodology

2.1. Study Design. A case of control study was conducted in 773 children ranging from 5 to 15 years old, from the children obesity cohort COIPIS (Cohorte de Obesidad Infantil-Proyecto Infancia Saludable) from Hospital Regional Lic, Adolfo López Mateos, ISSSTE (Instituto de Seguridad y Servicios Sociales de los Trabajadores del Estado) [15].

After parents signed an informed consent to include their children in this study, anthropometric measurements (weight, height, BMI, and BF%) were determined. BMI was calculated as weight (kg) divided by height (m) squared. Obesity was defined according to the World Health Organization (WHO) Child Growth Standard for children as BMI-for-age value over +2 SD [16] using the 95 percentile. The BF% was evaluated using a Body composition analyzer with an accurate stadiometer (InBody J10). A whole blood sample was taken to evaluate clinical parameters at fasting: glucose, glycated hemoglobin (HbA1c), creatinine, triglycerides (TAG), total cholesterol (CHOL), and high-density lipoprotein cholesterol (HDL).

An exclusion criterion was related to children with foreign parents and grandparents. This study was conducted in accordance with the Declaration of Helsinki and approved by the Hospital Regional Lic, Adolfo López Mateos Research, Ethics, and Biosafety Committees (registration number 245.2012).

2.2. DNA Extraction and Genotyping. Genomic DNA was obtained from 200 μl of the whole blood-EDTA with the InviMag Blood DNA Mini Kit, using an automated system (InviGenius, Stratec). For genotyping, we selected 18 SNPs (Table 1) which were amplified using predesigned 5’ exconuclease TaqMan genotyping assays on a 7500 series Real-Time PCR system (Applied Biosystems, Foster City, CA, USA).

3. Statistical Analysis

The results of continue variables are presented as mean ± standard deviation for Gaussian distribution and analyzed by the Student t test. For non-Gaussian distribution, the continuous variables are presented as median and interquartile range and analyzed by Mann-Whitney test. A chi-squared test was performed to estimate the Hardy-Weinberg equilibrium in controls. Pearson correlations (R) were used to assess
Table 2: Clinical and anthropometrical measurements according to obesity status.

|               | Nonobesity | Obesity | P       |
|---------------|------------|---------|---------|
| n             | 569        | 204     |         |
| Age           | 9.3 (6.1–12.9) | 11.3 (9.2–13.4) | <0.001  |
| Male n, (%)   | 275 (48.3) | 120 (58.8) | 0.032   |
| Body Fat (%)  | 23.9 ± 10.2 | 38.27 ± 8.7 | <0.001  |
| BMI           | 173 (15.7–19.7) | 24.98 (22.5–21.6) | <0.001  |
| Glucose       | 96 (90–103) | 98 (92–105) | 0.0199  |
| HbA1c         | 5.4 (5.1–5.6) | 5.44 (5.2–5.7) | 0.2182  |
| Creatinine    | 0.91 (0.77–1.07) | 0.98 (0.83–1.14) | 0.0018  |
| TAG           | 87.5 (66.5–113) | 120 (90–167) | <0.001  |
| CHOL          | 162 (142–180) | 170 (152–191) | 0.0023  |
| HDL           | 61.34 ± 12.34 | 56.77 ± 12.05 | <0.001  |

Data are presented as mean ± SD for continuous variables with normal distribution, median (interquartile range) for continuous variables with nonnormal distribution. BMI: body mass index (kg/m²), HbA1c: glycated hemoglobin, TAG: triglycerides, CHOL: cholesterol, and HDL: high density lipoprotein cholesterol.

Table 3: Logistic regression of polymorphisms studied.

| SNP            | Gene        | MAF  | Allele | OR (95% CI), Pdom | OR (95% CI), Padd |
|----------------|-------------|------|--------|-------------------|-------------------|
| rs34305371     | NEGR1       | 0.02 | G      | 0.92, (0.4-1.97), 0.841 | 0.88, (0.4-1.8), 0.746 |
| rs10913469     | SEC6B-RASAL2| 0.02 | C      | 1, (0.7-1.36), 0.902 | 1, (0.8-1.3), 0.826 |
| rs6548238      | TMEM18      | 0.05 | T      | 0.5, (0.3-0.83), 0.008 | 0.53, (0.3-0.9), 0.013 |
| rs7561317      | TMEM18      | 0.06 | A      | 0.52, (0.3-0.8), 0.008 | 0.5, (0.3-0.9), 0.012 |
| rs1143643      | IL-1beta    | 0.25 | T      | 0.93, (0.7-1.3), 0.711 | 0.88, (0.7-1.16), 0.372 |
| rs6444174      | ADIPOQ      | 0.03 | C      | 0.65, (0.3-1.3), 0.224 | 0.63, (0.3-1.2), 0.193 |
| rs16857402     | GNPDA2      | 0.12 | C      | 1.06, (0.7-1.6), 0.743 | 1.06, (0.7-1.5), 0.745 |
| rs1800750      | TNF-alpha   | 0.04 | A      | 1.8, (0.9-3.6), 0.068 | 1.75, (0.9-3.3), 0.087 |
| rs1524107      | IL-6        | 0.47 | T      | 0.96, (0.7-1.3), 0.847 | 0.94, (0.7-1.2), 0.670 |
| rs2069845      | IL-6        | 0.2  | G      | 1.1, (0.8-1.5), 0.551 | 1.08, (0.8-1.44), 0.606 |
| rs2167270      | LEP         | 0.49 | G      | 0.98, (0.7-1.4), 0.916 | 0.99, (0.8-1.25), 0.990 |
| rs10838738     | MTCH2       | 0.39 | G      | 1.3, (0.9-1.8), 0.126 | 1.1, (0.8-1.4), 0.379 |
| rs925946       | LGR4-LIN7C-BDNF | 0.14 | T      | 0.7, (0.5-1.03), 0.072 | 0.78, (0.6-1.1), 0.128 |
| rs7138803      | BCDIN3D-FAIM2| 0.23 | A      | 1.03, (0.7-1.45), 0.825 | 1.04, (0.8-1.36), 0.775 |
| rs62033400     | FTO         | 0.28 | G      | 1.02, (0.7-1.5), 0.873 | 1.02, (0.7-1.3), 0.880 |
| rs11872992     | MC4R        | 0.24 | A      | 1.2 (0.8-1.8), 0.290 | 1.2, (0.85-1.7), 0.276 |
| rs17782313     | MC4R        | 0.08 | C      | 0.8 (0.5-1.3), 0.454 | 1.02, (0.6-1.6), 0.912 |
| rs29942        | KCTD15      | 0.42 | A      | 0.77, (0.5-1.1), 0.158 | 0.9, (0.7-1.14), 0.402 |

OR adjusted by age, sex, and BMI. Inheritance models = dom: dominant; add: additive. MAF: minor allele frequency.

The findings of this study are available from the corresponding author upon request.

4. Results

Clinical and anthropometrical characteristics are summarized in Table 2. This study included 204 children with obesity and 569 children without obesity. BF%, glucose, creatinine, TAG, and CHOL levels were found higher in children with obesity. However, the levels of these clinical outcomes in the obesity group fall in normal ranges. Only HDL levels decreased in this group. There was no difference in HbA1c concentration between groups.

All the SNPs analyzed were in Hardy-Weinberg equilibrium. The allelic distribution of the SNPs is shown in Table 3. Only rs6548238 and rs7561317 SNPs in TMEM18 were
associated in the dominant and additive inheritance models. Both polymorphisms confer significant protection against obesity. These polymorphisms were in linkage disequilibrium in our studied population (\(r^2=0.87\)).

A marginal association was found between rs925946 in LGR4-LIN7C-BDNF and rs1800750 in TNF-\(\alpha\) with obesity but does not reach statistical significance. In this study we did not identify any association between ADIPOQ or any cytokine polymorphism with obesity (Table 3).

To evaluate the effects of the associated polymorphisms on BMI and BF\%, a linear regression was performed polymorphisms (Table 4). Both TMEM18 rs6548238 and rs7561317 loci were only associated BF\% after adjustment by age and gender. However, the rs7561317 polymorphism was significantly associated with BMI. There was no association between these polymorphisms and clinical parameters.

5. Discussion

It is well recognized that adipokines and cytokines homeostasis plays an important role in obesity condition. Additionally, GWAS in Caucasian populations revealed many common loci associated with adult obesity [18]. Here, we analyzed the effect of several of these polymorphisms, as well as adipokines (ADIPOQ) and cytokines (TNF-\(\alpha\), IL-1\(\beta\), and IL-6).

We observed a significant association between TMEM18 (rs7561317) and children obesity; however, we did not observe a significant association between adipokines and obesity in our study. A meta-analysis of the influence of adipokine polymorphisms in adipokine genes (LEP, ADIPOQ, IL-1\(\beta\), IL-6, and TNF-\(\alpha\)) in obesity susceptibility showed that there was no association with the risk of obesity and LEP variants in adults. Interestingly, the results of this meta-analysis suggested that polymorphisms in the adipokine genes, ADIPOQ, IL-1\(\beta\), IL-6, and TNF-\(\alpha\), increase the risk of obesity [19]. Our results are consistent with three studies in Mexican children that showed no contribution of several polymorphisms in ADIPOQ gene (rs2241766, rs182052, rs266729, and rs822393) in children obesity [9, 20, 21]. A similar effect was observed in TNF-\(\alpha\) and interleukin-10 [22], suggesting that genetic variants in adipokines do not have a significant effect in Mexican-Mestizo children obesity.

Polymorphisms in FTO and MC4R have been associated with obesity in children. A recent meta-analysis which included 13 articles published between 2011 and 2015, comprising 15,613 participants, shows that the polymorphism rs9939609 in FTO was significantly associated with an increased risk of obesity in children and adolescents in several populations, except Mexicans [23]. Another recent report exposed that the rs9939609 polymorphism interacts with the Native American-origin polymorphism rs9282541, in the ATP-binding cassette transporter (ABCA1). Also, in the presence of the rs9282541 risk allele, the rs9939609 variant was not associated with BMI in Mexican adults, but this interaction was not observed in children [24]. This lack of significance was attributed to the to sample size; however the study of Mejía-Benitez (REF) showed no association between several polymorphisms in FTO with obesity in Mexican children, using a larger sample size. We propose that large cohorts during extensive periods could help to understand the real contribution of FTO variants in Mexican children.

The TMEM18 rs7561317 variant has been widely studied. This polymorphism, together with rs10913469 in SEC16B gene, was associated with adult obesity in a Japanese population [25] and Caucasians children [26]. On the contrary, the minor allele of TMEM18 rs7561317 was related to underweight in Czech adolescents [27]. This polymorphism has also been associated with BMI in Mexican children [28]. In our study, after adjustment by age, sex, and BMI, we observed the same association. Thus, this report indicates that TMEM18 rs7561317 has an effect on obesity development at all stages of life development.

6. Conclusions

Here we report no association of adipokines and cytokines polymorphisms with children obesity. However, the rs7561317 polymorphism in TMEM18 gene is associated with BMI and BF\% in our sample of Mexican-Mestizo children.

Data Availability

Data (.dta) used to support the findings of this study are available from the corresponding author upon request.

Disclosure

Funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Conflicts of Interest

The authors declare no conflicts of interest.

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