Contribution of Common Genetic Variants to Obesity and Obesity-Related Traits in Mexican Children and Adults

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Abstract

Background: Several studies have identified multiple obesity-associated loci mainly in European populations. However, their contribution to obesity in other ethnicities such as Mexicans is largely unknown. The aim of this study was to examine 26 obesity-associated single-nucleotide polymorphisms (SNP) in a sample of Mexican mestizos.

Methods: 9 SNPs in biological candidate genes showing replications (PPARG, ADRB3, ADRB2, LEPR, GNB3, UCP3, ADIPOQ, UCP2, and NR3C1), and 17 SNPs in or near genes associated with obesity in first, second and third wave GWAS (INSIG2, FTO, MC4R, TMEM18, FAIM2/BCDIN3, BDNF, SH2B1, GNPD2A2, NEG1, KCTD15, SEC16B/RASAL2, NPC1, SRF10/EVT5, MAF, PRL, MITCH2, and PTER) were genotyped in 1,156 unrelated Mexican-Mestizos including 683 cases (441 obese class I/II and 242 obese class III) and 473 normal-weight controls. In a second stage we selected 12 of the SNPs showing nominal associations with obesity, to seek associations with quantitative obesity-related traits in 3 cohorts including 1,218 Mexican Mestizo children, 945 Mexican Mestizo adults, and 543 Indigenous Mexican adults.

Results: After adjusting for sex, age and admixture, significant associations with obesity were found for 6 genes in the case-control study (ADIPQ, FTO, TMEM18, INSIG2, FAIM2/BCDIN3 and BDNF). In addition, SH2B1 was associated only with class I/II obesity and MC4R only with class III obesity. SNPs located at or near FAIM2/BCDIN3, TMEM18, INSIG2, GNPD2A2 and SEC16B/RASAL2 were significantly associated with BMI and/or WC in the combined analysis of Mexican-mestizo children and adults, and FTO locus was significantly associated with increased BMI in Indigenous Mexican populations.

Conclusions: Our findings replicate the association of 8 obesity-related SNPs with obesity risk in Mexican adults, and confirm the role of some of these SNPs in BMI in Mexican adults and children.

Introduction

The prevalence of obesity is rapidly increasing worldwide [1]. According to the 2012 National Health and Nutrition Survey in the Mexican population, the prevalence of overweight and obesity is 71.2% in adults aged above 20 years and 34.4% in children [2]. Moreover, according to the 2002 Mexican Family Life survey, 59% of indigenous Mexican individuals are overweight or obese [3]. Hereditability of obesity has been estimated as high as 70% [4]. Using a biological candidate gene approach, more than 127 genes have been associated with obesity and/or obesity-related phenotypes [5]; while genome-wide association studies (GWAS) have identified more than 120 genes (the vast majority not
previously identified as biological candidates) associated with obesity mainly in European populations [6].

Although associations of many common genetic variants with obesity have been replicated mainly in several European and Asian populations [3,6] there are still few studies in the Mexican population [7–10]. This population resulted from recent admixture mainly of indigenous Mexican and European populations [11], and thus genetic variants that are common in Europeans are likely to be part of the genetic architecture of obesity in Mexicans.

Using a case control design, we sought to assess the contribution of two different sets of SNPs with obesity in Mexican Mestizo adults: 9 SNPs in biological candidate genes showing replications in at least 10 studies (PPARG, ADRB3, ADRB2, LEPR, GNB3, UCPS, ADIPOQ, UCP2, and NR3C1) [5] and 17 SNPs in or near genes associated with obesity in first, second and third wave GWAS (ADIPQ, FTO, MC4R, TME1M18, FAIM2/BCDIN3, BDNF, SH2B1, GNPDA2, NEGR1, KCTD15, SEC16B/RASAL2, NPC1, SFRF10/ETV5, MAIF, PRL, MTC12, and PTER) [12–18]. In a second stage we selected of the SNPs showing nominal associations with obesity, to seek associations with quantitative obesity-related traits in 3 distinct cohorts of Mexican Mestizo children and adults, as well as Indigenous Mexicans.

Research Design and Methods

Case-control Study

The case-control study included 1,156 unrelated Mexican-Mestizos. Control, class I and class II obesity individuals were workers from several Governmental Institutions in Mexico City, including Instituto Nacional de Neurologia y Neurociencias Manuel Velasco Suárez, Centro Médico Nacional Siglo XXI, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ), Universidad Nacional Autónoma de México, and Universidad Autónoma Metropolitana. Individuals with class III obesity were outpatients from Obesity Clinics at the INCMNSZ and the Dr. Rubén Leñero General Hospital. All participants were aged 18 to 82 years, without chronic disease that may compromise body weight (including cancer, HIV infection and thyroid disorders); 473 were non-diabetic normal weight subjects (BMI >18.5 and ≤25 kg/m²); 441 were obese class I/II individuals (BMI ≥30 kg/m² and <40 kg/m²), and 242 were obese class III individuals (BMI ≥40 kg/m²) described by Villalobos-Comparán et al. [9]. Anthropometric characteristics of the subjects are summarized in Table S1. All participants provided written informed consent prior to the inclusion in the study.

CoHORT Studies

This study analyzed three independent cohorts. The first group included 945 unrelated Mexican-mestizos aged 18–82 years, recruited at their work-sites at different governmental institutions in Mexico City (including the Instituto Nacional de Neurologia y Neurociencias Manuel Velasco Suárez, Centro Médico Nacional Siglo XXI, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Universidad Nacional Autónoma de México, and Universidad Autónoma Metropolitana-Iztapalapa), 788 described by Villalobos-Comparán et al. [8]. A total of 441 of these subjects were also included in the case-control study described above.

The second group included 1,218 healthy unrelated school-aged Mexican-Mestizo children (595 boys and 628 girls) aged 6–13 years, recruited from a summer camp for children of employees of the Mexican Health Ministry (Convivencia Infantil 2008, Secretaría de Salud) and from a public junior high school in Mexico City, previously described by Flores-Dorantes et al [19]. A parent or guardian of each child signed the consent form for participation.

The third group included 543 unrelated individuals aged over 18 years, belonging to 4 indigenous groups from rural communities: 77 Seris from Sonora located in Northern Mexico, 271 Nahua and 112 Tonotonas from Puebla in East-central Mexico, and 33 Zapotecs from Oaxaca in Southeastern Mexico. All individuals in this group, their parents and grandparents recognized themselves as indigenous, had been born and lived in their home communities, and spoke their native language. Blood samples were drawn with the permission of local authorities and a translator was used as needed.

This study was conducted according to the principles expressed in the Declaration of Helsinki and was approved by the Ethics Committees of participant institutions.

Anthropometric and Biochemical parameters

Anthropometric measurements were determined following the procedures recommended by Loehman et al. [20] and included weight, height, waist circumference (WC) and hip circumference. All instruments were calibrated following the standard methods of the manufacturers. BMI was calculated as weight in kilograms divided by the square of height in meters. In adults, obesity status was determined according to WHO (World Health Organization) criteria [21]. In children, BMI z-scores were calculated using age and sex specific BMI reference data, as recommended by the Centers for Disease Control and Prevention [22]. Biochemical parameters including fasting glucose, insulin, total cholesterol, HDL-C and triglycerides serum levels were measured as previously described [23]. Homeostasis model assessment of B-cell function (HOMA-B) was estimated using a computer model [24].

SNP selection and genotyping

Genomic DNA was isolated from peripheral blood white cells using a commercial kit based on the salt fractionation method (QiAmp 96 DNA Blood Kit, Quiagen, Hilden, Germany). A total of 26 SNPs in or near genes previously associated with obesity risk in other populations were genotyped in cases and controls: 12 SNPs were selected from 9 biological candidate genes including rs3556806 (PPARG), rs4994 (ADRB3), rs1042719 (ADRB2), rs1137101 (LEPR), rs5413 (GNB3), rs1800849 (UCP2), rs2241766 (ADIPOQ), rs659366 (UCP2) and rs6149945 (R3C1); while the remainder 17 SNPs were selected from first, second and third wave GWAS reports and included rs7566605 (INSIG2), rs9939609 (FTO), rs17782313 (MC4R), rs6548283 (TME1M18), rs7188803 (FAIM2/BCDIN3), rs6265 (BDNF), rs7498665 (SH2B1), rs10938397 (GNPDA2), rs2815752 (NEGR1), rs29941 (KCTD15), rs10913469 (SEC16B/RASAL2), rs1805081 (NPC1), rs7647305 (SFRS10/ETV5), rs1424233 (MAF), rs4712652 (PRL), rs10383738 (MTC12) and rs10508503 (PTER).

Because the Mexican-Mestizo population is admixed, ancestry informative markers (AIMs) were used to assess whether any association could be confounded by population stratification. A panel of 10 AIMs (rs42884, rs2695, rs17203, rs2962, rs3340, rs722098, rs203096, rs223630, rs1800498, and rs281478) distinguishing mainly Amerindian and European ancestry (β>0.44) [8] were genotyped in the case-control sample.

Genotyping was performed using TaqMan Probes (ABI Prism 7900HT Sequence Detection System; Applied Biosystems) and/or KASPAR assays (Kbioscience, U.K. http://www.kbioscience.co.uk/). Call rate exceeded 95% for all SNPs tested, with no discordant genotypes in 10% of duplicate samples. In addition, all
samples were genotyped for rs7566605 (INSIG2) using both methods, finding no discordant genotypes. Deviation from Hardy–Weinberg equilibrium was not observed for any SNPs in any group ($P>0.05$).

Statistical analyses

Associations of each SNP with obesity were tested using logistic regression analysis. The Admixture program was used to test the possible effect of population stratification on associations with obesity [25,26]. Because the Mexican-Mestizo population derived mainly from Amerindian and European (Spanish) populations, the model included two primary parental populations. Admixture fits a logistic regression model of the trait on individual admixture, and it allows the inclusion of covariates such as age and sex. All associations were tested for additive, dominant and recessive inheritance models, reporting the most significant. To assess the combined effect of risk alleles, we calculated genotype score counting the number of risk alleles through a logistic model adjusted for age, sex and admixture.

All obesity-related quantitative variables were transformed to normal distribution with a mean of zero and standard deviation (SD) of one in each study separately using inverse normal transformation. Effect sizes were compared across traits and age groups using linear regression analysis. Combined association tests for obesity quantitative traits in children and adult populations and among Indigenous populations were conducted using a Mantel–Haenszel-like model. The combined estimated effect was computed as weighted average of the individual estimated effects using weights proportional to the inverse of the square of the standard error [27]. Cochran’s Q test was used to analyze heterogeneity among study populations [28]. All statistical analyses were performed using SPSS (version 16.0; Chicago, IL). Because the majority of the SNPs analyzed were well validated variants, a $P$-value threshold <0.05 was used for declaring significant association.

Results

Case-control study

Of the 9 biological candidate variants, only NR3C1 rs56149945 showed a very low minor allele frequency (<0.01), and was thus excluded from the analyses. Only 2 of the 8 SNPs previously associated with obesity using this approach showed significant association in the Mexican population. ADIPOQ rs2241766 showed a nominal significant association with overall obesity (OR 2.34, $P_{rec} = 0.033$, recessive model), and UCP3 rs1800849 was significantly associated only with class I/II obesity (OR 1.33, $P_{add} = 0.050$, additive model; Table 1).

On the other hand, 5 of the 17 SNPs previously identified as obesity risk alleles by GWAS showed overall significant associations with obesity in the Mexican population (Table 1): FTO rs9939609 (OR 1.42, $P_{add} = 0.001$), TMEM18 rs6548238 (OR 1.57, $P_{add} = 0.003$), INSIG2 rs7566605 (OR 1.33 $P_{add} = 0.006$), FAIM2/BCDIN3 rs7138803 (OR 1.83, $P_{rec} = 0.034$), and BDNF rs6265 (OR 1.33 $P_{rec} = 0.044$). On stratifying by obesity class, SH2B1 rs7498665 was associated with class I/II obesity (OR 1.21, $P_{add} = 0.047$), and MC4R rs17782313 was associated with class III obesity (OR 1.85 $P_{add} = 0.003$). Interestingly, although FTO showed an overall association with obesity, the strongest and most significant association was observed for class III obesity ($P_{add} = 4 \times 10^{-16}$). Heterogeneity in effect sizes between class I/II and class III obesity was statistically significant only for FTO rs9939609 ($P_{add} = 0.031$) and borderline significant for MC4R rs17782313 ($P_{hot} = 0.050$). All risk alleles significantly associated with obesity were consistent with those previously reported, except for INSIG2 rs7566605 as the risk allele is “C” in Europeans and “G” in the Mexican population. All associations remained significant after adjusting for admixture, except for that with the UCP3 locus (Table 1).

When we examined the joint effects of the nine SNPs nominally associated with obesity, there was a significant increase in obesity risk with increasing mean number or risk alleles ($\geq 2$ SD) adjusted for age, sex and admixture. Mean number of risk alleles was lower in normal weight (5.04±1.49) than in class I/II obese (5.36±1.53) and class III obese individuals (5.49±1.39) (OR 1.16 95% CI 1.04–1.29, $P=0.007$ and OR 1.22 95% CI 1.06–1.40, $P=0.006$, respectively).

Cohort Studies: Associations with obesity-related traits in adults and children

We genotyped 12 SNPs in the 3 independent cohorts samples, including 9 SNPs showing significant association with obesity in the case-control study, plus 3 SNPs with $P<0.20$ in order to include polymorphisms which may have not reached nominal significance because of weaker effect and/or reduced sample size. Table 2 summarizes the results of associations with BMI and WC in each cohort and in the combined sample. Four loci (FAIM2/BCDIN3, TMEM18, INSIG2 and KCN15) were significantly associated with BMI in adults, while only 2 loci (FAIM2/BCDIN3 and GNPDA2) were significantly associated with BMI in children. In the combined analysis of Mexican-mestizo children and adults, 5 SNPs located at or near FAIM2/BCDIN3, TMEM18, INSIG2, GNPDA2 and SEC16/RASAL2 were significantly associated with BMI and/or WC ($P<0.05$). Three of these genes (FAIM2/BCDIN3, TMEM18, and INSIG2) also showed the strongest associations with obesity in the case-control study. FAIM2/BCDIN3 rs7138803 showed the strongest and most significant effect on BMI in both children and adults. The presence of two risk A allele copies represent an increase of 0.505 SD unit of BMI equivalent to 2.6 kg/m$^2$ in adults ($P_{rec} = 0.001$), and a increase of 0.334 SD unit of BMI equivalent to 1.48 kg/m$^2$ in children ($P_{rec} = 0.008$). Of note, FTO and MC4R variants were not significantly associated with BMI/WC variation in children, adults or in the combined analysis, which is consistent with their association mainly with class III obesity.

While there was no heterogeneity in effect size on BMI between adult and children populations, the effect size of TMEM18 was not significantly associated with waist circumference in children (Table 2). Interestingly, although SH2B1 rs7498665 was not significantly associated with waist circumference in children or adults, heterogeneity in effect size was statistically significant ($P_{hot} = 0.042$), as adults bearing the “G” allele showed increased waist circumference, while children bearing this allele showed decreased waist circumference.

Table S2 shows associations of the 12 gene variants analyzed with biochemical measurements in children and adults. No significant associations of these SNPs with biochemical parameters were observed in the combined analysis. However, in adults the MC4R rs17782313 obesity-risk allele was associated with increased glucose levels ($P_{add} = 0.034$), while FTO rs9939609 was associated with lower fasting insulin levels ($P_{add} = 0.013$ and 0.050, respectively), while FTO was associated with lower triglyceride levels ($P_{add} = 0.048$) and BDNF was associated with higher triglyceride levels ($P_{add} = 0.047$). In children, only INSIG2 and BDNF were significantly associated with higher and lower triglyceride levels ($P_{add} = 0.004$ and $P_{rec} = 0.006$, respectively).
Table 1. Associations of Candidate SNP loci with Obesity in the Mexican Population.

| Nearest gene | Chr | SNP | Ref allele | Test allele | Overall obese vs. normal weight | Class I/II obese vs. normal weight | Class III obese vs. normal weight |
|--------------|-----|-----|------------|-------------|---------------------------------|-----------------------------------|-----------------------------------|
| | | | | | OR (95% CI) | OR (95% CI) | OR (95% CI) |
| Biological candidates | | | | | aP | bP | aP | bP | aP | bP | P-Het |
| ADIPOQ | 3 | rs2241766 | G | G | 2.34 (1.07–5.12) | 0.033 | 0.033 | 2.76 (1.23–6.19) | 0.014 | 0.017 | 1.26 (0.37–4.22) | 0.713 | 0.722 | 0.289 |
| UCP3 | 11 | rs1808049 | C | C | 1.25 (0.97–1.62) | 0.085 | 0.086 | 1.33 (1.00–1.77) | 0.050 | 0.061 | 1.09 (0.77–1.56) | 0.632 | 0.667 | 0.398 |
| ADRB3 | 12 | rs4994 | T | C | 1.15 (0.89–1.48) | 0.297 | 0.316 | 1.16 (0.88–1.52) | 0.293 | 0.412 | 1.13 (0.81–1.58) | 0.480 | 0.341 | 0.910 |
| PPARγ | 3 | rs3856806 | C | T | 1.18 (0.86–1.62) | 0.313 | 0.315 | 1.14 (0.81–1.61) | 0.442 | 0.447 | 1.28 (0.85–1.94) | 0.240 | 0.268 | 0.678 |
| ADRB2 | 5 | rs1042719 | G | C | 1.09 (0.89–1.34) | 0.408 | 0.447 | 1.12 (0.90–1.39) | 0.326 | 0.421 | 1.03 (0.78–1.36) | 0.853 | 0.761 | 0.638 |
| UCP2 | 11 | rs659366 | C | T | 1.08 (0.89–1.33) | 0.436 | 0.438 | 1.06 (0.86–1.32) | 0.581 | 0.581 | 1.14 (0.87–1.49) | 0.357 | 0.365 | 0.709 |
| LEPR | 1 | rs1137101 | G | A | 1.05 (0.85–1.30) | 0.625 | 0.625 | 1.05 (0.83–1.31) | 0.700 | 0.729 | 1.06 (0.80–1.41) | 0.668 | 0.694 | 0.927 |
| GNBP3 | 12 | rs5443 | T | C | 1.02 (0.84–1.23) | 0.853 | 0.857 | 0.93 (0.76–1.15) | 0.517 | 0.513 | 1.21 (0.92–1.59) | 0.176 | 0.139 | 0.142 |
| GWAS candidates | | | | | | | | | | | | |
| FTO | 16 | rs9939609 | A | A | 1.42 (1.15–1.76) | 0.001 | 0.003 | 1.19 (0.93–1.52) | 0.174 | 0.146 | 1.88 (1.44–2.45) | 3.4 × 10⁻⁸ | 1.8 × 10⁻⁸ | 0.031 |
| TMEM18 | 2 | rs6548238 | C | C | 1.57 (1.17–2.12) | 0.003 | 0.001 | 1.74 (1.23–2.46) | 0.002 | 0.004 | 1.29 (0.87–1.91) | 0.200 | 0.028 | 0.260 |
| INSR2 | 2 | rs756605 | C | G | 1.33 (1.08–1.63) | 0.006 | 0.006 | 1.38 (1.10–1.74) | 0.005 | 0.006 | 1.23 (0.92–1.63) | 0.161 | 0.114 | 0.511 |
| FAIM2:BCDIN3 | 12 | rs7138803 | A | A | 1.88 (1.05–3.37) | 0.034 | 0.039 | 1.93 (1.03–3.61) | 0.040 | 0.024 | 1.76 (0.84–3.65) | 0.132 | 0.266 | 0.850 |
| BDNF | 11 | rs6265 | G | G | 1.33 (1.01–1.74) | 0.044 | 0.043 | 1.21 (0.89–1.63) | 0.222 | 0.231 | 1.59 (1.09–2.32) | 0.017 | 0.014 | 0.269 |
| SH2B1 | 16 | rs7489665 | G | G | 1.12 (0.94–1.33) | 0.195 | 0.175 | 1.21 (1.00–1.46) | 0.047 | 0.050 | 0.97 (0.78–1.22) | 0.799 | 0.955 | 0.230 |
| GNPD42 | 4 | rs10938397 | G | G | 1.13 (0.95–1.36) | 0.117 | 0.183 | 1.18 (0.97–1.44) | 0.097 | 0.070 | 1.05 (0.82–1.33) | 0.709 | 0.946 | 0.443 |
| MC4R | 18 | rs17782313 | C | C | 1.24 (0.89–1.72) | 0.198 | 0.206 | 0.98 (0.68–1.42) | 0.923 | 0.880 | 1.85 (1.23–2.80) | 0.003 | 0.012 | 0.050 |
| KCTD15 | 19 | rs29941 | C | C | 1.13 (0.92–1.38) | 0.237 | 0.163 | 1.14 (0.92–1.42) | 0.234 | 0.052 | 1.13 (0.88–1.46) | 0.342 | 0.828 | 0.958 |
| SEC16B/RASAL2 | 1 | rs10913469 | C | C | 1.10 (0.88–1.36) | 0.410 | 0.413 | 1.24 (0.98–1.56) | 0.072 | 0.111 | 0.79 (0.57–1.09) | 0.151 | 0.276 | 0.070 |
| Nearest gene | Chr | SNP  | Ref allele | Test allele | OR (95% CI) | aP | bP | OR (95% CI) | aP | bP | OR (95% CI) | aP | bP | P-Het |
|--------------|-----|------|------------|-------------|-------------|----|----|-------------|----|----|-------------|----|----|-------|
| NEGR1        | 1   | rs2815752 | T          | T           | 1.08 (0.88–1.31) | 0.468 | 0.451 | 1.08 (0.86–1.34) | 0.506 | 0.621 | 1.10 (0.84–1.43) | 0.495 | 0.342 | 0.923 |
| NPC1         | 18  | rs1805081 | A          | G           | 1.07 (0.85–1.35) | 0.585 | 0.567 | 1.15 (0.90–1.48) | 0.269 | 0.228 | 0.87 (0.64–1.18) | 0.396 | 0.338 | 0.130 |
| SFRF10/ETVS  | 3   | rs7647305 | C          | C           | 1.08 (0.81–1.32) | 0.601 | 0.607 | 1.19 (0.87–1.64) | 0.283 | 0.304 | 0.92 (0.64–1.32) | 0.646 | 0.729 | 0.293 |
| MAF          | 16  | rs1424233 | A          | A           | 1.04 (0.83–1.32) | 0.714 | 0.700 | 1.07 (0.84–1.37) | 0.567 | 0.516 | 0.92 (0.67–1.26) | 0.603 | 0.559 | 0.446 |
| PRL          | 6   | rs4712652 | A          | A           | 1.02 (0.82–1.26) | 0.891 | 0.958 | 1.10 (0.87–1.39) | 0.424 | 0.704 | 0.86 (0.64–1.15) | 0.310 | 0.554 | 0.195 |
| MTCH2        | 11  | rs10838738 | G          | G           | 1.03 (0.83–1.28) | 0.764 | 0.958 | 1.06 (0.84–1.39) | 0.651 | 0.651 | 1.00 (0.76–1.32) | 0.991 | 0.971 | 0.782 |
| PTER         | 10  | rs10508503 | C          | T           | 1.03 (0.55–1.93) | 0.919 | 0.918 | 1.17 (0.61–2.26) | 0.630 | 0.605 | 0.77 (0.30–1.94) | 0.572 | 0.613 | 0.960 |

Chr, chromosome; CI, confidence interval; OR, odds ratio; P-Het, P-heterogeneity. SNPs were ranked by P-values. Statistically significant associations are bold-faced. aP-values were adjusted for age and sex, and bP-values were further adjusted for admixture. All P-values were tested under an additive model (\(P_{add}\)), except those reported for \(ADIPOQ\), \(BCDIN3/FAIM2\) and \(BDNF\) genes which were analyzed under a recessive model (\(P_{rec}\)).

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Table 2. Associations of 12 loci with BMI and WC in Mexican Adults and Children.

| Trait          | Nearest gene | SNP            | Risk Chr allele | RAF Effect size (SE) | P     | RAFF Effect size (SE) | P     | Effect size (SE) | P     | P-Het |
|----------------|--------------|----------------|-----------------|----------------------|-------|-----------------------|-------|-----------------|-------|-------|
| BMI Adipoq     | rs2241766    | 3 G            | 17.6            | 0.14 (0.18)         | 0.420 | 18.2                  | 0.05 (0.16) | 0.757 | 0.09 (0.12) | 0.442 | 0.695 |
| BMI Ucp3       | rs1800849    | 11 T           | 12.8            | −0.01 (0.07)        | 0.855 | 11.2                  | 0.03 (0.061) | 0.597 | 0.01 (0.05) | 0.789 | 0.625 |
| BMI Fto        | rs9939609    | 16 A           | 19.4            | 0.05 (0.06)         | 0.392 | 18.1                  | 0.08 (0.05) | 0.125 | 0.07 (0.04) | 0.885 | 0.757 |
| BMI Tmem18     | rs6548238    | 2 C            | 92.5            | 0.25 (0.09)         | 0.005 | 91.1                  | 0.09 (0.07) | 0.149 | 0.15 (0.05) | 0.004 | 0.166 |
| BMI Insig2     | rs7566025    | 2 G            | 76.6            | 0.15 (0.06)         | 0.007 | 73.4                  | 0.03 (0.05) | 0.475 | 0.08 (0.04) | 0.024 | 0.095 |
| BMI Faim2/bcdin3 | rs7138803 | 12 A           | 20.8            | 0.51 (0.15)         | 0.001 | 21.2                  | 0.33 (0.13) | 0.008 | 0.40 (0.09) | 3.5×10⁻⁵ | 0.388 |
| WC Bdnf        | rs6265       | 11 G           | 86.4            | 0.03 (0.08)         | 0.684 | 84.6                  | 0.02 (0.06) | 0.728 | 0.03 (0.05) | 0.598 | 0.917 |
| WC Gnpda2      | rs10938397   | 4 G            | 35.6            | 0.08 (0.05)         | 0.106 | 35.2                  | 0.09 (0.04) | 0.021 | 0.09 (0.03) | 0.005 | 0.810 |
| WC Sh2B1       | rs7498665    | 16 G           | 49.9            | 0.06 (0.05)         | 0.181 | 50.3                  | −0.03 (0.04) | 0.447 | 0.01 (0.03) | 0.787 | 0.127 |
| WC Mcc4        | rs1773213    | 18 C           | 7.3             | −0.04 (0.09)        | 0.690 | 8.2                   | −0.04 (0.07) | 0.607 | −0.04 (0.06) | 0.515 | 0.993 |
| WC Kctd15      | rs29941      | 19 C           | 62.6            | 0.11 (0.05)         | 0.020 | 55.2                  | 0.02 (0.04) | 0.658 | 0.05 (0.03) | 0.072 | 0.121 |
| WC Sec16b/rala2 | rs10913469   | 1 C            | 20.4            | 0.07 (0.06)         | 0.210 | 20.5                  | 0.06 (0.05) | 0.243 | 0.06 (0.04) | 0.090 | 0.861 |
| WC Bdnf        | rs6265       | 11 G           | 86.4            | 0.03 (0.08)         | 0.684 | 84.6                  | 0.02 (0.06) | 0.728 | 0.03 (0.05) | 0.598 | 0.917 |
| WC Gnpda2      | rs10938397   | 4 G            | 35.6            | 0.11 (0.05)         | 0.021 | 35.2                  | 0.04 (0.04) | 0.307 | 0.02 (0.04) | 0.931 | 0.253 |
| WC Sh2B1       | rs7498665    | 16 G           | 49.9            | 0.07 (0.04)         | 0.106 | 50.3                  | −0.05 (0.04) | 0.229 | 0.01 (0.03) | 0.903 | 0.042 |
| WC Mcc4        | rs1773213    | 18 C           | 7.3             | 0.03 (0.09)         | 0.692 | 8.2                   | −0.03 (0.07) | 0.698 | −0.01 (0.05) | 0.908 | 0.639 |
| WC Kctd15      | rs29941      | 19 C           | 62.6            | 0.08 (0.05)         | 0.074 | 55.2                  | 0.03 (0.04) | 0.449 | 0.05 (0.03) | 0.082 | 0.365 |
| WC Sec16b/rala2 | rs10913469   | 1 C            | 20.4            | 0.09 (0.05)         | 0.099 | 20.5                  | 0.06 (0.05) | 0.193 | 0.07 (0.03) | 0.038 | 0.698 |

Abbreviations: Chr, chromosome; RAF, risk allele frequency; SE, standard error; P-Het, P-heterogeneity; BMI, body mass index; WC, waist circumference. Effect values are presented as effect size per allele copy, except for Adipoq, Bcdin3/Faim2 and Bdnf analyzed under a recessive model, where effect size is reported for two allele copies. P-values were adjusted for age and sex. Statistically significant associations are bold-faced.

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Association with obesity-related traits in Mexican Indigenous Populations

The 12 selected SNPs were genotyped in a total of 543 indigenous Mexicans. Overall, most obesity-risk alleles were less frequent in this group than in Mexican Mestizo and European populations. Comparisons of risk allele frequencies among European, Mestizo and Indigenous Mexican populations are shown in Table S3. Interestingly, only Fto rs9939609 was significantly associated with increased BMI, and each copy of the risk A allele increases 0.250 SD unit of BMI equivalent to 1.22 kg/m² (Pₚₛₜₑ = 0.045; Table 3).

Discussion

In the present study, we analyzed 26 SNPs previously associated with obesity in a case-control study in adult Mexican Mestizo subjects. In this population, only 2 of the 8 (25%) biological candidate genes (Adipoq and Ucp3) were associated with obesity, although only Adipoq remained significant after adjusting for admixture. Given the substantial number of obesity association replications in various ethnicities [3], it is noteworthy that the proportion of these biological candidate genes associated with obesity in the present study was low. However, this is consistent with several other studies failing to show associations with such genes, and the fact that these genes are not significantly associated with obesity in most GWAS [16–18]. In addition, 7 of the 17 (41.2%) GWAS-selected genes (Fto, Tmem18, Insr2, Faim2/ Bcdin3, Bdnf, Sh2b1 and Mc4r) were associated with obesity in this population. This proportion is similar to that reported in other studies seeking to replicate associations of these variants with obesity [29–31]. Thus, it is clear that some of the previously obesity-associated variants identified in Europeans also confer susceptibility to obesity in Mexican population. Interestingly, the significant ORs observed here were higher, although P-values were
lower than those reported in the original studies [12–18], which may be explained by the smaller sample size analyzed in this study.

**FTO, TMEM18 and INSL2 variants showed the most significant associations with obesity in this population. FTO and TMEM18 are probably the most replicated genes showing the strongest and most significant effects in various different populations [30,32–34]. One of the most notable results is the association of obesity with INSL2, as previous studies have shown conflicting results and few studies have replicated this association [35–38]. Moreover, in contrast to the original report of Herbert et al. [12], we found that the G and not the C allele confers risk for obesity. Linkage disequilibrium with other variants may explain this inverse association.

Interestingly, **FTO, MC4R and BDNF loci were most significantly associated with class III obesity. The association of FTO rs9939609 with class III obesity has been previously observed in the Mexican and several other populations [8,14,39,40]. On the other hand, MC4R and BDNF mutations are known to cause monogenic obesity [41,42], and common variation in these genes has also been previously associated with severe obesity [15–18]. The more significant association with class III obesity may be explained by the presence of rare functional variants in high LD with the variants studied here.

Seventeen of the 25 SNPs analyzed failed to show associations with obesity in the Mexican population. However, the trend of association for most GWAS-selected SNPs was in the same direction as the initially reported findings. Considering that ORs reported in Europeans were around 1.1 for these 21 SNPs [5,16–18], and that most risk alleles were less frequent in Mexican Mestizos than in Europeans, the lack of association observed here may be due to insufficient statistical power (range 6.3%–72.7% for 13 of these SNPs). Further studies in larger samples are necessary to confirm whether these 13 or other SNPs contribute to the risk of obesity in Mexicans.
Mexican Indigenous populations. Our results suggest that obesity risk loci identified in Europeans also confer risk for obesity and increased BMI in Mexicans, and that diverse ethnic groups share some degree of genetic predisposition to obesity.

Supporting Information

Table S1 Anthropometric characteristics of case and control subjects.

Table S2 Associations of 12 loci with Biochemical Characteristics in Mexican Adults and Children.

References

1. Kelly T, Yang W, Chen C-S, Reynolds K, He J (2008) Global burden of obesity in 2005 and projections to 2030. Int J Obes 32: 1431–1437.
2. Secretaría de Salud – Instituto Nacional de Salud Pública. (2012) Encuesta Nacional de Salud y Nutrición.
3. Mejía-Benítez A, Klünder-Klünder M, Yengo L, Meyre D, Aradillas C, et al. (2013) Analysis of the contribution of FTO, NPC1, ENPP1, NEGR1, GNPDA2 and MC4R genes to obesity in Mexican children. BMC Med Genet 1: 14–21.
4. Baadsgaard O, Edge J, Fagerli H, Flack J, Gerdts E, et al. (2007) Common body mass index-associated variants confer risk of extreme obesity. Nat Genet 39: 218–225.
5. Willer CJ, Speliotes EK, Roche AF (2010) Anthropometric standardization reference manual. Champaign, IL: Human Kinetics Books.
6. World Health Organization (WHO). (2000) Obesity: Preventing and managing the global epidemic. Report of a WHO consultation. Tech Rep Ser 894: 1–253.
7. Thorleifsson G, Walters GB, Gudbjartsson DF, Steinthorsdottir V, Sulem P, et al. (2009) Six new loci that associate with measures of obesity. Nat Genet 41: 18–24.
8. Levy JC, Matthews DR, Hermans MP (1998) Correct homeostasis model assessment (HOMA) evaluation uses the computer program. Diabetes Care 21: 2191–2192.
9. Kuczmarski RJ, Ogden CL, Guo SS, Grummer-Strawn LM, Flegal KM, et al. (2002) CDC growth charts for the United States: methods and development. Vital Health Stat 246: 1–190.
10. Villarreal-Molina MT, Aguilar-Salinas CA, Rodríguez-Cruz M, García-Ulloa AG, et al. (2008) The FTO gene is associated with adulthood obesity in the Mexican population. Obesity (Silver Spring) 16: 2296–2306.
11. Ng MC, Tam CH, So WY, Ho JS, Chan AW, et al. (2010) Implication of genetic variation of 15 obesity-associated genes in 4232 adults from Norway. Hum Mol Genet 19: R95–R106.

Table S3 Comparison of Risk Allele Frequencies of 12 SNPs in Mexican Indigenous, Mexican Mestizo and Caucasian populations.

Author Contributions

Conceived and designed the experiments: PLM HVR MVC SCQ. Performed the experiments: PLM HVR MVC. Analyzed the data: PLM HVR TVM SRH SCQ. Contributed reagents/materials/analysis tools: BLC RGV JVB LJAA CPR AGR BRN FCP VAA CAS. Wrote the paper: PLM HVR TVM CAS SCQ.
41. Farooqui IS, Yeo GS, Krogh JM, Aminian S, Jebb SA, et al. (2000) Dominant and recessive inheritance of morbid obesity associated with the melanocortin 4 receptor deficiency. J Clin Invest 106: 271–279.

42. Gray J, Yeo GS, Cox J, Morton J, Adlam AL, et al. (2006) Hyperphagia, severe obesity, impaired cognitive function, and, hyperactivity associated with functional loss of one copy of the brain-derived neurotrophic factor (BDNF) gene. Diabetes 55: 3366–3371.

43. Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, et al. (2001) The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat Med 7: 941–946.

44. Fleury C, Neverova M, Collins S, Raimbault S, Champigny O, et al. (1997) Uncouplin protein-2: a novel gene linked to obesity and hyperinsulinemia. Nat Genet 15: 269–272.

45. Mei H, Chen W, Jiang F, He J, Srinivasan S, et al. (2012) Longitudinal replication studies of GWAS risk SNPs influencing body mass index over the course of childhood and adulthood. PLoS One 7: e31470.

46. Dorajoo R, Blaquemore AI, Sim X, Ong RT, Ng DP, et al. (2012) Replication of 13 obesity loci among Singaporean Chinese, Malay and Asian-Indian population. Int J Obes (Lond) 36: 159–163.

47. Hardy R, Wills AK, Wong A, Elks CE, Wareham NJ, et al. (2010) Life course variations in the associations between FTO and MC4R gene variants and body size. Hum Mol Genet 19: 545–552.

48. Bastarrachea RA, Kent JW Jr, Rozada G, Cole SA, López-Alvarrenga JC, et al. (2007) Heritability and genetic correlations of metabolic disease-related phenotypes in Mexico: preliminary report from the GEMM Family Study. Hum Biol 79: 121–129.

49. Li X, Quiñonez MJ, Wang D, Bulnes-Alvarenga I, Jimenez X, et al. (2006) Genetic effects on obesity assessed by bivariate genome scan: the Mexican-American coronary artery disease study. Obesity (Silver Spring) 14: 1192–1200.

50. Rong R, Hanson RL, Ortiz D, Wiedrich C, Kubes S, et al. (2009) Association analysis of variation in/near FTO, CDKAL1, SLC30A8, HHEX, EXT2, IGF2BP2, LOC387761, and CDKN2B with type 2 diabetes and related quantitative traits in Pima Indians. Diabetes 58: 478–488.