**PCSK1 rs6232 Is Associated with Childhood and Adult Class III Obesity in the Mexican Population**

Marisela Villalobos-Comparán, Hugo Villamil-Ramírez, Teresa Villarreal-Molina, Elena Larrieta-Carrasco, Paola León-Mimila, Sandra Romero-Hidalgo, Leonor Jacobo-Albavera, Adriana E. Liceaga-Fuentes, Francisco J. Campos-Pérez, Blanca E. López-Contreras, Teresa Tusié-Luna, Blanca E. del Río-Navarro, Carlos A. Aguilar-Salinas, Samuel Canizales-Quinteros

1 Departamento de Biología, Facultad de Química, Universidad Nacional Autónoma de México (UNAM), Mexico City, Mexico, 2 Unidad de Biología Molecular y Medicina Genómica, Instituto Nacional de Ciencias Médicas y Nutrición “Salvador Zubirán” (INCMNSZ), Mexico City, Mexico, 3 Instituto Nacional de Medicina Genómica (INMEGEN), Mexico City, Mexico, 4 Clínica de Obesidad, Hospital Rubén Leñero, Mexico City, Mexico, 5 Instituto de investigaciones Biomédicas, Universidad Nacional Autónoma de México (UNAM), Mexico City, Mexico, 6 Departamento de Alergia e Inmunología Clínica, Hospital Infantil de México Federico Gómez, Mexico City, Mexico, 7 Departamento de Endocrinología y Metabolismo, INCMNSZ, Mexico City, Mexico

**Abstract**

**Background:** Common variants rs6232 and rs6235 in the PCSK1 gene have been associated with obesity in European populations. We aimed to evaluate the contribution of these variants to obesity and related traits in Mexican children and adults.

**Methodology/Principal Findings:** Rs6232 and rs6235 were genotyped in 2382 individuals, 1206 children and 1176 adults. Minor allele frequencies were 0.78% for rs6232 and 19.99% for rs6235. Rs6232 was significantly associated with childhood obesity and adult class III obesity (OR = 3.01 95%CI 1.64–5.53; P = 4.10⁻⁴ in the combined analysis). In addition, this SNP was significantly associated with lower fasting glucose levels (P = 0.01) and with increased insulin levels and HOMA-B (P = 0.05 and 0.01, respectively) only in non-obese children. In contrast, rs6235 showed no significant association with obesity or with glucose homeostasis parameters in any group.

**Conclusion/Significance:** Although rs6232 is rare in the Mexican population, it should be considered as an important risk factor for extreme forms of obesity.

**Citation:** Villalobos-Comparán M, Villamil-Ramírez H, Villarreal-Molina T, Larrieta-Carrasco E, León-Mimila P, et al. (2012) PCSK1 rs6232 Is Associated with Childhood and Adult Class III Obesity in the Mexican Population. PLoS ONE 7(6): e39037. doi:10.1371/journal.pone.0039037

**Editor:** Shengxu Li, Tulane School of Public Health and Tropical Medicine, United States of America

**Copyright:** © 2012 Villalobos-Comparán et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Funding:** This research was supported by grant 113861 from the Consejo Nacional de Ciencia y Tecnología (CONACyT, http://www.conacyt.mx/Paginas/default.aspx). MJC is in the PhD program from Ciencias Biomédicas at Universidad Nacional Autónoma de México (UNAM). MVC, HVR, PLM and LJA are recipients of the CONACyT scholarship number 210322, 244112, 234714 and 195399, respectively. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing Interests:** The authors have declared that no competing interests exist.

* E-mail: canil@servidor.unam.mx

**Introduction**

The prevalence of obesity has increased worldwide, including Mexico, where more than 70% of the adults and 26% of the children are overweight or obese [1]. Even though obesity has a strong genetic contribution, the identification of genes related to obesity risk has proven difficult [2–4]. The discovery of genes causing monogenic forms of obesity such as the prohormone convertase subtilisin/kevin type 1 gene (PCSK1) has greatly improved our understanding of the pathophysiology of obesity [2–4]. This gene encodes an enzyme expressed in neuroendocrine cells that converts inactive prohormones into functional key hormones that regulate central and/or peripheral energy metabolism. Although loss-of-function mutations in this gene causing childhood obesity and impaired glucose tolerance are rare [5–7], two common nonsynonymous variants (rs6232 and rs6235) were recently found to be strongly associated with childhood and adulthood obesity in European population [8]. Functional *in vitro* analysis of these variants revealed a significant impairment of the catalytic activity of the enzyme for rs6232 (N221D), but no enzyme activity alteration for rs6235 (S690T) [8].

Several studies have sought to replicate the association of these variants (mainly rs6235) with obesity and obesity-associated traits in Asian and European adult populations, with inconsistent results [9–15]. These population-based studies included only a reduced number of obese class III individuals and did not include children as the initial report of Benzinou et al. [8]. This may partially explain such inconsistencies, as genetic influences on BMI may be stronger precisely in children and individuals with class III obesity [2,16]. Thus, the aim of the present study was to analyze the association of rs6232 and rs6235 with obesity and related traits in a case-control analysis of Mexican-Mestizo adults and children.
Materials and Methods

Subjects

The study included 1206 non-related Mexican-Mestizo children aged 5 to 12 years (596 boys and 610 girls), recruited at a summer camp for children of employees of the Mexican Health Ministry (Convivencia Infantil 2008–2009, Secretaría de Salud) and the Hospital Infantil de México. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. BMI z-scores and percentiles were calculated using age and sex specific BMI reference data, as recommended by the Centers for Disease Control and Prevention [17]. The population was stratified based on percentile BMI, 802 children were non-obese (BMI percentile<95th) and 404 were obese (BMI percentile≥95th).

The study also included 1176 unrelated Mexican Mestizos aged 18–82 years (806 nonpregnant women and 370 men); 788 individuals described by Villalobos-Comparañ et al. [18] and 388 additional subjects recruited from Obesity Clinics at the INCENNS and Ruben Leñero Hospital. Two hundred and fifty four (21.59%) of these individuals had been diagnosed with type 2 diabetes (T2D) according to WHO (World Health Organization) criteria [19]. Individuals were grouped according to BMI: 562 were non-obese (BMI <30 kg/m2), 390 had class I/II obesity (30≤ BMI <40 kg/m2), and 234 had class III obesity (BMI ≥40 kg/m2). Biochemical parameters were measured in blood samples obtained after 12-h fast as previously described [20]. Homeostasis model assessment of beta-cell function (HOMA-B) and insulin sensitivity (HOMA-S) as measures of beta-cell function and insulin sensitivity were estimated using a computer model [21]. The characteristics of the children and adult populations are shown in Table 1.

Table 1. Characteristics of the cases and controls in the children and adult populations.

|            | Non-obese | Obese |
|------------|-----------|-------|
| N (1206)   | 802       | 404   |
| Males (%)  | 354 (44.13)| 236 (58.41) |
| Age (yrs)  | 9.40±1.92 | 10.25±2.27 |
| z-score BMI| 0.31±1.00 | 2.08±0.28 |
| Glucose (mmol/L) | 5.00±0.45 | 5.01±0.52 |
| Insulin (pmol/L) | 39.5 (23.6, 59.0) | 76.3 (48.6, 108.3) |
| HOMA-B (%) | 76.3 (57.1, 102.7) | 125 (84.8, 158.7) |
| HOMA-S (%) | 136.5 (90.7, 222.1) | 69.1 (49.8, 107.6) |

|            | Non-obese | Obese |
|------------|-----------|-------|
| N (1176)   | 562       | 614   |
| Males (%)  | 191 (33.98)| 179 (29.15) |
| Age (yrs)  | 47.40±14.73 | 41.91±12.68 |
| BMI (Kg/m2) | 24.20±2.67 | 38.51±7.65 |
| Glucose (mmol/L) | 5.88±2.44 | 6.20±2.46 |
| Insulin (pmol/L) | 61.1 (39.5, 104.1) | 75.0 (45.1, 123.6) |
| HOMA-B (%) | 93.7 (66.3, 131.4) | 99.8 (61.2, 136.0) |
| HOMA-S (%) | 83.2 (50.3,130.5) | 71.0 (41.2, 116.8) |

As part of the eligibility criteria, subjects with thyroid gland disease or showing weight instability three months prior to the study were excluded, as well as elderly subjects with signs of dementia by Mini-Mental state examination [22]. Only individuals born in Mexico whose parents and grandparents identified themselves as Mexican Mestizos were included. This project was approved by the Institutional Committee of Biomedical Research in Humans of the INCENNS. All adult participants and parents of the children provided written informed consent prior to their inclusion in the study.

Single Nucleotide Polymorphism Genotyping

rs6232 and rs6235 were genotyped using TaqMan assays (ABI Prism 7900HT Sequence Detection System; Applied Biosystems, Foster City, CA). Genotyping call rate exceeded 97% per SNP and no discordant genotypes were observed in 25 duplicate samples. In addition, because the Mexican-Mestizo population is admixed, we analyzed 10 ancestry informative markers to rule out population stratification [18]. Genotyping was performed using K Biosciences (Hertfordshire, UK) using a KASPar assay system. Genotyping call rates of each ancestry informative marker exceeded 95%, and no discordant genotypes were observed in 54 duplicate samples. Deviation from Hardy–Weinberg equilibrium was not observed for rs6232 and rs6235 in any group (P>0.66 and P>0.21, respectively).

Statistical Analysis

Logistic regression was used to test for associations between the rs6232 and rs6235 SNPs and obesity. Children and adult combined odds ratios were estimated using the Mantel-Haenszel method. The AdmMixMap program was used to test the possible effect of population stratification on associations of rs6232 with obesity only in the adult population [23]. Generalized linear regression was applied to test for associations of rs6232 and rs6235 with quantitative traits only in individuals without T2D. Because fasting serum insulin levels and HOMA indices were not normally distributed, they were log transformed for analysis. Interactions between the SNPs and age or gender were tested by including a two-way interaction term (SNP*age or SNP*gender) in the model. All analyses were adjusted for age and gender, and with other covariates as appropriate. The reported P-values are nominal and two-sided. Association analyses were performed with SPSS V15.0, statistical package; Chicago, IL.

Pairwise linkage disequilibrium (LD) between both SNPs was estimated using Haploviz V3.2. (http://www.broad.mit.edu/mpg/haploviz). Power calculations were performed using QUANTO software (http://hydra.usc.edu/gxe/).

Results

The study included a total of 2382 individuals, 1176 adults and 1206 children. Minor allele frequencies (MAFs) for rs6232 and rs6235 SNPs were 0.78%, and 19.99%, respectively. Both variants are in weak linkage disequilibrium (r^2 = 0.30). Because risk genotypes frequencies showed no significant differences in lean and overweight individuals (children or adults; P>0.40), both groups were considered together as non-obese subjects for the analyses. SNP rs6232 was significantly associated with obesity in children (OR = 3.78, 95%CI 1.42–9.88; P = 7×10⁻³) and was significantly associated with class III obesity in adults (OR = 2.61, 95%CI 1.10–6.19; P = 0.02) showing a trend of association after adjusting for admixture (P = 0.07). The odds ratio estimated in the combined analysis was 3.01 (95%CI 1.64–5.53; P = 4×10⁻³, Table 2). In contrast, the rs6235 variant was not associated with
Association with Obesity

Discussion

Association with Obesity

Obesity is a complex disorder involving both genetic and environmental factors [2]. Although obesity is highly prevalent in Mexico, both in children and adults, studies on the genetic component of this disease in the Mexican population are scarce [18,20,24]. Recently, N221D (rs6232) and S690T (rs6235) PCSK1 nonsynonymous polymorphisms were found to contribute to the etiology of polygenic obesity in European populations [8]. In the present case-control study, the PCSK1 rs6235 was common but not associated with obesity in Mexican children or adults. This result is consistent with several recent studies in European and Chinese populations [9–14]. In contrast, rs6232 was infrequent in the Mexican population (0.78% as compared to 4–8% in Europeans), but was significantly associated with obesity in both children and adults. In fact, the association with obesity for G allele carriers was significant only in extreme phenotypes (childhood and adult class III obesity), and was higher than the risk previously reported (OR = 3.01 vs. OR = 1.34, respectively) [8]. Thus, it is likely to provide only a weak population-attributable risk for common obesity and not to be a major contributor to obesity in the general population of Mexico. However, the presence of this functional variant should be considered as a serious risk factor for extreme forms of obesity in the Mexican population, as has been recently reported for heterozygous PCSK1 mutations in Europeans [25].

A limitation of the study was low statistical power. For the combined analysis (children and adults), the present study had only 13.6% and 78.3% statistical power to detect previously reported associations of rs6232 and rs6235 with obesity, respectively [8]. Because statistical power to detect obesity subclasses was lower, we cannot rule out the possibility of rs6235 associations with extreme forms of obesity in this population.

Associations with Glucose Homeostasis

Both rs6235 and rs6232 have been recently associated with glucometabolic traits. Rs6235 was found to be associated with decreasing fasting glucose levels and increased HOMA-B [13,26], while rs6232 was associated with decreased circulating post-prandial glucose and elevated glucagon levels in a Danish population-based study [27], and discordantly associated with decreased fasting insulin levels and reduced insulin sensitivity in German adults with increased risk of T2D [26]. In the present study rs6235 was not associated with glucometabolic traits in any group; however the rs6232 G-allele was significantly associated with decreased circulating insulin levels only in non-obese children (Table 3). No significant associations between rs6235 and glucose homeostasis parameters were found in children or adults (Table 4).

Table 2. Association of the rs6232 and rs6235 with obesity in children and adult populations.

| Genotype (%) | rs6232 (encoding N221D) | rs6235 (encoding S690T) |
|-------------|-------------------------|-------------------------|
| **Children**|                         |                         |
| Genotype (%)| AA AG GG G allele frequency | GG GC CC C allele frequency | P-value | P-value |
| Non-obese  | 795 (99.1) 7 (0.9) 0 0.44 | 528 (66.6) 234 (29.5) 31 (3.9) 18.66 |
| Obese      | 392 (97.0) 12 (3.0) 0 1.57 | 374 (63.9) 172 (31.7) 24 (4.4) 20.26 |
| **Adults** |                         |                         |
| Non-obese  | 550 (97.9) 12 (2.1) 0 1.07 | 347 (63.9) 172 (31.7) 24 (4.4) 20.26 |
| Obese      | 595 (96.9) 19 (3.1) 0 1.54 | 368 (61.5) 209 (34.9) 21 (3.5) 20.73 |

Data are n (%). All odds ratios and P-values were calculated by logistic regression analyses using non-obese individuals as reference group, adjusting for age, sex and DT2. Padd: P-values for the additive model.

doi:10.1371/journal.pone.0039037.t002

oS

Acknowledgments

We thank Salvador Ramı´rez-Jimenez and Luz E. Guillén-Pineda for their technical assistance.
Table 3. Glucose homeostasis parameters in nonobese children and adult populations according to PCSK1 rs6232.

|       | Non-obese | Obese |
|-------|-----------|-------|
|       | Children  | Adults |
|       | AA        | AG    | P-value | AA    | AG    | P-value |
| N (1206) | 795      | 392   | 12      |
| Age (yrs)     | 9.42±1.92 | 10.26±2.29 | 0.70    | 10.23±1.90 | 0.97 |
| z-score BMI   | 0.31±1.01 | 0.05±1.09 | 0.60    | 2.08±0.29 | 2.08±0.24 | 0.98 |
| Glucose (mmol/L) | 5.01±0.45 | 5.01±0.52 | 0.01    | 4.96±0.32 | 0.72 |
| Insulin (pmol/L) | 38.8 (23.6, 59.0) | 76.3 (48.6, 109.0) | 0.05    | 118.9 (78.7, 168.2) | 0.99 |
| HOMA-A (%)   | 76.2 (57.1, 102.5) | 125.3 (85.3, 159.3) | 0.01    | 122.7 (101.3, 166.2) | 0.16 |
| HOMA-S (%)   | 136.5 (90.5, 222.1) | 101.2 (92.8, 167.8) | 0.06    | 69.0 (49.7, 107.3) | 0.78 |
|       | 432      | 463   | 17      |
| Age (yrs)     | 45.29±14.36 | 40.61±12.38 | 0.46    | 36.59±15.10 | 0.20 |
| BMI (kg/m²)   | 23.77±2.49 | 23.92±2.68 | 0.56    | 41.20±7.66 | 0.18 |
| Glucose (mmol/L) | 5.02±0.57 | 4.99±0.72 | 0.88    | 5.38±0.60 | 5.49±0.43 | 0.39 |
| Insulin (pmol/L) | 43.1 (29.9, 61.1) | 105.6 (69.1, 156.9) | 0.45    | 136.1 (59.0, 164.3) | 0.46 |
| HOMA-A (%)   | 83.8 (63.9, 108.5) | 79.8 (56.5, 98.3) | 0.78    | 130.8 (101.6, 167.7) | 0.26 |
| HOMA-S (%)   | 125.0 (86.2, 177.2) | 125.3 (109.6, 190.8) | 0.58    | 50.8 (34.8, 77.3) | 38.5 (33.4, 94.9) | 0.54 |

Data are means ± s.d. or medians (interquartile range). P-values were calculated by generalized linear regression. BMI was adjusted for age and gender. Plasma glucose/insulin levels and HOMA indices were adjusted for age, gender and BMI. HOMA-B, homeostasis model assessment of beta-cell function; HOMA-S, homeostasis model assessment of insulin sensitivity.
doi:10.1371/journal.pone.0039037.t003

Table 4. Glucose homeostasis parameters in nonobese children and adult populations according to PCSK1 rs6235.

|       | Non-obese | Obese |
|-------|-----------|-------|
|       | Children  | Adults |
|       | GG        | GC    | CC    | P-value | GG    | GC    | CC    | P-value |
| N (1189) | 528      | 318   | 31    | 0.47    | 249   | 132   | 15    |
| Age (yrs)     | 9.45±1.90 | 8.96±1.84 | 0.20    | 10.28±2.42 | 10.44±2.02 | 0.63 |
| z-score BMI   | 0.32±1.01 | 0.35±0.89 | 0.88    | 2.08±0.27 | 2.07±0.22 | 0.72 |
| Glucose (mmol/L) | 5.04±0.47 | 5.05±0.53 | 0.16    | 5.03±0.51 | 4.98±0.55 | 5.04±0.28 | 0.71 |
| Insulin (pmol/L) | 40.2 (24.3, 59.7) | 45.1 (21.1, 69.1) | 0.77    | 74.3 (50.8, 106.0) | 85.0 (53.8, 118.9) | 0.17 |
| HOMA-A (%)   | 76.4 (58.5, 101.0) | 73.3 (51.5, 97.6) | 0.87    | 125.6 (87.5, 156.2) | 122.7 (101.3, 166.2) | 0.16 |
| HOMA-S (%)   | 135.6 (87.6, 213.2) | 116.0 (75.2, 245.9) | 0.63    | 73.0 (51.9, 106.4) | 65.60 (54.8, 100.3) | 0.34 |
| N (893)      | 267      | 133   | 22    | 283    | 169   | 19    |
| Age (yrs)     | 45.7±14.7 | 45.45±17.69 | 0.40    | 41.0±12.1 | 41.0±11.5 | 0.26 |
| BMI (kg/m²)   | 23.7±2.4 | 23.83±2.55 | 0.31    | 38.5±7.8 | 38.6±6.56 | 0.40 |
| Glucose (mmol/L) | 5.7±2.3 | 6.15±2.38 | 0.86    | 6.1±2.2 | 6.3±2.7 | 0.59 |
| Insulin (pmol/L) | 56.2 (36.9,92.8) | 60.7 (42.3, 78.4) | 0.23    | 74.6 (45.8, 132.4) | 89.9 (64.7, 130.5) | 0.14 |
| HOMA-A (%)   | 89.7 (67.6, 124.6) | 88.2 (58.5, 107.5) | 0.79    | 106.7 (64.4, 141.0) | 111.1 (49.3, 161.5) | 0.11 |
| HOMA-S (%)   | 92.0 (53.9, 137.9) | 83.8 (51.7, 126.5) | 0.24    | 70.9 (39.7, 116.8) | 61.6 (38.4, 77.8) | 0.65 |

Data are means ± s.d. or medians (interquartile range). P-add values were calculated by generalized linear regression using an additive model. BMI was adjusted for age and gender. Plasma glucose/insulin levels and HOMA indices were adjusted for age, gender and BMI. HOMA-B, homeostasis model assessment of beta-cell function; HOMA-S, homeostasis model assessment of insulin sensitivity.
doi:10.1371/journal.pone.0039037.t004

Author Contributions
Conceived and designed the experiments: MVC SCQ. Performed the experiments: MVC HVR ELC PLM. Analyzed the data: MVC SRH TVM SCQ. Contributed reagents/materials/analysis tools: LJA AELF EFJP BELC TTL BERN CAAS SCQ. Wrote the paper: MVC TVM SRH CAAS SCQ.
References

1. Secretaría de Salud, Instituto Nacional de Salud Pública. (2006) Encuesta Nacional de Salud y Nutrición (ENSANUT).

2. Bell CG, Walley AJ, Froogd P (2005) The genetics of human obesity. Nat Rev Genet 6: 221–234.

3. Farooqi S, O’Rahilly S (2006) Genetics of obesity in humans. Endocr Rev 27: 710–718.

4. Ramachandrapa S, Farooqi IS (2011) Genetic approaches to understanding human obesity. J Clin Invest 121: 2080–2086.

5. Jackson RS, Creemers JW, Ohagi S, Raffin-Sanson ML, Sanders L, et al. (1997) Obesity and impaired prohormone processing associated with mutations in the human prohormone convertase 1 gene. Nat Genet 16: 303–306.

6. Jackson RS, Creemers JW, Farooqi IS, Raffin-Sanson ML, Varro A, et al. (2003) Small-intestinal dysfunction accompanies the complex endocrinopathy of human proprotein convertase 1 deficiency. J Clin Invest 112: 1550–1560.

7. Farooqi IS, Volders K, Stanhope R, Heuschkel R, White A, et al. (2007) Hyperphagia and early-onset obesity due to a novel homozygous missense mutation in prohormone convertase 1/3. J Clin Endocrinol Metab 92: 3369–3373.

8. Benzinou M, Creemers JW, Choquet H, Lobbers S, Dina C, et al. (2008) Common nonsynonymous variants in PCSK1 confer risk of obesity. Nat Genet 40: 943–945.

9. Kilpeläinen TO, Bingham SA, Khaw KT, Wareham NJ, Loos RJ (2009) Association of variants in the PCSK1 gene with obesity in the EPIC-Norfolk study. Hum Mol Genet 18: 3496–3501.

10. Renstrom F, Payne F, Nordstrom A, Brito EC, Rolandsson O, et al. (2009) Replication and extension of genome-wide association study results for obesity in 4923 adults from northern Sweden. Hum Mol Genet 18: 1489–1496.

11. Sandholt CH, Sparsø T, Grarup N, Albrechtsen A, Almind K, et al. (2010) Combined Analysis of 20 Common obesity susceptibility variants. Diabetes 59: 1667–1673.

12. Qi Q, Li H, Loos RJ, Liu C, Hu FB, et al. (2010) Association of PCSK1 rs6234 with obesity and related traits in a Chinese Han population. PLoS One 5: e10590.

13. Chang YC, Chiu YF, Shih KC, Lin MW, Sheu WH, et al. (2010) Common nonsynonymous variants in PCSK1 associate with obesity in the Chinese population. Obesity 18: 1404–1409.

14. Wen W, Cho YS, Zheng W, Dorajoo R, Kato N, et al. (2012) Meta-analysis identifies common variants associated with body mass index in east Asians. Nat Genet 44: 307–311.

15. Strawbridge Rj, Dupuis J, Prokopenko I, Barker A, Ahlqvist E, et al. (2011) Genome-wide association identifies nine common variants associated with fasting proinsulin levels and provides new insights into the pathophysiology of type 2 diabetes. Diabetes 60: 2624–2634.

16. Wardle J, Cattell S, Haworth CM, Plomin R (2006) Evidence for a strong genetic influence on childhood adiposity despite the force of the obesogenic environment. Am J Clin Nutr 87: 398–404.

17. 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.

18. Villalobos-Comparán M, Flores-Dorantes MT, Villarreal-Molina MT, Rodriguez-Cruz M, García-Ulloa AC, et al. (2008) The FTO gene is associated with adulthood obesity in the Mexican population. Obesity 16: 2286–2301.

19. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (2003). Diabetes Care 26: s3–s20.

20. Villarreal-Molina MT, Aguilar-Salinas CA, Rodriguez-Cruz M, Riaño D, Villalobos-Comparán M, et al. (2007) The ATP-binding cassette transporter A1 R250C variant affects HDL cholesterol levels and BMI in the Mexican population: association with obesity and obesity-related comorbidities. Diabetes 56: 1081–1087.

21. Levy JC, Matthews DR, Hermans MP (1998) Correct homeostasis model assessment (HOMA) evaluation uses the computer program. Diabetes Care 21: 2191–2192.

22. Folstein MF, Folstein SE, McHugh PR (1975) “Mini-mental state”: a practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 12: 189–198.

23. Hoggart CJ, Shriver MD, Kittles RA, Clayton DG, McKeigue PM (2004) Design and analysis of admixture mapping studies. Am J Hum Genet 74: 965–976.

24. Canizales-Quinteros S, Aguilar-Salinas CA, Ortiz-López MG, Rodriguez-Cruz M, Villarreal-Molina MT, et al. (2007) Association of PPARG2 Pro12Ala variant with larger body mass index in Mestizo and Amerindian populations of Mexico. Hum Biol 79: 111–119.

25. Creemers JW, Choquet H, Stijnen P, Vatin V, Digeyre M, et al. (2012) Heterozygous Mutations Causing Partial Prohormone Convertase 1 Deficiency Contribute to Human Obesity. Diabetes 61: 303–309.

26. Gjesing AP, Vestmar MA, Jørgensen T, Henri M, Holt JJ, et al. (2011) The effect of PCSK1 variants on waist, waist-hip ratio and glucose metabolism is modified by sex and glucose tolerance status. PLoS One 6: e23907.

27. Henri M, Haupt A, Schäfer SA, Ketterer C, Thamer C, et al. (2010) Association of obesity risk SNPs in PCSK1 with insulin sensitivity and proinsulin conversion. BMC Med Genet 11: 86.