Contribution of Common Genetic Variation to the Risk of Type 2 Diabetes in the Mexican Mestizo Population

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Several studies have identified nearly 40 different type 2 diabetes susceptibility loci, mainly in European populations, but few of them have been evaluated in the Mexican population. The aim of this study was to examine the extent to which 24 common genetic variants previously associated with type 2 diabetes are associated in Mexican Mestizos. Twenty-four single nucleotide polymorphisms (SNPs) in or near genes (*KCNJ11*, *PPARG*, *TCF7L2*, *SLC30A8*, *HHEX*, *CDKN2A/2B*, *CDKAL1*, *IGF2BP2*, *ARHGEF11*, *JAZF1*, *CDC123/CAMK1D*, *FTO*, *TSPAN8/LGR5*, *KCNQ1*, *THADA*, *ADAMTS9*, *NOTCH2*, *NXPH1*, *RORA*, *UBQLNL*, and *RALGPS2*) were genotyped in Mexican Mestizos. A case-control association study comprising 1,027 type 2 diabetic individuals and 990 control individuals was conducted. To account for population stratification, a panel of 104 ancestry-informative markers was analyzed. Association to type 2 diabetes was found for rs13266634 (*SLC30A8*), rs7923837 (*HHEX*), rs10811661 (*CDKN2A/2B*), rs4402960 (*IGF2BP2*), rs12779790 (*CDC123/CAMK1D*), and rs2237892 (*KCNQ1*). In addition, rs7754840 (*CDKAL1*) was associated in the nonobese type 2 diabetic subgroup, and for rs7903146 (*TCF7L2*), association was observed for early-onset type 2 diabetes. Lack of association for the rest of the variants may have resulted from insufficient power to detect smaller allele effects. ***Diabetes*** **61:3314–3321, 2012**

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Received 25 April 2011 and accepted 6 June 2012.

DOI: 10.2337/db11-0550

This article contains Supplementary Data online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db11-0550/-/DC1.

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The prevalence of type 2 diabetes is rapidly increasing worldwide (1). For the Mexican population aged older than 20 years, type 2 diabetes prevalence increased from 7.5% in 2000 to 14.4% in 2006 ($\sim 7.3 \times 10^6$ individuals) (2,3). Furthermore, the proportion of patients diagnosed before age 40 years also showed a steady increase, with 21.5% of patients diagnosed before age 45, imposing a significant public health burden due to substantial disability and premature death. Several studies in the Mexican population have established polygenic early-onset type 2 diabetes as a clinically and metabolically distinct entity from late-onset type 2 diabetes (4,5).

Various genome-wide association studies (GWASs) have identified close to 40 type 2 diabetes susceptibility loci mainly in European populations, including *TCF7L2*, *SLC30A8*, *HHEX/KIF1/IDE*, *EXT2*, *CDKN2A/CDKN2B*, *IGF2BP2*, *CDKAL1*, *FTO* (6–9), and more recently *JAZF1*, *CDC123/CAMKID*, *THADA*, *ADAMTS9*, *NOTCH2*, *TSPAN8* (10), *KCNQ1* (11,12), *NXPH1*, *RORA*, *UBQLNL*, and *RALGPS2* (13). Moreover, the *PPARG* and *KCNJ11* genes, initially associated with type 2 diabetes through candidate genes studies, were confirmed through European GWASs (7–9). The transcription factor-7-like 2 (*TCF7L2*), extensively associated with type 2 diabetes in various populations, has also been found associated in Mexican Mestizos (14–18).

Although associations of many common variants to type 2 diabetes have been replicated in several European and some Asian populations (19–21), there are still few studies in Mexican Mestizos, where lack of association may be due to limited sample size (18,22). The Mexican Mestizo population resulted from a recent admixture of European, Amerindian, and African populations, having estimated average proportions of ~50, ~45, and ~5%, respectively (23). Therefore, in addition to susceptibility variants that may derive exclusively from Amerindian ancestry (24), genetic variants that are common in Europeans are likely to be part of the genetic architecture of type 2 diabetes in Mexicans.

To assess the contribution of previously identified type 2 diabetes risk alleles in Mexican Mestizos, we analyzed 24 SNPs in or near 21 genes and evaluated their role in type 2 diabetes susceptibility in our population. Most of the examined gene variants correspond to those identified through the analysis of candidate genes and the "first-generation" GWAS type 2 diabetes risk alleles reported and further replicated in other populations. In addition, gene variants identified in Mexican Americans and Pima

Indians were included for analysis in the study due to the substantial Amerindian genetic component of our population.

RESEARCH DESIGN AND METHODS

Subjects. This study was approved by the ethics committees of all participating institutions. All individuals gave informed consent before they were included in the study. Only unrelated Mexican individuals whose parents and grandparents were self-identified as Mexican Mestizos were included.

Diagnosis of type 2 diabetes was based on American Diabetes Association criteria such as fasting plasma glucose values >125 mg/dL, current treatment with a hypoglycemic agent, or casual glucose values ≥200 mg/dL. Included were 1,027 type 2 diabetic individuals: 529 (216 men and 313 women) recruited at the Department of Endocrinology and Lipid Metabolism of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ) and three other reference clinics in Mexico City, and 498 additional type 2 diabetic individuals (151 men and 347 women) identified from the 2000 National Health Survey (a nation-wide population-based probabilistic survey). Of the latter, 160 (32%) were previously unaware of their condition at the time of the survey (25). From the total type 2 diabetic individuals, 643 were nonobese (BMI <30 kg/m^2), and 384 were obese (BMI ≥30 kg/m^2; Supplementary Table 1). Early- or late-onset of diabetes was defined using age 45 years as a cutoff, as described in different genetic studies for type 2 diabetes (24,26). Individuals with early-onset type 2 diabetes did not require insulin at least during the first 2 years after diagnosis and had no family history of maturity-onset diabetes of the young. In addition, to reduce the possibility of including control individuals who might develop type 2 diabetes later in life, the control group also included 990 (392 men and 598 women; 692 nonobese and 298 obese) healthy, normoglycemic individuals (glucose ≤100 mg/dL), aged 45 years or older, with no first-degree relatives or grandparents with type 2 diabetes (Table 1).

Biochemical measurements. The Endocrinology and Lipid Metabolism Department of INCMNSZ performed all biochemical laboratory measurements with commercially available standardized methods. This laboratory is certified by the External Comparative Evaluation of Laboratories Program of the College of American Pathologists. Blood samples were obtained after a 9- to 12-h fast. Glucose was measured using the glucose oxidase method, and serum total cholesterol, HDL cholesterol (HDL-C), and triglycerides were measured using a Synchron autoanalizer (Beckman Co). LDL-C was calculated as described by Friedwald (1972) (27).

SNP selection and genotyping. The studied gene variants correspond to those identified through the analysis of candidate genes and most of the "first-generation" GWAS type 2 diabetes reported and further replicated in other populations (6–11). In addition, gene variants identified in Mexican Americans and Pima Indians were included for analysis because Mexican Mestizos have a high Amerindian genetic component (13,28).

Genomic DNA was extracted from total blood samples using a commercial kit (QIAamp 96 DNA Blood kit, Qiagen). A total of 24 SNPs previously associated with type 2 diabetes risk in other populations were genotyped in all individuals: rs5219 (*KCNJ11*), rs1801282 (*PPARG*), rs7903146 (*TCF7L2*), rs1111875 and rs7923837 (*HHEX/KIF1/IDE*), rs13266634 (*SLC30A8*), rs10811661 (*CDKN2A/2B*), rs7754840 and rs10484634 (*CDKAL1*), rs945508 (*ARHGEF11*), rs864745 (*JAZF1*), rs12779790 (*CDC123/CAMK1D*), rs8050136 (*FTO*), rs7578597 (*THADA*), rs2237892 (*KCNQ1*), rs4607103 (*ADAMTS9*), rs10923931 (*NOTCH2*), rs1470579 and rs4402960 (*IGF2BP2*), rs7961581 (*TSPAN8*), rs757705 (*NXPH1*), rs7164773 (*RORA*), rs979752 (*UBQLNL*), and rs2773080 (*RALGPS2*).

Genotyping was performed using KASPAR assays (Kbioscience, U.K.). A randomly selected subset of samples was tested in duplicate to confirm genotyping results. In addition, 104 ancestry informative markers (AIMs) were genotyped to account for population stratification in 1,372 individuals (Supplementary Table 4). AIMs were genotyped at the Biomedical Genomics Center, University of Minnesota. Four multiplex PCR assays containing 28, 27, 26, and 26 markers, respectively, were performed, followed by single-base primer extensions using iPLEX enzyme and buffers (Sequenom, San Diego, CA). Primer extension products were measured with the MassARRAY Compact System (Sequenom) and analyzed by mass spectra using TYPER software (Sequenom) to generate genotype calls.

Statistical analysis. Median and the 25th and 75th percentiles of baseline characteristics were calculated and compared using the Kruskal-Wallis test. Also, χ^2 tests were used to compare genotype frequencies between groups and to determine Hardy-Weinberg equilibrium. Pairwise linkage disequilibrium (LD) between SNPs was calculated using R 2.7.1 software.

Individual ancestry was estimated using data from evenly distributed AIMs using STRUCTURE 2.2 software (29,30). Means and correlations among the three vectors of ancestry (Native American, European, and African) were assessed. Because total ancestry marker information was available in a subset of 1,372 subjects, we first imputed the missing Native American ancestry values for the rest of the study sample (n = 645). Briefly, we used a two-step imputation model, as reported by Weissglas-Volkov et al. (31), in which we assessed the relationship between Amerindian ancestry and glucose value through a linear regression. However, although the coefficient (β = 96.27) for this model was statistically significant ($P < 0.001$), the determination coefficient was very low (R^2= 0.023). Even when we tested for other clinical or biochemical parameters and used saturated models, including several covariates, the proportion of variability accounted for the statistical model did not exceed 25%; thus, we decided the model was not accurate enough to predict ancestry.

As an alternative, the association between each SNP and type 2 diabetes was assessed through two logistic models exclusively on those subjects genotyped for ancestry. The first model was adjusted for age, sex, and BMI. The second model was also adjusted for Native American ancestry. These analyses led us to identify a group of nine SNPs for which the odds ratio (ORs) or the P value did not change drastically due to the effect of ancestry when comparing the ancestry adjusted versus nonadjusted association analyses (a threshold of less than 10% variation for ORs was set). For these nine SNPs, ancestry was not a covariate in the final models, so the whole set of 2,017 subjects was included.

For the 12 SNPs, where the risk effect changed more than 10% or the P value changed drastically, only the subjects with ancestry information were included, and the final models were adjusted for this variable. Associations between genotypes and type 2 diabetes were tested under an additive disease model. A stratified association analysis was also conducted according to obesity status and age of onset (defining age 45 years as a cutoff). A Woolf test was used to assess the heterogeneity between ORs in the stratified analysis as well as between Mexican and European ancestry (32).

Because the prior probability of association is highly based on studies in Europeans and further replicated in other populations, it is unlikely to detect effects due to statistical fluctuation only. Therefore, correction by multiple comparisons was not applied, and an association was considered statistically significant if at a nominal P value ≤0.05.

The association between SNPs with quantitative outcomes was done through a stratified linear regression analysis conditional to type 2 diabetic status. For SNPs rs13266634, rs10811661, rs7754840, and rs12779790, models were adjusted for age, sex, and BMI in the entire case-control sample. For rs7903146, rs7923837, rs4402960, and rs2237892, the model was also adjusted for ancestry. In all cases, risk alleles are those previously reported as associated with type 2 diabetes in other populations (8,10,11,13,28).

To assess the combined effect of the studied loci, we calculated three genotype scores, counting the number of risk alleles: the first was constructed with the 21 SNPs analyzed, the second was obtained with the 17 variants that consistently replicated in Europeans (excluding *NXPH1*, *RORA*, *UBQLNL* and *RALGPS2* gene variants), and the third was obtained by excluding from these 17 the 8 gene variants showing significant association to type 2 diabetes in our sample. The combined effect of the studied loci on type 2 diabetes was assessed through a logistic model adjusted for age, sex, BMI, and ancestry.

Calculations were performed using STATA/SE 10.0 software (StataCorp LP, College Station, TX).

RESULTS

Differences in clinical, anthropometric, and biochemical measurements between case and control individuals are described in Table 1. Overall, individuals with type 2 diabetes had significantly higher BMI, waist circumference, systolic and diastolic blood pressure, glucose and triglyceride levels, and lower total cholesterol, HDL-C, and LDL-C levels. Waist circumference and systolic and diastolic blood pressure also showed statistically significant differences between the subgroups with early- and late-onset type 2 diabetes. Insulin treatment was given to 12.6% of patients, but no statistical differences were observed between early- and late-onset groups. In contrast, significant differences were observed for age, sex, and systolic and diastolic blood pressure between obese and nonobese type 2 diabetic patients (Supplementary Table 1).

Type 2 diabetes association analyses and the effect of population stratification. To investigate the role of different type 2 diabetes allelic variants, we studied 24 SNPs in or near 21 genes, most of which had been replicated in other populations (20,33,34). Although all tested SNPs

TABLE 1
General characteristics of the studied sample and stratification by age of onset

	Control subjects	Type 2 diabetic patients		
		Total case subjects	Early-onset	Late-onset
N	990	1,027	510	517
Males (%)	39.60	35.74	38.04	33.46
Age (years)	53 (49–62)	53 (45–62)*	45 (38–52)†	60 (54–66)
Age at diagnosis (years)	—	46 (37–52)	37 (31–42)†	52 (49–59)
BMI (kg/m^2)	27.5 (25.3–30.5)	28.7 (26–32)*	28.7 (25.7–32.1)	28.7 (26.4–31.7)
Waist circumference (cm)	92.9 (86.5–101)	100 (92–108.2)*	98 (90–108)†	101 (93–108.5)
Blood pressure (mmHg)				
Systolic	120 (110–131)	130 (120–140)*	122 (112–135)†	130 (120–145)
Diastolic	80 (70–88)	80 (75–90)*	80 (73–90)†	83 (76–90)
Glucose (mg/dL)	88.5 (82–95)	221 (147.5–315.5)*	224 (150–316)	220 (145–312)
Cholesterol (mg/dL)				
Total	213 (189–241)	203 (175–237)*	202 (173–237)	205 (177–238)
HDL-C	44 (37–51.5)	38 (32–45)*	38 (31–44)	38 (32–45)
LDL-C	133 (113–156.5)	111 (86–138)*	110 (86–139)	112 (86–138)
Triglycerides (mg/dL)	164 (119–237)	233 (157–373)*	231 (154–370)	233.5 (161–375)

Median (25th–75th percentiles) values of baseline characteristics are shown. *$P < 0.05$ comparisons between overall type 2 diabetic patients and controls subjects. †$P < 0.05$ comparisons between early-onset and late-onset type 2 diabetes: early-onset subjects diagnosed before age 45 years; late-onset subjects diagnosed at age 45 years or older.

showed genotyping call rates above 95%, only 21 of the variants in or near 20 genes were used for the analysis: SNPs rs10484634 (*CDKAL1*) and rs75785597 (*THADA*) had a minor allele frequency lower than 0.05, and SNP rs1470579 (*IGF2BP2*) did not reach Hardy-Weinberg equilibrium in control individuals (Supplementary Table 5). However, variant rs4402960 (*IGF2BP2*) in LD with rs1470579 in the total sample was included in the analysis ($r^2 = 0.89$).

In this sample, mean Native American ancestry was 58.9% for type 2 diabetic subjects and 51.1% in control individuals (Kruskal-Wallis test $P < 0.01$). Because ancestry marker information was available only in a subset of 1,372 subjects, Table 2 shows the association analysis for the case-control individuals with or without ancestry adjustment. Through this analysis we identified 12 of 21 SNPs where the ORs or the *P* value was drastically affected by ancestry. For all reported associations we considered the case-control sample that had AIMS data. However, for three of the studied variants, not drastically affected by ancestry, stronger *P* values were obtained when the

TABLE 2
Association analyses with type 2 diabetes in Mexican Mestizos and the confounder effect of ancestry

			868 case and 504 control subjects adjusted for ancestry		868 case and 504 control subjects without ancestry correction		1,027 case and 990 control subjects without ancestry	
Nearest gene	SNP	Risk allele	OR (95% CI)*	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
KCNJ11	rs5219	*T*	1.16 (0.97–1.37)	0.099	1.17 (0.98–1.38)	0.076	1.10 (0.96–1.26)	0.154
PPARG	rs1801282	*C*	1.19 (0.92–1.55)	0.180	1.08 (0.84–1.39)	0.524	1.10 (0.90–1.34)	0.342
TCF7L2	rs7903146	*T*	1.21 (0.97–1.51)	0.087	1.04 (0.84–1.28)	0.735	—	—
SLC30A8	rs13266634	*C*	**1.21 (1.01–1.46)**	**0.040**	1.22 (1.02–1.46)	0.034	**1.22 (1.05–1.41)**	**0.009**
HHEX	rs1111875	*C*	0.95 (0.80–1.12)	0.554	0.93 (0.79–1.10)	0.384	1.01 (0.89–1.16)	0.859
	rs7923837	*G*	**1.21 (1.02–1.44)**	**0.025**	1.10 (0.94–1.30)	0.238	—	—
CDKN2A/2B	rs10811661	*T*	**1.47 (1.14–1.89)**	**0.003**	1.59 (1.24–2.04)	<0.001	**1.42 (1.15–1.75)**	**0.001**
CDKAL1	rs7754840	*C*	1.16 (0.97–1.38)	0.104	1.14 (0.96–1.35)	0.148	1.13 (0.98–1.30)	0.081
IGF2BP2	rs4402960	*T*	**1.24 (1.01–1.53)**	**0.042**	1.12 (0.92–1.37)	0.265	—	—
ARHGEF11	rs945508	*A*	1.05 (0.87–1.28)	0.604	0.91 (0.76–1.09)	0.319	—	—
JAZF1	rs864745	*T*	1.11 (0.93–1.32)	0.253	1.24 (1.04–1.47)	0.015	—	—
CDC123/CAMK1D	rs12779790	*G*	**1.26 (1.02–1.58)**	**0.036**	1.25 (1.01–1.55)	0.042	**1.24 (1.05–1.47)**	**0.013**
FTO	rs8050136	*A*	1.03 (0.84–1.26)	0.762	0.90 (0.74–1.09)	0.278	—	—
TSPAN/LGR5	rs7961581	*C*	1.15 (0.90–1.47)	0.252	0.93 (0.73–1.17)	0.516	—	—
KCNQ1	rs2237892	*C*	**1.36 (1.13–1.64)**	**0.001**	1.18 (0.99–1.42)	0.062	—	—
ADAMTS9	rs4607103	*C*	1.01 (0.84–1.20)	0.952	1.03 (0.87–1.23)	0.726	1.05 (0.91–1.20)	0.521
NOTCH2	rs10923931	*T*	1.05 (0.78–1.42)	0.752	0.99 (0.73–1.33)	0.931	1.04 (0.82–1.32)	0.731
NXPH1	rs757705	*G*	1.17 (0.98–1.39)	0.080	1.25 (1.05–1.48)	0.010	—	—
RORA	rs7164773	*T*	0.95 (0.80–1.38)	0.600	1.08 (0.91–1.28)	0.357	—	—
UBQLNL	rs979752	*C*	0.88 (0.70–1.11)	0.290	1.04 (0.84–1.30)	0.700	—	—
RALGPS2	rs2773080	*A*	1.03 (0.84–1.28)	0.760	0.90 (0.74–1.10)	0.315	—	—

Risk allele is defined as previously reported associated to type 2 diabetes risk in other populations. *P* values are nominal *P* values. Statistically significant observations are bold-faced. All analyses were based on additive models, and logistic models were adjusted for age, sex, and BMI. *Logistic models were also adjusted for ancestry.

total sample was included for the association analysis: rs13266634 (*SLC30A8*; OR 1.22, $P = 0.009$), rs10811661 (*CDKN2A/2B*; OR 1.42, $P = 0.001$), and rs12779790 (*CDC123/CAMK1D*; OR 1.24, $P = 0.013$; Table 2).

It is important to mention that although the ancestry correction included more case than control individuals (868 vs. 504), selection of subjects for AIMs analysis was random, and ethnicity could not be obvious before the AIMs genotyping. Furthermore, when comparing genotype frequencies from case or control individuals with or without AIMs information, we did not find significant differences, except for rs10811661 (*CDKN2A/2B*) and rs864745 (*JAZF1*) in type 2 diabetic individuals and for rs7961581 (*TSPAN/LGR5*) in control individuals. Thus, potential selection bias is unlikely.

In contrast, the allelic variants of genes *KCNJ11* (rs5219), *PPARG* (rs1801282), *HHEX* (rs1111875), *ARHGEF11* (rs945508), *JAZF1* (rs864745), *FTO* (rs8050136), *TSPAN/LGR5* (rs7961581), *ADAMTS9* (rs4607103), *NOTCH2* (rs10923931), *NXPH1* (rs757705), *RORA* (rs7164773), *UBQLNL* (rs979752), and *RALGPS2* (rs2773080) failed to show association in the studied sample. In addition, variants rs1111875 and rs7923837 (*HHEX*) were not in LD in our sample ($r^2 = 0.099$).

Supplementary Table 2 summarizes the comparisons of the risk alleles frequencies between Mexican and European populations.

Differential contributions of type 2 diabetes alleles in distinct phenotypic subgroups. Table 3 reports the association analysis when the sample was stratified by age of onset or obesity status. Despite a reduced sample size, rs7903146 (*TCF7L2*) was associated with early-onset type 2 diabetes (OR 1.39, $P = 0.024$). However, a test of heterogeneity failed to demonstrate a significant difference between subgroups with early- and late-onset type 2 diabetes. In addition, when stratifying by obesity, rs7754840 (*CDKAL1*) showed a significant association in the nonobese type 2 diabetic subgroup (1.25 [95% CI 1.06–1.49], $P = 0.009$) and heterogeneity was significant ($P = 0.04$).

We also explored the association of SNPs with type 2 diabetes-related quantitative traits, including insulin levels and homeostasis model assessment-insulin resistance (HOMA-IR) and HOMA-β. An association was detected for rs7903146 (*TCF7L2*) in normoglycemic subjects where risk allele carriers (CT+TT) showed significantly lower HOMA-β values than noncarriers (CC; OR 109.6 [95% CI 104.1–115.2] vs. 121.8 [116.9–126.6], respectively; $P = 0.015$). Interestingly, rs8050136 (*FTO*) was associated with HOMA-β in normoglycemic subjects. Risk allele carriers (CA+AA) showed significantly lower HOMA-β values than noncarriers (CC; 109.8 [104.6–115] vs. 121.6 [116.5–126.7], respectively $P = 0.014$). No other significant differences were observed according to genotype for HOMA-β, HOMA-IR, or glucose or insulin levels. In addition, only rs8050136 (*FTO*) showed an association to obesity in the total sample (1.25 [1.06–1.47], $P = 0.008$).

Assessment of cumulative effect of studied loci. We first determined the genotype score including the 21 SNPs (ranging from 10 to 30 risk alleles). The mean (± SD) genotype score was 19.4 ± 2.7 for control individuals and 19.9 ± 2.9 for case subjects. Also, in a logistic model adjusted for age, sex, BMI, and ancestry, the obtained OR of 1.09 (95% CI 1.04–1.14) was statistically significant ($P < 0.01$). For the second genotype score, which included 17 SNPs (ranging from 7 to 24 risk alleles, and excluding variants from the Mexican-American population), we obtained an OR of 1.11 (1.06–1.17; $P < 0.01$). Finally, the third genotype score (ranging from 2 to 13 risk alleles) was performed excluding from these 17 the 8 associated variants with nominal significance, and an OR of 1.03 (0.95–1.12; $P = 0.45$) was obtained.

DISCUSSION

Because of its ethnic composition, common type 2 diabetes susceptibility variants of European and Amerindian origin are expected to contribute to some degree to the genetic susceptibility of type 2 diabetes in the Mexican Mestizo population. In this report, we analyzed whether 21 type 2 diabetes risk variants, most of which were previously identified and replicated in different European and Asian populations, were associated with type 2 diabetes in Mexican Mestizos. Although the European component of our type 2 diabetic case subjects is close to 40%, not all the alleles conferring type 2 diabetes risk in Europeans seem to be associated with type 2 diabetes in Mexican Mestizos. Given that all ORs identified are consistent with those seen in studies of Europeans, providing all detected associations involved the same risk allele and were in the same direction, we showed significant replication for 8 of 21 studied loci.

Interestingly, from the identified associated variants, rs2237892 (*KCNQ1*) and rs10811661 (*CDKN2A/2B*) showed the strongest effects on type 2 diabetes risk in the studied sample, with ORs of 1.36 ($P = 0.001$) and 1.42 ($P = 0.001$), respectively. *KCNQ1* (initially found associated to type 2 diabetes in Asians) was not associated in Mexican Americans or Mexicans in two separate GWA studies, and only a marginal association to this locus was found through a meta-analysis merging data from these two populations (35). The association we found for rs10811661 near the *CDKN2A/2B* gene region is consistent with the fact that this locus has shown replication for type 2 diabetes and related traits such as decreased glucose-stimulated insulin secretion and decreased β-cell function across populations (7–9,20,34,36). In addition, in a meta-analysis that included Mexican Americans, Mexican Mestizos, and Europeans, SNP rs1333051 (in LD with rs10811661 [$r^2 = 0.75$] in Europeans), showed the strongest association to type 2 diabetes (35). More recently, this locus was also found associated with coronary heart disease in type 2 diabetic patients (37).

Also of interest is that the associations found for *TCF7L2* and to *CDKAL1* were evident upon stratification of the sample by age of onset or obesity status. That these associations are artificial is unlikely, because the *TCF7L2* and *CDKAL1* loci have been extensively associated with type 2 diabetes in several populations (14–16,21,37), and despite the size of the sample, a significant association was detected for rs7903146 (*TCF7L2*; $P = 0.024$) in the group with early-onset type 2 diabetes. Similarly, upon stratification of the sample by obesity status, a significant association was detected for the rs7754840 variant (*CDKAL1*; $P = 0.019$).

It is therefore possible that the observed associations may be the result of a differential role of the *TCF7L2* and *CDKAL1* gene variants on insulin secretion in these two type 2 diabetic subgroups. For instance, the association found for rs7903146 (*TCF7L2*) in the early-onset type 2 diabetic subgroup may reflect a larger proportion of type 2 diabetic subjects with reduced β-cell function, and we were able to find significantly lower HOMA-β values for

TABLE 3
Association analyses with type 2 diabetes in Mexican Mestizos according to age of onset and obesity status

		Early-onset		Late-onset			Nonobese		Obese		
Nearest gene	SNP	OR (95% CI)	*P*	OR (95% CI)	*P*	Het test	OR (95% CI)	*P*	OR (95% CI)	*P*	Het test
KCNJ11	rs5219*	1.12 (0.93–1.34)	0.233	1.13 (0.96–1.34)	0.153	0.94	1.14 (0.97–1.34)	0.119	1.01 (0.80–1.28)	0.923	0.43
PPARG	Rs1801282*	1.12 (0.86–1.47)	0.394	1.07 (0.83–1.37)	0.602	0.81	1.12 (0.88–1.43)	0.361	1.04 (0.74–1.48)	0.811	0.98
TCF7L2	Rs7903146†	**1.39 (1.04–1.85)**	**0.024**	1.13 (0.86–1.48)	0.376	0.43	1.30 (0.99–1.70)	0.060	1.13 (0.77–1.64)	0.539	0.36
SLC30A8	Rs13266634*	**1.39 (1.13–1.72)**	**0.002**	1.18 (0.98–1.42)	0.075	0.25	**1.23 (1.02–1.48)**	**0.027**	1.23 (0.95–1.59)	0.110	0.76
HHEX	Rs1111875*	0.99 (0.82–1.19)	0.913	1.02 (0.87–1.21)	0.793	0.81	1.00 (0.85–1.18)	0.997	1.04 (0.83–1.32)	0.715	0.81
	Rs7923837†	1.07 (0.85–1.34)	0.567	1.22 (0.99–1.50)	0.062	0.41	**1.37 (1.11–1.70)**	**0.003**	0.92 (0.72–1.31)	0.850	0.16
CDKN2A/2B	Rs10811661*	**1.46 (1.10–1.95)**	**0.009**	**1.45 (1.11–1.88)**	**0.006**	0.97	1.38 (1.07–1.78)	0.013	**1.49 (1.03–2.14)**	**0.033**	0.81
CDKAL1	Rs7754840*	1.11 (0.92–1.34)	0.290	1.07 (0.90–1.27)	0.435	0.78	**1.25 (1.06–1.49)**	**0.009**	0.90 (0.71–1.15)	0.392	0.04
IGF2BP2	Rs4402960†	1.04 (0.79–1.37)	0.768	**1.35 (1.05–1.74)**	**0.019**	0.17	1.23 (0.95–1.58)	0.114	1.30 (0.89–1.91)	0.188	0.59
ARHGEF11	rs945508†	1.12 (0.88–1.44)	0.354	1.00 (0.79–1.28)	0.974	0.37	0.95 (0.75–1.21)	0.696	1.28 (0.91–1.81)	0.154	0.06
JAZF1	rs864745†	1.20 (0.94–1.52)	0.143	1.03 (0.83.1.29)	0.790	0.94	1.13 (0.91–1.41)	0.261	1.06 (0.77–1.45)	0.724	0.55
CDC123/CAMK1D	rs12779790*	**1.33 (1.05–1.68)**	**0.017**	1.15 (0.93–1.43)	0.194	0.37	**1.29 (1.05–1.59)**	**0.015**	1.16 (0.85–1.58)	0.351	0.67
FTO	rs8050136†	1.07 (0.82–1.39)	0.642	0.95 (0.74–1.23)	0.704	0.53	1.07 (0.83–1.38)	0.625	0.95 (0.68–1.34)	0.787	0.96
TSPAN8/LGR5	rs7961581†	1.00 (0.72–1.39)	0.994	1.27 (0.94–1.72)	0.116	0.48	1.14 (0.85–1.52)	0.393	1.23 (0.77–1.96)	0.392	0.19
KCNQ1	rs2237892†	1.27 (0.99–1.62)	0.051	**1.37 (1.09–1.74)**	**0.008**	0.43	**1.37 (1.09–1.73)**	**0.007**	**1.39 (1.01–1.91)**	**0.046**	0.73
ADAMTS9	rs4607103*	1.07 (0.88–1.29)	0.516	1.02 (0.86–1.22)	0.809	0.96	1.08 (0.91–1.28)	0.374	0.99 (0.78–1.28)	0.977	0.62
NOTCH2	rs10923931*	1.19 (0.85–1.66)	0.303	0.99 (0.73–1.33)	0.949	0.42	1.00 (0.75–1.33)	0.980	1.12 (0.73–1.71)	0.616	0.62
NXPH1	rs757705†	1.22 (0.96–1.54)	0.106	1.17 (0.94–1.45)	0.163	0.81	1.20 (0.97–1.49)	0.090	1.09 (0.80–1.49)	0.571	0.53
RORA	rs7164773†	0.98 (0.78–1.24)	0.880	1.05 (0.84–1.31)	0.674	0.77	0.94 (0.76–1.17)	0.584	0.97 (0.72–1.31)	0.859	0.80
UBQLNL	rs979752†	0.91 (0.67–1.24)	0.568	0.94 (0.71–1.24)	0.648	0.45	0.91 (0.69–1.21)	0.523	0.80 (0.54–1.20)	0.287	0.34
RALGPS2	rs2773080†	0.94 (0.71–1.25)	0.680	1.04 (0.80–1.34)	0.795	0.41	1.18 (0.91–1.52)	0.222	0.81 (0.56–1.17)	0.254	0.27

Het test, heterogeneity (Woolf) test. *P* values are nominal *P* values. Statistically significant observations are bold-faced. *All analyses were based on an additive model, and logistic models were adjusted for age, sex, and BMI. †Logistic models were also adjusted for ancestry.

rs7903146 risk allele carriers versus noncarriers in normoglycemic individuals ($P = 0.02$), as previously reported (39–41). In addition, although we were unable to demonstrate significantly lower HOMA-β values in rs7754840 (*CDKAL1*) risk allele carriers versus noncarriers, there are previous reports where SNPs rs7756992 and rs10946398 in the *CDKAL1* gene are associated with an insulin secretory defect and impaired insulin response in oral and intravenous glucose tolerance tests (38,42,43).

The three rs7754840, rs7756992, and rs10946398 variants are all within intron 5, and rs7756992 and rs7754840 are in LD in European ($r^2 = 0.73$) and Hispanic populations ($r^2 = 1.0$) (44,45). Moreover, accounting for the excess of men in the nonobese group, we also tested for a potential sex-specific association; however, similar ORs and nominal *P* values were observed for both sexes.

Modulation of type 2 diabetes risk by obesity has been reported for other susceptibility gene variants. Cauchi et al. (44) reported that genetic variants modulating insulin action may have an increased effect on type 2 diabetes susceptibility in the presence of obesity, whereas genetic variants acting on insulin secretion may have a greater effect on type 2 diabetes susceptibility in nonobese individuals. In this regard, the association found for rs7754840 (*CDKAL1*) in nonobese type 2 diabetic patients in our study supports impaired insulin secretion as an important mechanism underlying type 2 diabetes in the nonobese type 2 diabetic subgroup.

A potential constraint of the current study is the lack of AIMs data for all of the subjects. However the *P* values for the reported associated variants (rs13266634, rs7923837, rs10811661, rs4402960, rs12779790, and rs2237892) were significant when the analysis was performed exclusively in individuals with AIMs data. Furthermore, an even stronger effect was observed for three of these variants (rs13266634, rs10811661, and rs12779790) by the inclusion of the entire sample set. A special case is that of variants rs864745 (*JAZF1*) and rs757705 (*NXPH1*): when individuals with AIMs data were used in the analyses without considering ancestry as a variable, a significant *P* value was obtained. However, these values were rendered nonsignificant when corrected by ancestry. Consequently, these two variants were reported as not associated with type 2 diabetes in our sample.

For those loci that showed replication to type 2 diabetes in our population, we compared the effect sizes between Mexicans and Europeans. In the case of *KCNQ1*, the comparison was also made with Asians because this locus has not been extensively replicated in Europeans (45). The ORs obtained for the *KCNQ1* variant were not significantly different from those reported for Europeans or Asians (Supplementary Table 3). Similar OR and *P* values were obtained for the multilocus genotype score for the analysis with all 21 analyzed gene variants as well as for the analysis excluding the *NXPH1*, *RORA*, *UBQLNL*, and *RALGPS2* type 2 diabetes risk alleles described in Mexican Americans. In contrast, a nonsignificant *P* value was obtained when excluding the eight associated variants from the analysis, which would be consistent with the association being driven entirely by the nominally significant variants.

Variants rs5219 (*KCNJ11*), rs1111875 (*HHEX*), rs8050136 (*FTO*), rs864745 (*JAZF1*), rs7961581 (*TSPAN/LGR5*), rs4607103 (*ADAMTS9*), and rs10923931 (*NOTCH2*), with reported OR values below 1.2 in European populations, failed to show an association with type 2 diabetes in Mexican Mestizos. In this regard, risk alleles frequencies for *KCNJ11*, *ARHGEF11 TSPAN/LGR5*, *ADAMTS9*, *NOTCH2*, and *FTO* are significantly lower in Mexicans than in Europeans (Supplementary Table 2). This observation has to be taken into account in future association studies, because a much larger sample would be required to detect potential risk effects of these loci.

Even though the prevalence of type 2 diabetes in Mexico is one of the highest worldwide, very few studies have reported an association of type 2 diabetes risk variants in the Mexican population (17,18,22,46). Previous reports studied smaller samples, did not exclude individuals with a family history of type 2 diabetes from the control group, and some excluded obese individuals or did not analyze the data stratifying by type 2 diabetic subgroups. These factors may explain the lack of association previously reported for rs7754840 (*CDKAL1*) (18) and rs13266634 (*SLC30A8*) (22). The current study included a larger sample size; however, the lack of association of variants *KCNJ11* (rs5219), *PPARG* (rs1801282), *JAZF1* (rs864745), *FTO* (rs8050136), *TSPAN/LGR5* (rs7961581), *ADAMTS9* (rs4607103), *NOTCH2* (rs10923931), *NXPH1* (rs757705), *RORA* (rs7164773), *UBQLNL* (rs979752), and *RALGPS2* (rs2773080) may result from still insufficient power to detect smaller allele effects. However, it is interesting that *FTO* (rs8050136) was found independently associated with obesity (OR 1.25, $P = 0.008$). We also showed that this variant was significantly associated with HOMA-β in normoglycemic subjects ($P = 0.014$). Both findings are consistent with previous results obtained in Mexican population (47).

Also of interest is that of the two *HHEX/KIF1/IDE* SNPs tested, only rs7923837 was associated with type 2 diabetes in our study, suggesting a narrow LD region in Mexicans, where rs7923837 may be closer to the functional variant(s).

Overall, our results underscore the importance of considering the phenotypic heterogeneity of the disease as well as the admixed component of the Mexican Mestizo population in future case-control association studies, because some of the previously type 2 diabetes risk alleles may be relatively uncommon or may have a modest effect on type 2 diabetes susceptibility. Therefore, high-density genome-wide association studies in this admixed population, as well as in larger cohorts, are still needed to dissect the type 2 diabetes genetic component not only in Mexican Mestizos but also in other admixed populations of Amerindian descent.

ACKNOWLEDGMENTS

This project was supported by the Dirección General de Asuntos del Personal Académico IN227007-2 and IT214711-3, the Fondo Sectorial de Investigación en Salud 14495, and National Institutes of Health Grant 5K12 DK-083021 to S.C., A.H.-C., M.A.G.-M., P.V.-C., and M.T.G.-G. received support through graduate fellowships provided by the Consejo Nacional de Ciencia y Tecnología (CONACyT).

No potential conflicts of interest relevant to this article were reported.

M.A.G.-M. and A.H.-C. acquired, analyzed, and interpreted the data, performed statistical analysis, and drafted the manuscript. H.M.-M. analyzed and interpreted the data, performed statistical analysis, and made critical revision of the manuscript for important intellectual content. P.V.-C. analyzed and interpreted the data and performed statistical analysis. M.L.O.-S. and M.R.-T. contributed to sample preparation and quality control and acquired the data.

R.R.-G. contributed to sample preparation and quality control. L.R. recruited participants; contributed to clinical characterization of samples, sample preparation and quality control, biochemical profiles, and database handling; handled funding and supervision; drafted the manuscript, and made critical revision of the manuscript for important intellectual content. M.T.G.-G. analyzed and interpreted the data. L.E.G.-P. contributed to biochemical profiles and database handling. S.C. conceived and designed the research, performed statistical analysis, drafted the manuscript, and made critical revision of the manuscript for important intellectual content. L.d.B.-P. conceived and designed the research and made critical revision of the manuscript for important intellectual content. S.C.-Q. recruited participants and contributed to clinical characterization of samples. G.P.-O. contributed to sample preparation and quality control and acquired the data. F.E.-A. and A.P. recruited participants, contributed to clinical characterization of samples, and made critical revision of the manuscript for important intellectual content. I.L.-G. recruited participants, contributed to clinical characterization of samples, analyzed and interpreted the data, and made critical revision of the manuscript for important intellectual content. C.A.A.-S. conceived and designed the research, recruited participants, contributed to clinical characterization of samples, handled funding and supervision, and made critical revision of the manuscript for important intellectual content. M.T.T.-L. conceived and designed the research, analyzed and interpreted the data, handled funding and supervision, and drafted the manuscript. M.T.T.-L. and C.A.A.-S. are the guarantors of this work and, as such, had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

The authors thank Salvador Ramírez Jiménez and Angélica Flores Álvarez, from the Unidad de Biología Molecular y Medicina Genómica, Universidad Nacional Autónoma de México/Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, for providing technical assistance; Dr. Kenneth B. Beckman, from the Biomedical Genomics Center, University of Minnesota, for the genotyping of AIMs; and the Instituto Nacional de Salud Pública and all participants in this study from the Encuesta Nacional de Salud (ENSA) 2000 National Survey and all other institutions.

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