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# Genetic variants in *KCNJ11*, *TCF7L2* and *HNF4A* are associated with type 2 diabetes, BMI and dyslipidemia in families of Northeastern Mexico: A pilot study

HUGO LEONID GALLARDO-BLANCO[1], JESUS ZACARÍAS VILLARREAL-PEREZ[2], RICARDO MARTIN CERDA-FLORES[3], ANDRES FIGUEROA[4], CELIA NOHEMI SANCHEZ-DOMINGUEZ[5], JUANA MERCEDES GUTIERREZ-VALVERDE[3], IRIS CARMEN TORRES-MUÑOZ[1], FERNANDO JAVIER LAVALLE-GONZALEZ[2], ESTHER CARLOTA GALLEGOS-CABRIALES[3] and LAURA ELIA MARTINEZ-GARZA[1]

[1]Department of Genetics, School of Medicine; [2]Department of Endocrinology, University Hospital 'José Eleuterio González'; [3]School of Nursing, Autonomous University of Nuevo León, Monterrey, Nuevo León, CP 64460, Mexico; [4]Department of Computer Science, University of Texas Rio Grande Valley, Edinburg, TX 78539, USA; [5]Department of Biochemistry and Molecular Medicine, School of Medicine, Autonomous University of Nuevo León, Monterrey, Nuevo León, CP 64460, Mexico

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**Abstract.** The aim of the present study was to investigate whether genetic markers considered risk factors for metabolic syndromes, including dyslipidemia, obesity and type 2 diabetes mellitus (T2DM), can be applied to a Northeastern Mexican population. A total of 37 families were analyzed for 63 single nucleotide polymorphisms (SNPs), and the age, body mass index (BMI), glucose tolerance values and blood lipid levels, including those of cholesterol, low-density lipoprotein (LDL), very LDL (VLDL), high-density lipoprotein (HDL) and triglycerides were evaluated. Three genetic markers previously associated with metabolic syndromes were identified in the sample population, including *KCNJ11, TCF7L2* and *HNF4A*. The *KCNJ11* SNP rs5210 was associated with T2DM, the *TCF7L2* SNP rs11196175 was associated with BMI and cholesterol and LDL levels, the *TCF7L2* SNP rs12255372 was associated with BMI and HDL, VLDL and triglyceride levels, and the *HNF4A* SNP rs1885088 was associated with LDL levels (P<0.05).

*Correspondence to:* Dr Jesus Zacarías Villarreal-Perez, Department of Endocrinology, University Hospital 'José Eleuterio González', Autonomous University of Nuevo León, Francisco I, Madero Avenue and José Eleuterio González Avenue, Colonia Mitras Centro, Monterrey, Nuevo León, CP 64460, Mexico
E-mail: zacvilla@yahoo.com.mx

Dr Hugo Leonid Gallardo-Blanco, Department of Genetics, School of Medicine, Autonomous University of Nuevo León, Francisco I, Madero Avenue and José Eleuterio González Avenue, Colonia Mitras Centro, Monterrey, Nuevo León, CP 64460, Mexico
E-mail: hugo.gallardobl@uanl.edu.mx

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## Introduction

Previous studies employing family-based association tests (FBAT) have identified numerous genes that may have a role in diabetes and obesity (1-3). In addition, more than 330 genes, 161 candidate regions and 103,077 single nucleotide polymorphisms (SNPs) have been associated with type 2 diabetes mellitus (T2DM) in European, African-American, Asian and Latino population (4). However, only ~40 candidate genes have been validated (5).

Detailed studies of population structure with geographical data are required to assess the frequency and the prevalence of genetic diseases in populations of European and Amerindian descent as these are genetically diverse; these and Mexican native populations are ethnically diverse across Mexico (6). The ancestry informative markers (AIMs) may be applied to determinate the population structure in the European/Amerindian populations in association studies (7). The use of AIMs in population structure studies reduce population heterogeneity in complex populations, and may reduce the genetic heterogeneity for specific traits, false positives in multifactorial diseases and multifactorial traits. Sixty four AIMs are sufficient to determine the genetic contribution of Amerindian contribution in Mexican American populations (using r2>0.8 as the threshold to define a high correlation) (7).

The present study aimed to determine whether 63 SNPs, including 37 genes and four intergenic regions, that have previously been associated with T2DM, body mass index (BMI) and dyslipidemia (5), could be identified in the Northeastern Mexican population.

## Materials and methods

*Study design*. A total of 37 families (178 individuals) were enrolled in the present study between June 2010 and June 2011. The present study was approved by the Committee for Ethics, Research and Biosecurity at the School of Nursing (Autonomous University of Nuevo León, Monterrey, Mexico; registry no. FAEN-0-449). In order for a family to be included in the present study, at least one parent had to have been diagnosed with T2DM. Conversely, a family was excluded from the present study if a parent had been diagnosed with type 1 diabetes. Written informed consent was obtained.

*Anthropometric and biochemical parameters*. Body composition was assessed by air impedance plethysmography (Bod Pod Gold Standard; Cosmed, Concord, CA, USA). The BMI was calculated according to World Health Organization guidelines (8). In order to assess biochemical parameters, 30-ml venous blood samples were collected following a 12-14 h fasting period. The blood glucose level was determined using the glucose oxidase method, and the total levels of cholesterol (mg/dl), high-density lipoprotein (HDL; mg/dl), low-density lipoprotein (LDL; mg/dl), very LDL (VLDL; mg/dl), glycated hemoglobin (HbA1c; mg/dl) and triglycerides (mg/dl) in serum or plasma, depending on the kit used, were examined. HbA1c and oral glucose tolerance (OGTT) standardized fasting was performed in all undiagnosed parents. T2DM was confirmed using the American Diabetes Association criteria (9), as follows: i) A 2-h oral glucose tolerance test (OGTT120) glucose level ≥200 mg/dl (≥11.1 mmol/l); and/or ii) HbA1c ≥6.5%.

*Nucleic acid extraction*. DNA was extracted from 200 $\mu$l ethylenediaminetetraacetic acid-treated whole blood samples, using the QIAamp® DNA Blood Mini kit and the automated QIAcube system (cat nos. 51106 and 9001292; Qiagen GmbH, Hilden, Germany). Purified DNA was collected at a final volume of 150 $\mu$l and stored at -20˚C prior to analysis.

*SNP selection*. A total of 63 SNPs that have previously been associated with T2DM in other populations, and 61 ancestry informative markers (AIMs) (10) were genotyped. The 63 SNPs associated with T2DM were present in the following genes: *ADAMTS9, CAPN10, CD36, CDKAL1, ENPP1, EPHX2, FABP2, FTO, HHEX, HNF1B, HNF4A, IGF2BP2, JAZF1, KCNJ11, KCNQ1, LEPR, MAPK1, MAPK14, MTHFR, NEUROD1, SCAF4, NOTCH2, PCK1, PON1, PPARG, RCAN1, RPTOR, SLC2A2, SLC30A8, TCF7L2, THADA, TNF, UCP3, USF1, VLDR, WNT5B* and *WSF1*. In addition, certain SNPs were present in the *CDKN2A-CDKN2B*, *CDC123-CAMK1D*, *FAT3-MTNR1B* and *TSPAN8-LGR5* intergenic regions.

*Genotype analyses*. Molecular analyses were performed using TaqMan® Assays (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA), and analyzed using an OpenArray® NT Genotyping System (Applied Biosystems; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocols. Briefly, DNA was diluted to a concentration of 50 ng/$\mu$l, mixed with Master mix (cat no. 4404846; Applied Biosystems; Thermo Fisher Scientific, Inc.) in a 384-well plate and transferred to the TaqMan OpenArray plate using an autoloader (250 copies/33 nl of the human haploid genome for each through-hole reaction). In addition, a non-template control (NTC) consisting of DNA-free and DNase-free double-distilled $H_2O$ was added to the plate. The plate was filled with immersion fluid, and sealed with glue. The multiplex TaqMan assay reactions were conducted in a Dual Flat Block GeneAmp PCR System 9700 (Applied Biosystems; Thermo Fisher Scientific, Inc.), under the following cycling conditions: Initiation at 93˚C for 10 min, followed by 50 cycles of 95˚C for 45 sec, 94˚C for 13 sec and 53˚C for 14 sec. This was followed by termination at 25˚C for 2 min, and storage at 4˚C. The plate was designed to analyze 32 TaqMan assays for each sample. Allele analysis was performed using the TaqMan® Genotyper software, version 1.0 (Applied Biosystems, Thermo Fisher Scientific, Inc.) and using default parameters, according to the manufacturer's protocol. The accuracy of the genotyping was assessed by comparison with concordance calls generated for 15 samples genotyped three times.

*Statistical methods*. Prior to conducting FBAT genetic analyses, genotype statistics by marker and sample were performed. Families with only one parent, Mendelian errors (non-paternity, non-maternity) and inbreeding were excluded, as were samples with a call rate (SNPs per sample/the total number of SNPs in the dataset) of <87%, and/or a Hardy-Weinberg equilibrium of P<0.01. FBAT was conducted using the PBAT package (http://www.hsph.harvard.edu/fbat/pbat.htm) with the FBAT-PC test statistic parameter in the SNP & Variation Suite (SVS) version 8 (goldenhelix.com/products/SNP_Variation/index.html), which includes an FBAT extension (FBAT-PC) for longitudinal phenotypes, repeated measurements and correlated phenotypes (11). This method was applied to maximize the genetic component of the overall phenotypes and to minimize the phenotypic/environmental variance. The threshold for genome-wide significance was set at $P<4x10^{-4}$, which considers a significance level of 0.05 and 124 SNPs. The analyses were tested under additive, dominant, recessive and heterozygous advantage models, with a maximum pedigree size of 14, and no linkage or association as the null hypothesis. A probability-probability plot, linkage disequilibrium (LD) plots and box-and-whisker plots were generated using the SVS, version 8. A Composite Haplotype Method test (CHM) was applied to calculate the linkage disequilibrium, using SVS.

## Results

*Clinical and biochemical data*. Among 173 patients (following 5 exclusions), 94 (54.3%) were T2DM patients, 103 (59.5%) were women and 133 (77%) had a BMI ≥25 kg/m$^2$. Regarding lipid levels, 113 subjects (65.3%) had LDL levels ≥100 mg/dl and 94 (54.3%) had triglyceride levels ≥150 mg/dl. For further clinical and biochemical data see Table I.

*Genotype analyses*. A total of 61 AIMs and 63 candidate SNPs that had been previously associated with T2DM in other populations were eligible for statistical analysis; acceptable quality control values and a minor allelic frequency of >0.01 was used, in accordance with previous studies (12). The *SFRS15*-rs2833483 SNP was not in Hardy-Weinberg equilibrium (P<0.01) and thus was excluded from the present study.

Table I. Clinical and biochemical characteristics of subjects, reported as mean ± standard deviation.

| Parameter | Females with T2DM | Females without T2DM | Males with T2DM | Males without T2DM |
|---|---|---|---|---|
| n (%) | 58 (33.53%) | 45 (26.01%) | 36 (20.81%) | 34 (19.65%) |
| Age (years) | 46.53±17.13 (n=58) | 43.64±16.43 (n=45) | 53.92±16.71 (n=35) | 47.29±20.25 (n=34) |
| OGTT120 (mg/dl) | 175.70±90.35 (n=11) | 132.68±34.26 (n=32) | 208.85±87.05 (n=12) | 125.13±37.70 (n=30) |
| $HbA_{1c}$ (%) | 8.81±2.52 (n=58) | 5.78±0.57 (n=43) | 8.13±1.91 (n=36) | 5.78±0.40 (n=34) |
| BMI ($kg/m^2$) | 29.93±5.27 (n=57) | 29.92±5.52 (n=44) | 27.56±4.18 (n=35) | 28.53±6.04 (n=34) |
| Cholesterol (mg/dl) | 211.30±48.24 (n=57) | 193.52±42.01 (n=44) | 196.36±45.13 (n=36) | 191.15±47.98 (n=33) |
| TG (mg/dl) | 212.52±146.59 (n=56) | 161.98±77.56 (n=44) | 279.56±235.88 (n=34) | 185.8±118.60 (n=33) |
| LDL (mg/dl) | 125.67±39.21 (n=55) | 120.43±32.30 (n=44) | 107.59±41.16 (n=32) | 108.70±42.09 (n=33) |
| VLDL (mg/dl) | 39.74±21.11 (n=55) | 32.39±15.51 (n=44) | 47.62±33.62 (n=32) | 37.18±23.71 (n=33) |
| HDL (mg/dl) | 43. 60±13.92 (n=55) | 45.41±11.05 (n=44) | 41.23±14.95 (n=32) | 45.27±16.22 (n=33) |

T2DM, type 2 diabetes mellitus; OGTT120, oral glucose tolerance test at 120 min; $HbA_{1c}$, glycated hemoglobin; BMI, body mass index; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.

Table II. Association between *KCNJ11*, *HNF4A* and *TCFL2* variants and aspects of the metabolic syndrome.

| Gene | SNP | R/NR | Allele | Freq[a] | HW[b] | Freq[c] | HW[d] | Model[e] | Associated trait | NIF[f] | P-value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *KCNJ11* | rs5210 | NR | A | 0.387 | 0.520 | 0.352 | 0.746 | 0 | T2DM | 23 | $4.0x10^{-4}$ |
| *KCNJ11* | rs5210 | R | G | 0.613 | 0.520 | 0.648 | 0.746 | 0 | T2DM | 23 | $9.6x10^{-5}$ |
| *HNF4A* | rs1885088 | NR | A | 0.192 | 0.675 | 0.213 | 0.089 | 1 | LDL | 13 | $2.8x10^{-4}$ |
| *HNF4A* | rs1885088 | R | G | 0.808 | 0.675 | 0.787 | 0.089 | 2 | LDL | 13 | $2.8x10^{-4}$ |
| *TCF7L2* | rs11196175 | NR | C | 0.090 | 0.816 | 0.098 | 0.394 | 1 | BMI, cholesterol | 10 | $1.3x10^{-4}$ |
| *TCF7L2* | rs11196175 | R | T | 0.910 | 0.816 | 0.902 | 0.394 | 2 | BMI, cholesterol | 10 | $1.3x10^{-4}$ |
| *TCF7L2* | rs11196175 | NR | C | 0.090 | 0.816 | 0.098 | 0.394 | 3 | BMI, LDL | 10 | $2.7x10^{-4}$ |
| *TCF7L2* | rs11196175 | R | T | 0.910 | 0.816 | 0.902 | 0.394 | 3 | BMI, LDL | 10 | $2.7x10^{-4}$ |
| *TCF7L2* | rs12255372 | NR | G | 0.890 | 0.216 | 0.877 | 0.274 | 0 | BMI, TG, HDL, LDL, VLDL | 14 | $1.9x10^{-3}$ |
| *TCF7L2* | rs12255372 | R | T | 0.110 | 0.216 | 0.123 | 0.274 | 0 | BMI, TG, HDL, LDL, VLDL | 14 | $2.2x10^{-4}$ |

[a]Allelic frequency of families. [b]Hardy-Weinberg equilibrium of families. [c]Allelic frequency of parents. [d]Hardy-Weinberg Equilibrium of parents; [e]0, additive; 1, Dominant; 2, Recessive; 3, Heterozygous advantage. [f]Number of informative families. SNP, single nucleotide polymorphism; R/NR, risk/non-risk; T2DM, type 2 diabetes mellitus; LDL, low-density lipoprotein; BMI, body mass index; HDL, high-density lipoprotein; TG, triglycerides.

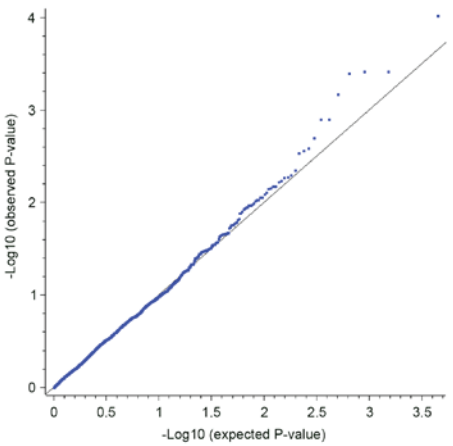


Figure 1. Expected vs. observed P-values in a P-P plot.

No significant inflation was detected between the observed and expected P-values (Fig. 1). The *HNF4A*-rs1885088 SNP was associated with LDL plasma level ($P=2.8x10^{-4}$), with G as the risk allele in the dominant genetic model (Table II and Fig. 2). The rs5210 (Glu23Lys) variant of the *KCNJ11* gene was associated with T2DM ($P=9.6x10^{-5}$), with G as the risk allele (with OGTT120 as adjusted predictor variable) in the additive genetic model (Table II and Fig. 2). Two *KCNJ11* SNPs were in LD when using the Composite Haplotype Method (CHM): rs5210 and rs5219 ($r^2=0.348697$ and D'=1); rs5210 and rs5218 ($r^2=0.461$ and D'=1) (Fig. 3).

Among the five *TCF7L2* SNPs analyzed, two (rs11196175 and rs12255372) were associated with biochemical or clinical markers of metabolic syndrome ($P<2.8x10^{-4}$). In particular, rs11196175 was associated with BMI and blood cholesterol

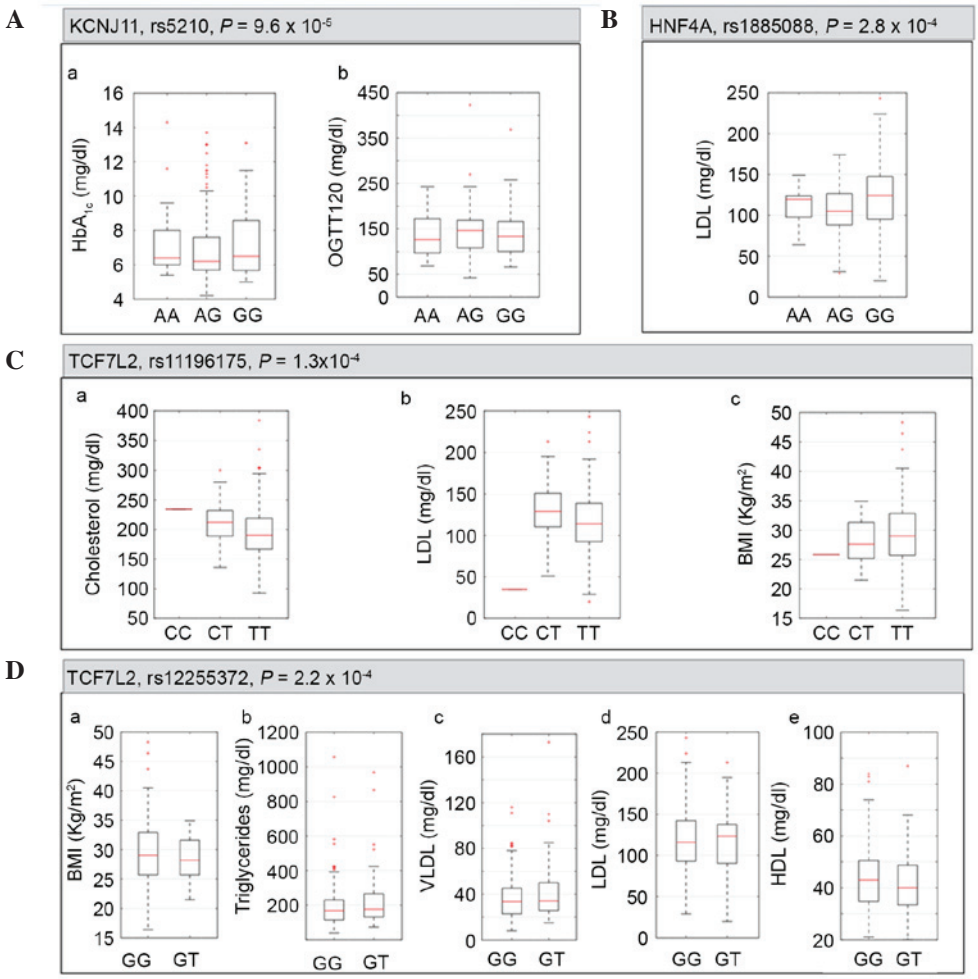


Figure 2. Effects of *KCNJ11*, *HNF4A* and *TCF7L2* genotypes on quantitative traits. (A) *KCNJ11* rs5210 SNP for (a) HbA1c and (b) OGTT120. (B) *HNF4A* rs1885088 SNP LDL levels. (C) *TCF7L2* rs11196175 SNP on (a) cholesterol, (b) LDL and (c) BMI. (D) *TCF7L2* rs12255372 SNP on (a) BMI, (b) triglycerides, (c) VLDL, (d) LDL and (e) HDL. SNP, single nucleotide polymorphisms; LDL, low-density lipoprotein; BMI, body mass index; VLDL, very low density lipoprotein; HDL, high-density lipoprotein.

levels in the dominant and recessive genetic model, whereas it was associated with BMI and LDL in the heterozygous advantage genetic model. In both cases, the recessive T allele was associated with the increased risk (Table II and Fig. 2). Furthermore, rs12255372 was associated with BMI and HDL, LDL, VLDL and triglyceride levels in the additive genetic model, with the T allele posing the risk (Table II and Fig. 2). LD was detected for the following *TCF7L2* SNPs: rs7903146 and rs12255372 (CHM, $r^2$=0.394019, D'= 0.978698); rs11196175 and rs12255372 (CHM, $r^2$=0.5720679, D'=0.8342954); rs11196175 and rs7903146 (CHM, $r^2$=0.2004928, D'=0.7584948) (Fig. 4).

## Discussion

The present study demonstrated that the G allele in the *HNF4A* rs1885088 SNP was associated with a risk for increased circulating LDL levels. Similarly, the *HNF4A* rs1800961 SNP has previously been associated with altered HDL levels (13). A previous study demonstrated that a reduction in the activity of the hepatocyte nuclear factors (HNFs)-4α and -1α led to an increased level of hepatic LDC receptors and, concordantly, lower levels of circulating LDL (14).

Numerous SNPs of the *HNF4A* gene have previously been associated with T2DM in various populations; the rs6017317 SNP, which is located in the *FITM2-R3HDML-HNF4A* region, in East Asians (15), the rs1884613 SNP in Ashkenazian Jews (16), and the rs6031558, rs2071197 and rs3212183 SNPs, although not rs1885088, in Pima Indians (17). Furthermore, rs1885088 was associated with T2DM in a dominant model; however, this association did not remain significant following a genome-wide association study using the summary association statistics from the Diabetes Genetics Initiative and Wellcome Trust Case-Control Consortium studies. Differences in the clinical characteristics of the case-control populations and ancestral genetic background may have accounted for these results (18).

Notably, sulfonylurea sensitivity has been described as a feature of *HNF1A*- and *HNF4A*-associated maturity-onset diabetes in the young (19). The sulfonyl-urea receptor-1 subunit of the pancreatic β-cell ATP-sensitive potassium (KATP) channel is encoded by the *ABCC8* gene, which is located 4,200 bp upstream of the *KCNJ11* gene. These findings suggested that the functions of *HNF4A* and *HNF1A* in T2DM may be associated with obesity and lipid metabolism. In the present study, 77% of participants had a BMI of ≥25 kg/m$^2$,

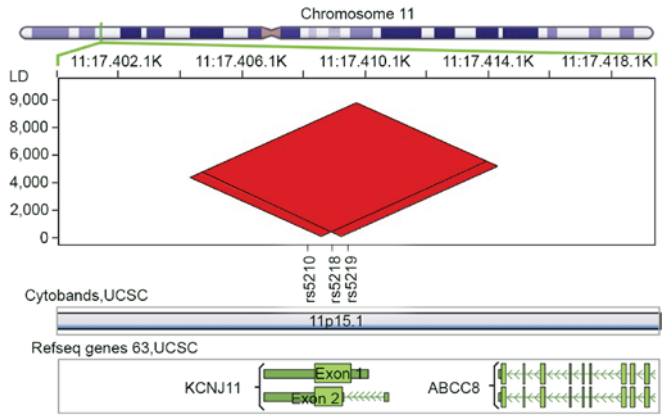


Figure 3. Linkage disequilibrium and block structure for the *KCNJ11* single nucleotide polymorphisms rs5210, rs5218, and rs5219.

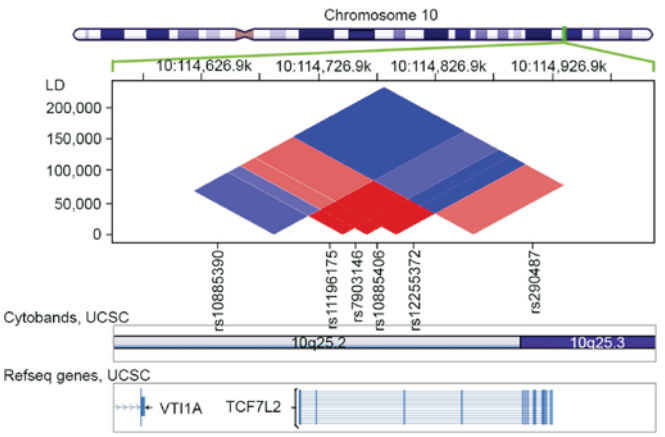


Figure 4. Linkage disequilibrium and block structure for the *TCF7L2* single nucleotide polymorphisms rs10885390, rs11196175, rs7903146, rs10885406, rs12255372 and rs290487.

and 54.3% suffered from T2DM (Table I). In addition, it was demonstrated that the *HNF4A* locus was directly correlated with LDL levels, but not with T2DM.

In the Northeastern Mexican population analyzed in the present study, the *KCNJ11* rs5210 SNP [minor allele frequency (MAF)=0.373] was associated with T2DM; however, the *KCNJ11* SNPs that have previously been associated with T2DM in European populations (rs5218 and rs5219), were not associated with T2DM in the present study. In the presently analyzed population, rs5218 had a MAF of 0.209 and rs5219 had a MAF of 0.329. Similarly, two previous studies analyzing the Mestizo population of Mexico City were unable to identify an association between rs5219 and T2DM (20,21). In particular, the frequency of the risk allele was shown to be too low to reach the power in order to detect an association (20). Furthermore, a previous study analyzed 9.2 million SNPs in Mexican (n=8,214; Mexico City) and Latin American (n=3,848) patients with T2DM and in 4,366 non-diabetic control (22). These studies detected an association between T2DM and *TCF7L2*, *KCNQ1* and *SLC16A11* loci, but did not identify an association between T2DM and rs5219 (genome-wide significance, $P<1x10^{-8}$). A systematic meta-analysis of the effect of the *KCNJ11* rs5219 SNP (23) in 48 published studies (T2DM cases, 56,349; controls, 81,800; family trios, 483) reported that rs5216 was significantly associated with an increased risk of T2DM ($P<10^{-5}$) when using the heterozygous and homozygous model (20). The low frequency of *KCNJ11* risk alleles in case-control studies may explain the inability to associate them with T2DM.

*TCF7L2* (24), *HNF4A* (25) and *KCNJ11* (26,27) have been associated with sulfonylurea sensitivity in previous studies, and the present study demonstrated an association with levels of LDL (*HNF4A*) and T2DM (*KCNJ11*).

The *TCF7L2* gene has previously been associated with T2DM, insulin sensitivity and resistance (28). In addition, the rs7903146 SNP has been reported to be a risk factor of non-alcoholic fatty liver disease and of various metabolic disorders involving glucose and lipoprotein homeostasis (29,30). In the Northeastern Mexican population, the CC and CT genotypes of the rs11196175 (*TCF7L2*) SNP were associated with a lower BMI and elevated levels of cholesterol and LDL, as compared with the TT genotype. The rs11196175 SNP has previously been associated with a variety of diseases/phenomena, including cancer (31) and metabolic syndrome in women with polycystic ovary syndrome (European Caucasian ancestry); however, this finding did not remain statistically significant following correction for multiple testing, and this was likely due to sample size (32), smoking (33) and adaptation to climate (34).

In the present study, the *TCF7L2* rs12255372 GG genotype was associated with a higher BMI and HDL levels, and lower levels of VLDL and LDL, as compared with the GT genotype. However, the *TCF7L2* rs12255372 SNP has been extensively studied and has previously been associated with T2DM (35) in numerous populations, including Iranian (36), Indian (37), Japanese (38), Chinese (39) and South Asian (40) populations, whereas it has been associated with BMI in Pima Indians (41) and the Mexico City population (with ADMIXMAP adjustment) (42). Furthermore, this SNP has been associated with the expression of proinsulin in pancreatic islets when applying the additive genetic model (43). In addition, it has been associated with gestational diabetes mellitus and it was shown, in additive and dominant models, to interact with adiposity to alter insulin secretion in Mexican Americans (44). Applying the same genetic model, the present study demonstrated an association with dyslipidemia, although not with T2DM. These results suggested that ethnic background, lipid metabolism, obesity and T2DM may be interlinked; however, the consensus is an T2DM association (39).

It has previously been reported that the Mexican population is genetically diverse (6); therefore, a more detailed study of population structure alongside geographical data is required in order to assess the frequency and the prevalence of genetic diseases in native and Mestizo Mexican populations (6). The Mexican population is an interesting model for genetic studies due to the great ethnic diversity within native and Mestizo populations. A previous study reported significant differences according to geographic region in Mexico (6), and this significant genetic variation highlights the need for a thorough analysis of Mexican populations. Once this information is collected, it may be used as a reference for Mexican genetic studies.

In conclusion, the present study identified SNPs associated with T2DM, BMI and dyslipidemia, in 39 families from

Northeastern Mexico. In particular, FBAT analyses (without population stratification) identified an association between the *KCNJ11, TCF7L2* and *HNF4A* genes and T2DM, dyslipidemia and obesity. These associations between *HNF4A* and *TCF7L2* and lipid homeostasis and obesity, and between *KCNJ11* and T2DM, form a complex model in which insulin resistance/sensitivity is a common factor. Due to the significant genetic diversity of the Mexican population, case-control studies enrolling >2,000 subjects are required in order to confirm the associations identified in the present study.

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