Candidate gene association study conditioning on individual ancestry in patients with type 2 diabetes and metabolic syndrome from Mexico City


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Abstract

Background Type 2 diabetes (T2D) is influenced by diverse environmental and genetic risk factors. Metabolic syndrome (MS) increases the risk of cardiovascular disease and diabetes. We analysed 14 cases of polymorphisms located in 10 candidate loci, in a sample of patients with T2D and controls from Mexico City.

Methods We analysed the association of 14 polymorphisms located within 10 genes (TCF7L2, ENPP1, ADRB3, KCNJ11, LEPR, PPARγ, FTO, CDKAL1, SIRT1 and HHEX) with T2D and MS. The analysis included 519 subjects with T2D defined according to the ADA criteria, 389 with MS defined according to the AHA/NHLBI criteria and 547 controls. Association was tested with the program ADMIXMAP including individual ancestry, age, sex, education and in some cases body mass index (BMI), in a logistic regression model.

Results The two markers located within the TCF7L2 gene showed strong associations with T2D (rs7903146, T allele, odd ratio (OR) = 1.76, p = 0.001 and rs12255372, T allele, OR = 1.78, p = 0.002), but did not show significant association with MS. The non-synonymous rs4994 polymorphism of the ADRB3 gene was associated with T2D (Trp allele, OR = 0.62, p = 0.001) and MS (Trp allele, OR = 0.74, p = 0.018). Nominally significant associations were also observed between T2D and the SIRT1 rs3758391 SNP and MS and the HHEX rs5015480 polymorphism.

Conclusions Variants located within the gene TCF7L2 are strongly associated with T2D but not with MS, providing support to previous evidence indicating that polymorphisms at the TCF7L2 gene increase T2D risk. In contrast, the non-synonymous ADRB3 rs4994 polymorphism is associated with T2D and MS. Copyright © 2010 John Wiley & Sons, Ltd.

Keywords candidate genes; ancestry informative markers; type 2 diabetes; metabolic syndrome; Mexican population

Introduction

Type 2 diabetes (T2D) is a complex multifactorial and polygenic metabolic disorder and its pathogenesis is influenced by diverse environmental and genetic risk factors. T2D is characterized by hyperglycaemia, with variable degrees of insulin resistance, impaired insulin secretion and increased hepatic glucose production [1,2]. The worldwide prevalence of T2D is increasing rapidly and is predicted to increase to 225 million by the end of the decade.
and 300 million in the year 2025 [3]. This dramatic increase coincides with a higher prevalence of obesity and reduced levels of physical activity. In Mexico, the prevalence of diabetes has increased dramatically, and it has been estimated that 10% of adults have the disease [4,5]. T2D and its complications are the first cause of death in Mexican women, and the second cause in men [5].

Changes in lifestyle have been associated, not only with T2D, but also with several metabolic disorders, including the metabolic syndrome (MS). MS is a combination of metabolic disorders that increase the risk of cardiovascular disease and diabetes. Several criteria have been proposed to define MS, such as those proposed by the OMS, ATP III, AHA/NHLBI and IDF. All of them share some categorical cutpoints such as fasting glucose $\geq 5.5$ mmol/L ($\geq 6.1$ mmol/L for ATP III), blood pressure $\geq 130/85$ mmHg, HDL-C $< 1.04$ mmol/L in men and $< 1.3$ mmol/L in women, triglycerides $\geq 1.7$ mmol/L, but they may include other particular parameters [6–10]. In Mexico, MS and impaired glucose tolerance identify nearly 70% of subjects with high T2D risk [11]. The prevalence of MS in Mexico is 26.6% or 21.4% if those with diabetes are excluded.

There have been important advances in our understanding of the genetics of T2D. These advances have been driven by the application of genome-wide association studies. Currently, at least 16 candidate loci have been unequivocally associated with T2D using this approach: FTO, CDKAL1, SLC30A8, CDKN2A/CDKN2B, HHEX, TCF7L2, EXT2, KCNJ11, JAZF1, CDC123-CAMK1D, TSPAN8-LGR5, THADA, ADAMTS9, NOTCH2-ADAM30 and KCNQ1 [12–22]. Additionally, other genes have been associated with T2D or MS traits, including the genes ENPP1 [23–25], FTO [26], ADRB3 [27], LEPR [28–31] and SIRT1 [32,33]. It is important to note that most of these loci have been identified and studied in populations of European ancestry, and the number of studies in non-European populations has been much more limited. It is therefore critical to expand this type of studies to other populations, in order to understand the population distribution of allele frequencies and to replicate the results observed in previous reports. In this sense, some published works have shown that there is substantial allele frequency differences between populations for some of the polymorphisms associated with T2D. The variants within the gene KCNQ1 are an excellent example. These variants were recently identified in two Japanese studies using relatively small sample sizes [21,22]. Interestingly, this gene was not identified in any of the larger GWA studies in European populations, because there are large frequency differences between East Asian and European populations for these variants, and the low levels of polymorphisms observed for these markers in Europe resulted in a dramatic reduction in statistical power [34]. Similarly, there are relatively large frequency differences between population groups for the rs7903146 polymorphism within the TCF7L2 gene [35,36].

In this study, we analysed 14 cases of polymorphisms located in 10 different loci (TCF7L2, ENPP1, ADRB3, KCNJ11, LEPR, PPARY, FTO, CDKAL1, SIRT1 and HHEX) in a sample of patients with T2D and controls from Mexico City. We also compared the distribution of allele frequencies in the case and control sample with those observed in a sample of subjects with MS collected in the same City.

Materials and methods

Study participants and phenotype definition

In a case–control design using Mexico City’s inhabitants, 519 subjects with T2D (defined according to the ADA criteria [37] and 389 with MS, defined according to the criteria of the AHA/NHLBI (American Heart Association/National Heart, Lung and Blood Institute Scientific Statement) [8], were compared to 547 controls aged 35–65 years. The AHA/NHLBI criteria are waist circumference $\geq 102$ cm in men and $\geq 88$ cm in women; fasting glucose $\geq 5.5$ mg/dL; systolic and diastolic blood pressure $\geq 130$ mmHg and $\geq 85$ mmHg, respectively; HDL-C $< 1.04$ mmol/L in men and $< 1.3$ mmol/L in women, and triglycerides $\geq 1.7$ mmol/L. Diabetes cases were selected from several Family Medical Units of the Social Security Mexican Institute (IMSS), located in the southern part of Mexico City. They all had diagnosis of and treatment for T2D, and they were invited to participate in this research. Donors of the National Medical Center Blood Bank that covers all medical units in the southern part of Mexico City were invited to participate in the study, and criteria was the absence of family history of diabetes among parents, brothers, sisters and/or siblings. All donors were assessed for the MS, and those who satisfied the criteria were classified as so. Among those who had no traits of the MS, were selected as controls for the study, under the assumption that they provided from the same population source from where patients with diabetes or the MS had been selected. Written consent was obtained from the participants and the protocol was approved by the National Ethical Committee of the IMSS.

Biochemical profile analyses

The biochemical profile in blood drawn before any medication included fasting glucose (mmol/L); insulin (pmol/L); insulin sensitivity (HOMA-IR); total cholesterol (mmol/L); LDL (mmol/L); HDL (mmol/L), and triglycerides (mmol/L). These parameters were determined using the ILab 350 Clinical Chemistry System (Instrumentation Laboratory, Barcelona Spain). Anthropometric measurements included weight (kg); height (cm); waist circumference; waist to hip ratio and body mass index (BMI in kg/m²) using the Body Composition Analyzer.
Genotyping

DNA was extracted from a peripheral blood sample using a QIAamp (Qiagen, Germany) kit, and analysed by electrophoresis in 0.8% agarose gels stained with ethidium bromide and visualized in a Gel Doc 2000 (BIORAD CA, USA). DNA concentration was determined using a VICTOR3 1420 spectrophotometer (Perkin–Elmer, Germany). The SNP analyses were made using real time PCR using the TaqMan method (7900HT Applied Biosystems, Foster City, CA, USA), following standard protocols. For each SNP in the groups in study, a concordance of 100% was observed when 30–50% of the samples were genotyped in duplicate.

The candidate genes (and the SNPs genotyped in each candidate gene) were selected based on previous reports of association with T2D or T2D-related phenotypes. The genes and SNPs analysed were TCF7L2 (rs7903146 and rs12255372), ENPP1 (rs1044498), ADRB3 (rs4994), KCNJ11 (rs5210 and rs5215), LEPR (rs1137100), PPARγ (rs17793693), FTO (rs9993609), CDKAL1 (rs7754840, rs946587 and rs10946398), SIRT1 (rs3758391) and HHEX (rs5015480). In order to control for the potential effect of population stratification (e.g. variation of individual admixture proportions in the samples), we also genotyped 50 ancestry informative markers (AIMs). Sixteen of the 50 AIMs (rs2814778, rs6003, rs2752, rs3287, rs17203, rs3309, rs3340, rs2763, rs2695, rs594689, rs1042602, rs1900404, rs2862, rs4646, rs2816 and rs16383) were described previously by Bonilla et al. [38,39]. Parental frequencies for these AIMs are available for several Native American (Nahua and Maya from Mexico, Native Americans from the Southwestern US), European (Germany, Spain), and West African (Nigeria, Sierra Leone, Central African Republic) population samples. Detailed information regarding these markers can be accessed at dbSNP using PSUANTH as the submitter handle. Thirty-three additional markers (rs723822, rs1506069, rs1435090, rs1344870, rs768324, rs1465648, rs719776, rs1112828, rs1403454, rs2077681, rs1935946, rs2396676, rs2341823, rs1320892, rs983271, rs1373302, rs1908088, rs1327805, rs1594335, rs2207782, rs1891760, rs1487214, rs726391, rs717091, rs2078588, rs724729, rs766479, rs292932, rs1074075, rs1369290, rs386569, rs718092, rs878825) were identified as excellent AIMs in a study using the Affymetrix GeneChip Mapping 10K array [40]. In this study, several relevant parental populations were genotyped, including Nahua from Mexico, Spanish from Valencia and Mende from Sierra Leone. The usefulness of these AIMs was further confirmed for all but two markers (rs2078588 and rs292932) by analysing independent Nahua, Spanish and West African samples. Finally, one marker was selected based on the information previously available in the literature (rs1008984). This panel of 50 AIMs is highly informative: the average allele frequency difference between European and Native American populations is 44%, the average allele frequency between European and West African populations is 42%, and the average allele frequency between Native American and West African populations is 51%. In the sample of subjects with MS, due to limited availability of DNA, we genotyped 27 AIMs (rs2814778, rs723822, rs1008984, rs1435090, rs17203, rs768324, rs1935946, rs1112828, rs1403454, rs3340, rs2077681, rs1935946, rs1320892, rs1373302, rs2695, rs1908088, rs1327805, rs2207782, rs1487214, rs2078588, rs724729, rs292932, rs1369290, rs386569, rs718092, rs878825 and rs16383). In previous studies, we have shown that 25–50 highly informative markers successfully control for confounding due to stratification in admixed populations [41]. Information on the parental frequencies for all the AIMs is available in a previous report from our group [42]. The AIMs were genotyped using a modified allele-specific PCR method with universal energy transfer-labelled primers by the company Prevention Genetics (Marshfield, WI, USA).

Statistical analysis

Deviations of genotype proportions from Hardy–Weinberg expectations were evaluated using an exact test available at the Institut fur Humangenetik Website (http://bih2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl, September 2009 accessed). The extent of linkage disequilibrium (LD) between genetic markers was estimated using an expectation maximization algorithm implemented in the program EMLD (available at http://cge.manderson.org/~quang/Software/pub.htm, September 2009 accessed). To test for association of the markers located within the T2D candidate genes with the traits under study, we used the program ADMIXMAP. This is a general purpose program for modelling population admixture with genotype and phenotype data, based on a combination of bayesian and classical methods. For this analysis, the Mexican Mestizo population was modelled as formed by admixture between three subpopulations: European, Native American and West African. The program fits a hierarchical model for the distribution of admixture proportions in the population, the admixture proportions of each parental gamete, and the ancestry of the gene copies at each locus. The variation between three states of ancestry on chromosomes of mixed descent is modelled as the outcome of three independent Poisson arrival processes. This requires only one extra parameter – the sum of the intensities of the arrival processes – to be specified in the model. Allele and haplotype frequencies are estimated by combining information from unadmixed and admixed population samples (using the posterior distribution of allele frequencies obtained
from data on unadmixed individuals as a prior distribution for the corresponding ancestry specific allele frequencies in the admixed population. A generalized linear model is specified for the relation of the dependent variable to individual admixture and other covariates such as age, sex and socioeconomic variables. For categorical variables such as T2D, this is a logistic regression model. The model is specified as a bayesian full probability model, in which all unobserved variables – such as haplotypes, ancestry states at each locus, gamete admixture proportions and population level parameters – are ‘missing data’. Noninformative prior distributions are specified for the distribution of admixture proportions in the population, and for the parameters of the regression model. The posterior distribution of the missing data, given the observed data, is then generated by Markov chain Monte Carlo simulation.

Inference about the parameters of the regression model is based on the posterior distribution. In large samples, the posterior means and 95% central posterior intervals (‘95% credible intervals’) are asymptotically equivalent to maximum likelihood estimates and 95% confidence intervals (95% CI). Score tests for allelic association with the trait, conditional on individual admixture and any other covariates, are constructed as described previously [41]. The parameter tested is the coefficient $b$ for the effect of the allele under study (coded as 0, 1 or 2 copies) in a regression model that includes admixture and other covariates such as age and sex. For each SNP, a positive score value indicates association of the trait with the allele being tested. To test the null hypothesis that $b = 0$, the score (gradient of the log-likelihood) and the observed information (curvature of the log-likelihood) at $b = 0$ are calculated by averaging over the posterior distribution of the missing data (the haplotypes and individual admixture values). The score test correctly allows for uncertainty about haplotype assignments and estimation of individual admixture proportions, because it is based on the likelihood of the observed data as a function of the parameter ($b$) that is being tested. ADMIXMAP has been described in detail [41] and is freely available at http://homepages.ed.ac.uk/pmckeigu/admixmap/index.html (September 2009 accessed).

**Estimation of false discovery probabilities**

To examine the likelihood that our results were false-positive findings, we employed two Bayesian methods to assess whether the associations observed in our study are ‘noteworthy’. Both methods require defining a priori probabilities of association and thresholds for noteworthiness. The Bayesian False Discovery Probabilities were estimated using the method described by Wakefield [43]. The BFDP cut-off for a noteworthy value was set as 0.75 (cost of a false non-discovery is three times as great as the cost of a false discovery) and the upper OR as 2. Given that we analysed genes with prior evidence of association with T2D in numerous studies, we set the prior probability of an association between each SNP and T2D at 0.1. Additionally, we also applied the False Positive Discovery Probability, using a cut-off of 0.5 for a noteworthy value and three different ORs: 1.3, 1.5 and 1.7 [44].

**Power analysis**

We used the program Quanto (http://hydra.usc.edu/gxe/, September 2009 accessed) to estimate the statistical power of our study using an additive model and a range of allele frequencies and allelic effects. Figure 1 shows the power calculations for the T2D/control sample and the MS/control sample.

**Results**

Table 1 shows the general characteristics of the groups analysed (T2D, MS and controls). Mean age of controls was lower than the mean age of those with MS or with T2D. Patients with T2D were characterized by hyperglycaemia, hyperinsulinemia and insulin resistance, while

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Figure 1. Power analysis using the program Quanto: (A) type 2 diabetes (T2D); (B) metabolic syndrome (MS)
those with MS showed obesity, hypertension, hyperinsulinemia, low HDL levels and hypertriglyceridemia. Patients with T2D had lower education levels than the other two groups. The three groups showed similar ancestry proportions, although the T2D sample showed a slightly higher Native Amerindian component than the other two groups (67% vs. 64%). Figure 2 shows a triangular representation depicting the admixture proportions of the individuals of the sample. There is a wide variation of European and Native American admixture proportions, but a limited West African contribution in the sample. There were no significant departures of the genotype frequencies of the 14 polymorphisms within the candidate genes from Hardy–Weinberg proportions, except for a slight excess for rs17793693 genes from Hardy–Weinberg proportions, except for a

Table 1. Characteristics of diabetic and metabolic syndrome (MS) subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>T2D (519)</th>
<th>MS (389)</th>
<th>Controls (547)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>53.44 ± 7.42*</td>
<td>45.0 ± 7.09*</td>
<td>43.60 ± 6.63</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.25 ± 4.76</td>
<td>30.53 ± 4.16</td>
<td>27.50 ± 3.55</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>118.55 ± 13.82</td>
<td>126.40 ± 12.64</td>
<td>116.27 ± 9.51</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>76.13 ± 8.65</td>
<td>78.20 ± 8.84</td>
<td>73.97 ± 7.23</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>10.15 ± 4.36</td>
<td>5.28 ± 0.65</td>
<td>4.78 ± 0.45</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>14.20 ± 10.04</td>
<td>12.76 ± 7.92</td>
<td>9.41 ± 5.05</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>6.25 ± 4.97*</td>
<td>3.00 ± 2.00</td>
<td>2.42 ± 6.27</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.72 ± 1.66*</td>
<td>5.32 ± 1.03*</td>
<td>5.12 ± 1.02</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>3.59 ± 1.00*</td>
<td>3.25 ± 0.90</td>
<td>3.30 ± 0.88</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.27 ± 0.39*</td>
<td>0.97 ± 0.25*</td>
<td>1.14 ± 0.29</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>2.66 ± 1.90*</td>
<td>2.93 ± 1.69*</td>
<td>1.88 ± 1.05</td>
</tr>
<tr>
<td>Ancestral contribution</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>African</td>
<td>0.026 ± 0.022</td>
<td>0.031 ± 0.025</td>
<td>0.026 ± 0.019</td>
</tr>
<tr>
<td>European</td>
<td>0.30 ± 0.10</td>
<td>0.33 ± 0.10</td>
<td>0.33 ± 0.12</td>
</tr>
<tr>
<td>Amerindian</td>
<td>0.67 ± 0.11</td>
<td>0.64 ± 0.11</td>
<td>0.64 ± 0.13</td>
</tr>
<tr>
<td>Gender (%)</td>
<td>F</td>
<td>71.37*</td>
<td>24.7</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>26.79</td>
<td>63.30*</td>
</tr>
<tr>
<td>Education (%)</td>
<td>Primary or less</td>
<td>57.8</td>
<td>19.53</td>
</tr>
<tr>
<td></td>
<td>Middle school (secondary)</td>
<td>24.28</td>
<td>28.65</td>
</tr>
<tr>
<td></td>
<td>High school (upper school)</td>
<td>7.9</td>
<td>28.39</td>
</tr>
<tr>
<td></td>
<td>University or above</td>
<td>10.02*</td>
<td>23.44</td>
</tr>
</tbody>
</table>

The results appear like average ± standard deviation. F: female, M: male, mean ± SD. *p < 0.05 [controls versus type 2 diabetes (T2D) or MS].

Figure 2. Admixture proportions of the individuals of the sample also the frequencies in three reference samples (HapMap East Asians, Europeans and West Africans). Table 2 also shows the results of the association tests for T2D and MS. The analysis was carried out with the program ADMIXMAP, and we report the ORs and p-values of the logistic regression analysis controlling for age, sex, education and ancestry, and also for age, sex, education, ancestry and BMI. An unrestricted (co-dominant) analysis using the program STATA gives similar results (data not shown). For T2D, when controlling for age, sex, education and ancestry, the strongest effects are observed for the two markers located within the gene TCF7L2 (rs7903146, T allele, OR = 1.76, p = 0.001 and rs12255372, T allele, OR = 1.78, p = 0.002). Another marker showing a strong effect is rs4994, which is located in the ADRB3 gene (Trp allele, OR = 0.62, p = 0.001). Finally, we also observed a nominally significant effect for the SIRT1 marker rs3758391 (T allele, OR = 1.32, p = 0.031). Adding BMI to the model does not substantially change the results and the ORs and p-values are very similar to those observed without BMI in the model (Table 2). The findings for the markers within the genes TCF7L2 and ADRB3 were assessed as noteworthy using both the BFDP and FPDP methods (BFDP values of 0.235 for rs7903146, 0.323 for rs12255372 and 0.168 for rs4994; FPDP values lower than 0.3 for the three markers for each of the ORs considered in the analysis). However, there was less support for the SIRT1 rs3758391 SNP (BFDP higher than the 0.75 cut-off). For MS, we observed nominally significant results for ADRB3 rs4994 (Trp allele, OR = 0.74, p = 0.018) and HHEX rs5015480 (T allele, OR = 0.80, p = 0.038) and the results remained significant after adding BMI (Table 2). The strength of the association for ADRB3 rs4994 was assessed as noteworthy using both the BFDP and FPDP methods (BFDP is 0.663 and FPDP lower than 0.3 for the three markers for each of the ORs considered in the analysis). As for SIRT1 and T2D, there is...
Table 2. Allele frequencies and logistic regression results using the ADMIXMAP program

<table>
<thead>
<tr>
<th>Gene</th>
<th>Allele frequency in our study</th>
<th>Reference allele frequency</th>
<th>Controlling for age, sex, education and ancestry</th>
<th>Controlling for age, sex, education, BMI and ancestry</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T2D</td>
<td>MS</td>
<td>Controls</td>
<td>Eur</td>
</tr>
<tr>
<td>LEPR (A/G)</td>
<td>rs1137100</td>
<td>A</td>
<td>0.669</td>
<td>0.687</td>
</tr>
<tr>
<td>PPARG (A/C)</td>
<td>rs17793693</td>
<td>A</td>
<td>0.357</td>
<td>0.295</td>
</tr>
<tr>
<td>CDKAL1 (A/C)</td>
<td>rs10946398</td>
<td>C</td>
<td>0.317</td>
<td>0.288</td>
</tr>
<tr>
<td>CDKAL1 (C/G)</td>
<td>rs7754840</td>
<td>C</td>
<td>0.316</td>
<td>0.287</td>
</tr>
<tr>
<td>CDKAL1 (C/T)</td>
<td>rs9465871</td>
<td>C</td>
<td>0.255</td>
<td>0.226</td>
</tr>
<tr>
<td>ENPP1 (A/C)</td>
<td>rs1044498</td>
<td>C</td>
<td>0.200</td>
<td>0.189</td>
</tr>
<tr>
<td>ADRB3 (Arg/Trp)</td>
<td>rs49394</td>
<td>Arg</td>
<td>0.257</td>
<td>0.223</td>
</tr>
<tr>
<td>SIRT1 (C/T)</td>
<td>rs3758391</td>
<td>T</td>
<td>0.643</td>
<td>0.580</td>
</tr>
<tr>
<td>HHEX (C/T)</td>
<td>rs5015480</td>
<td>C</td>
<td>0.355</td>
<td>0.415</td>
</tr>
<tr>
<td>TCF7L2 (C/T)</td>
<td>rs7903146</td>
<td>T</td>
<td>0.197</td>
<td>0.153</td>
</tr>
<tr>
<td>TCF7L2 (G/T)</td>
<td>rs12255372</td>
<td>T</td>
<td>0.153</td>
<td>0.130</td>
</tr>
<tr>
<td>KCNJ11 (C/T)</td>
<td>rs5215</td>
<td>T</td>
<td>0.595</td>
<td>0.613</td>
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<tr>
<td>KCNJ11 (A/G)</td>
<td>rs5210</td>
<td>A</td>
<td>0.324</td>
<td>0.352</td>
</tr>
<tr>
<td>FTO (A/T)</td>
<td>rs9939609</td>
<td>A</td>
<td>0.252</td>
<td>0.183</td>
</tr>
</tbody>
</table>

BMI, body mass index.

aData obtained from International HapMap Project.

bPima and Maya from Mexico, T2D: type 2 diabetes.

cData obtained from http://spsmart.cesga.es.
less support for the association observed between HHEX and MS (BFDP higher than the 0.75 cut-off).

Discussion

We carried out a case–control study in Mexico City to test the association between 14 SNPs located within 10 different genes and T2D. We also genotyped a sample of individuals with MS, defined using the AHA/NHLBI criteria. In our sample, the controls are slightly younger than the subjects with MS (43.6 vs. 45) and approximately 10 years younger than the patients with T2D (Table 1). However, it is important to note that none of the controls had a family history of T2D. This was verified using questionnaires and a clinical interview and we confirmed by laboratory tests that none of the controls had MS or undiagnosed T2D. The statistical analysis was carried out with the program ADMIXMAP, controlling for confounding due to population stratification and other important factors, such as education, sex and age (BMI was also included in some models, in order to evaluate if the association of the markers with T2D was mediated through effects on obesity). In previous studies, we have shown that the Mexican Mestizo population has substantial stratification due to variation in admixture proportions [42]. Therefore, in this population it is critical to control for the effect of admixture, particularly for markers, such as TCF7L2, that show substantial differences in frequency between the parental populations [36]. Socioeconomic status also has an important effect on T2D risk, and in Mexico socioeconomic status is also associated with genetic ancestry [42,45]. So we also included education as a proxy for socioeconomic status in our statistical analysis. Given the size of our TD2/control sample, our study has adequate power (>80% power) to identify risk alleles with ORs higher than 1.3 for alleles with frequencies higher than 30%, but the power decreases for low-frequency alleles (for alleles with a frequency of 15%, there is 60% power to identify an effect of 1.3 or higher). The statistical power is slightly lower for the MS/control sample, due to the reduced size of the MS sample (Figure 1). We observed a strong association of two polymorphisms within the gene TCF7L2 and T2D (rs7903146, OR = 1.76, p = 0.001 and rs12255372, OR = 1.78, p = 0.002, Table 2). This result is in agreement with numerous studies in other population groups [46] and also with a previous study by our research group in a smaller Mexican sample [36]. TCF7L2 is the gene showing the strongest effect so far described for T2D, with a meta-analysis indicating a pooled OR of 1.46 for the rs7903146 polymorphism [46]. Interestingly, we did not find an association of the two TCF7L2 polymorphisms with MS (rs7903146, OR = 1.13, p = 0.384 and rs12255372, OR = 1.22, p = 0.214). These results are in general agreement with previous evidence indicating that polymorphisms at the TCF7L2 gene increase T2D risk primarily through effects on insulin secretion, rather than insulin action [47–51].

The non-synonymous rs4994 polymorphism located within the ADRB3 gene has been previously associated with T2D, obesity, insulin resistance and hypertension [52–56]. This gene belongs to the family of beta adrenergic receptors and is involved in the regulation of lipolysis and thermogenesis. We also observed a strong association of rs4994 and T2D in the sample from Mexico City. In agreement with the previous studies, the Arg variant confers increased T2D risk and the Trp allele has a protective effect (Trp allele, OR = 0.62, p = 0.001, Table 2). It is interesting to note that the signal remains significant in the model controlling for BMI, indicating that the association of this polymorphism is not mediated exclusively through its effects on overall obesity. We also observed a significant association of ADRB3 (rs4994) and MS, indicating a potential role of this polymorphism in key components of the MS, such as insulin resistance, dyslipidemia or diastolic blood pressure, in accordance to what has been described in previous studies [53,57,58]. There is evidence indicating that the Arg variant alters beta-3 adrenoreceptor function by decreasing agonist sensitivity [59] but further research is necessary to decipher the mechanisms through which this missense polymorphism has an impact on metabolic traits.

We also observed a significant association of SIRT1 rs3758391 and T2D, although the OR (T allele, OR = 1.32, p = 0.031) is lower than those observed for the TCF7L2 and ADRB3 genes (Table 2). The association remained significant after controlling for BMI, but we found no evidence of association between this marker and MS. The SIRT1 gene plays an important role in glucose and lipid metabolism [32]. One potential explanation for the significant association observed between rs3758391 and T2D, but not MS, is that this polymorphism, which is located in the promoter region of the SIRT1 gene, could alter the function of SIRT1 as a regulator of gluconeogenesis and insulin secretion [32,60–63].

Finally, for the marker rs5015480 located near the HHEX gene, we observed a significant association with MS, but not with T2D (MS, T allele, OR = 0.78, p = 0.032 and T2D, T allele, OR = 0.98, p = 0.869, Table 2). Several markers in the HHEX gene, including rs5015480, have been associated with T2D in several genome-wide association studies [14,15,19] in European populations, and these results have been replicated in other population groups [64,65]. The allele associated with MS in our sample (C allele) is the same allele described in previous studies. There is evidence indicating that the HHEX variants may influence T2D risk primarily through effects on beta cell function [66–70]. However, a recent study also indicates that this gene has a strong effect on insulin sensitivity (e.g. HOMA-IR) [70].

In summary, we report that markers located within the genes TCF7L2 (rs7903146 and rs12255372) and ADRB3 (rs4994) are strongly associated with T2D in a sample from Mexico City. These associations are still significant after using the conservative Bonferroni multiple test correction and are also supported by the BFDP and FPDP analyses. As described above, TCF7L2 is the gene with
The strongest effect so far described for T2D, and it has been associated with T2D in many population groups. However, although the ADRB3 gene has been associated with T2D in previous candidate gene studies, it was not reported as one of the significant genes identified in recent genome-wide association studies carried out in European populations. Studies of this gene in other population groups, including the Mexican population, have been quite limited, so it would be important to characterize this gene in other samples from Mexico and other populations in order to confirm the results of our study. The ADRB3 rs4994 polymorphism is also associated with MS in the Mexico City's sample, but this is not the case for the two SNPs in the TCF7L2 gene. We also observed evidence of association between the SNP rs3758391 at the SIRT1 gene and T2D, and the SNP rs5015480 located near the HHEX gene and MS. However, these results are not significant after multiple test correction and do not have strong support using the BFDP approach, and should be interpreted with caution.

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Conflict of interest

The authors declare that there is no conflict of interest. The supporting source had no involvement in study design, in the collection, analysis and interpretation of data or in the writing of the report and in the decision to submit the report for publication.

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