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ORIGINAL ARTICLE

Exploring single nucleotide polymorphisms previously related to obesity and metabolic traits in pediatric-onset type 2 diabetes

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Abstract
Aims To evaluate the association of 64 obesity-related polymorphisms with pediatric-onset type 2 diabetes and other glucose- and insulin-related traits in Mexican children.
Methods Case–control and case–sibling designs were followed. We studied 99 patients with pediatric-onset type 2 diabetes, their siblings ($n = 101$) without diabetes, 83 unrelated pediatric controls and 137 adult controls. Genotypes were determined for 64 single nucleotide polymorphisms, and a possible association was examined between those genotypes and type 2 diabetes and other quantitative traits, after adjusting for age, sex and body mass index.
Results In the case–pediatric control and case–adult control analyses, five polymorphisms were associated with increased likelihood of pediatric-onset type 2 diabetes; only one of these polymorphisms (*CADM2*/rs1307880) also showed a consistent effect in the case–sibling analysis. The associations in the combined analysis were as follows: *ADORA1*/rs903361 (OR 1.9, 95% CI 1.2; 3.0); *CADM2*/rs13078807 (OR 2.2, 95% CI 1.2; 4.0); *GNPDA2*/rs10938397 (OR 2.2, 95% CI 1.4; 3.7); *VEGFA*/rs6905288 (OR 1.4, 95% CI 1.1; 2.1) and *FTO*/rs9939609 (OR 1.8, 95% CI 1.0; 3.2). We also identified 16 polymorphisms nominally associated with quantitative traits in participants without diabetes.
Conclusions *ADORA*/rs903361, *CADM2*/rs13078807, *GNPDA2*/rs10938397, *VEGFA*/rs6905288 and *FTO*/rs9939609 are associated with an increased risk of pediatric-onset type 2 diabetes in the Mexican population.

Keywords Type 2 diabetes · Metabolic syndrome · Children · Adolescents · Single nucleotide polymorphisms

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Introduction

Type 2 diabetes (T2D) occurs most commonly in adults aged 40 years or older. However, in recent years the incidence of pediatric T2D has increased, and this increase may be related to the ongoing childhood obesity epidemic. In Mexico, 9.17% of adults have diabetes, and T2D is most prevalent form [1]. In the Mexican population, the disease is observed in younger people, sometimes even before the age of nineteen [2, 3]. Although the prevalence of pediatric T2D is still low, it is predicted that in some ethnic groups it will become the predominant form of diabetes within the next decade [4].

T2D is a multifactorial disease caused by a complex interplay between genetic and environmental factors.

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Although the major environmental factors, such as an unhealthy diet and physical inactivity, are well known, identification of the genetic factors involved in pediatric T2D has been challenging. It has been hypothesized that because the genetic risk is higher among younger affected people [5], they are less tolerant of environmental factors.

A strong familial history of the disease suggests the involvement in genetic factors, and we have previously observed that the heritability of pediatric-onset T2D in Mexican youths reaches 0.50 [6]. This high heritability points to an important role for genetic factors, but defining the molecular genetics of T2D is difficult because of the small number of subjects available for study.

There is some evidence suggesting a role of epigenetic factors in the complex interplay between genes and the environment. However, this is still poorly understood [7] and is a field of active research [8].

Currently, approximately 153 variants, mapping to >120 loci, have been associated with T2D in adults. These variants explain less than 20% of the heritability of T2D [9]. These genetic studies have been carried out almost exclusively in adults, and the findings have been widely replicated mostly in European and Asian populations. The investigation of genetic risk factors in people of Hispanic descent, including Mexicans, has not been as extensive.

The genetic risk factors for adult T2D have also been associated with other metabolic traits in individuals without T2D. These traits include glucose levels, beta cell function, and insulin sensitivity and resistance [10]. Some studies have shown that loci associated with fasting glucose in adults also have a detectable association in children; however, whether the same is true for T2D loci is still an open question [11].

The most important diabetes susceptibility variants reported to date are single nucleotide polymorphisms (SNPs) in the *TCF7L2* gene, which have strong associations with T2D in multiple ethnic populations [12]. Dabelea et al. [13] identified *TCF7L2* variants associated with an increased risk of T2D among African-American youths but did not find a significant association in non-Hispanic white youths. We recently reported the association between SNPs in *SLC16A11* (rs13342232) and pediatric-onset T2D in Mexican population. We also analyzed SNPs in *TCF7L2* (rs7903146 and rs12255372) and *ABCA1* (rs9282541), but we did not have sufficient power to affirm or reject the association [14]. To our knowledge, these are the only studies that have evaluated the association of genetic variants with the presence of pediatric-onset T2D, so further genetic association studies are required to uncover other T2D susceptibility genes in children, in whom the genetic contribution may be larger. The identification of genetic variants increasing the risk of T2D early in life could also provide a better understanding of the genetic determinants of the disease in later stages in life.

The purpose of this study was to evaluate the association of 64 SNPs, previously related to obesity and/or metabolic traits in adults, and the presence of both pediatric-onset T2D and other glucose- and insulin-related traits in Mexicans.

Methods

Participants

Ninety-nine patients were recruited through the Diabetes Clinic in Mexico's Children's Hospital Federico Gómez between January 2011 and June 2014. Previously, we reported the association of the SNP rs13342232 in the *SLC16A11* gene with pediatric-onset T2D in this cohort [14]. Briefly, we include patients under 18 years of age and had a recent (<1 month) diagnosis of T2D. The presence of the disease was determined by (1) a previous diagnoses or oral glucose tolerance test (OGTT) according to the criteria of the American Diabetes Association [15]; (2) the absence of anti-glutamic acid decarboxylase and anti-insulin antibodies; (3) levels of C-peptide ≥0.45 ng/mL, and none of the following clinical features to consider MODY (a) individuals who have mild stable fasting hyperglycemia; and (b) a multiple family members with diabetes not characteristic of type 1 (no islet autoantibodies, low or no insulin requirements 5 years after diagnosis) or type 2 diabetes (marked obesity, acanthosis nigricans) [15, 16].

For control subjects, we enrolled siblings of these patients ($n = 101$) as well as unrelated population controls ($n = 83$ children and $n = 137$ adults). The unrelated controls were enrolled by requesting the participation of friends, neighbors or family acquaintances of the index cases. These unrelated controls reported no known family history of diabetes and were confirmed to be diabetes-free using an OGTT. Unrelated pediatric controls were matched to patients by age and sex. Unrelated adult controls were used to reduce the chances of misclassification compared with the use of children, whom might later develop early type 2 diabetes, as controls.

All participants lived within Mexico City's metropolitan area, and they, as well as their parents and grandparents, were born in Mexico. The Ethics, Research and Biosafety Committees of Mexico's Children's Hospital Federico Gómez approved this study (HIM 2014-041). Written assent was obtained from all children and adolescents, in conjunction with written consent from their parents.

Anthropometric and biochemical measurements

Measurements were taken by trained personnel. Participants were measured without shoes and with light clothing. Weight was measured with a digital scale (Seca® 884, Hamburg, Germany) to 0.1 kg accuracy; height was determined with a stadiometer (Seca® 225, Hamburg, Germany) to 0.1 cm precision; and body mass index was calculated from these two measurements.

Participants were studied following a 12-h fast, and blood samples were obtained for the following tests: glucose (mg/dL, hexokinase method Dimension RXL.MAX, Siemens), insulin (mU/mL, chemiluminescence IMMULITE 1000, Siemens, Euro, DPC, Llanberis, UK), C-peptide (ng/dL, chemiluminescence IMMULITE 1000, Siemens, Euro, DPC, Llanberis, UK), and hemoglobin A1c (HbA1c % and mmol/mol, Dimension RXL.MAX Siemens immunoassay). In participants without diabetes, an OGTT was done with 1.75 g/kg of anhydrous glucose (up to 75 g), with glucose and insulin measurements 2 h after glucose administration. To assess insulin resistance, we calculated the HOMA-IR index ([fasting glucose in mg/dL × fasting insulin in mU/mL]/405) [17, 18], and the evaluation of the secretory capacity of beta cells was determined with HOMA-B ([fasting insulin in mU/mL × 20]/[fasting glucose in mmol/L − 3.5]) [18]. Finally, insulin sensitivity was obtained with QUICKI (1/[log fasting insulin in μU/mL] + [log fasting glucose in mg/dL]) [19].

SNP selection

We examined a total of 64 SNPs (for gene names, functions, and associated traits, see Table 1). Most of them were previously associated with obesity or BMI [10], and two of these associations were reported by this research group [20]. Other SNPs were previously reported by others to be associated with metabolic glucose traits or lipids [10]. All these SNPs could be related, either directly or indirectly, to glucose pathways, and therefore to T2D, and our goal was to investigate novel candidate SNPs in early-onset T2D.

Genotyping

DNA was extracted from peripheral blood mononuclear cells using the QIAamp DNA Blood Mini/Kit (Qiagen), following the manufacturer's instructions. Purity and concentration were measured using absorbance at 260 and 280 nm (Epoch spectrophotometer, BioTek Instruments, Winooski, VT, USA), and integrity was checked in a 0.8% agarose gel. SNP genotyping was performed using a TaqMan® OpenArray® system (Applied Biosystems, Foster City, CA, USA), which provides PCR-based endpoint analysis, according to the manufacturer's instructions. Two hundred and fifty control samples were genotyped in duplicate for quality control purposes.

Statistical analysis

Database management and statistical analyses were performed using STATA (version 11.0/SE; STATA Corp, College Station, TX). SNPs were filtered out if the minor allele frequency (MAF) was less than 0.08 (due our small sample size) or if a deviation from Hardy–Weinberg equilibrium (HWE, $p < 0.05$) was observed among controls.

Anthropometric and metabolic data were tested for normal distribution using skewness and kurtosis. Comparisons between continuous variables and groups were made with Student's t test or Mann–Whitney U test according to data distribution. For categorical data, the Chi-squared test was used.

The association between pediatric-onset T2D and each SNP was analyzed using logistic regression comparing allele and genotype frequencies between case–pediatric control and case–adult control. A family-based test of association among siblings discordant for diabetes was conducted using conditional logistic regression. To increase statistical power, the p value was calculated combining the p value from logistic regression of the case–pediatric control and case–adult control with the p value from the conditional logistic regression for the case–sibling analysis. We estimated ORs and 95% CIs under additive, dominant and recessive genetic models, and we calculated the smallest p value from up to three models tested. In the additive model, a numeric value (0, 1, or 2) was assigned based on the number of referent alleles. The analysis of the comparison of cases with pediatric controls and with siblings was adjusted by age, sex and body mass index (BMI), whereas the comparison with adult controls was adjusted only by sex and BMI. The significance threshold was set at $p < 0.05$, with a Bonferroni correction for the number of SNPs remaining after filtering by MAF and HWE.

Additionally, among controls, we explored the association of genetic variants with quantitative glucose- and insulin-related traits adjusting for age, sex and BMI. The following traits were available: fasting glucose, 2-h glucose, fasting insulin, 2-h insulin, C-peptide, HbA1c, HOMA-IR, HOMA-B and QUICKI. Analyses were performed using an additive model to calculate the β coefficient per risk allele.

Statistical power

Statistical power was calculated with Quanto (v. 1.2.4). Assuming an OR $\geq$1.8; an MAF of 0.1, 0.2 and 0.5; a log-

Table 1 SNPs assessed for association

SNP	Chr	Reported gene	Functional class	A	a	MAF	HWE (*p*)	Obesity[b]	Gluc/Ins[c]	Lipids[d]
rs903361	1	*ADORA1*	Intron	A	G	0.284	0.253			
rs1011731	1	*DNM3*	Intron	A	G	0.264	0.318	*		
rs1514175	1	*TNNI3 K*	Intron	A	G	0.329	0.082	*		
rs1555543	1	*PTBP2*	Intergenic	C	A	0.390	0.412	*		
rs2605100	1	*LYPLAL1*	Intergenic	G	A	0.456	0.759	*		
rs2815752	1	*NEGR1*	Intergenic	A	G	0.217	0.822	*		
rs984222	1	*TBX15*	Intron	G	C	0.383	0.739	*		
rs10913469	1	*SEC16B*	Intron	T	C	0.222	0.929	*		
rs340874	1	*PROX1*	5 prime UTR	T	C	0.324	0.902		*	
rs2890652	2	*LRP1B*	Intergenic	T	C	0.229	0.759	*		
rs713586[a]	2	*ADCY3*	Intergenic	T	C	0.289	0.035	*		
rs887912[a]	2	*FANCL*	Intergenic	C	T	0.068	0.155	*		
rs560887[a]	2	*G6PC2*	Intron	C	T	0.061	0.406		*	
rs10195252	2	*GRB14*	Intron	T	C	0.277	0.564	*		*
rs780094	2	*GCKR*	Intron	C	T	0.329	0.288		*	*
rs6784615[a]	3	*NISCH*	Intron	T	C	0.036	0.630	*		
rs6795735[a]	3	*ADAMTS9-AS2*	Intron	T	C	0.164	0.047	*		
rs13078807	3	*CADM2*	Intron	A	G	0.095	0.638	*		
rs7647305	3	*SFRS10*	Intron	C	T	0.171	0.396	*		
rs11920090	3	*SLC2A2*	Intron	T	A	0.284	0.333		*	
rs11708067	3	*ADCY5*	Intron	A	G	0.362	0.316		*	
rs10938397	4	*GNPDA2*	Intergenic	A	G	0.326	0.856	*		
rs13107325[a]	4	*SLC30A8*	Missense	C	T	0.037	0.192	*		*
rs6232	5	*PCSK1*	Intron	T	C	0.166	0.260			
rs2112347	5	*POC5*	Upstream	T	G	0.296	0.855	*		
rs1294421	6	*LY86*	Intergenic	G	T	0.345	0.078	*		
rs206936	6	*RPS10-NUDT3*	Intron	A	G	0.348	0.387	*		
rs6905288	6	*VEGFA*	Downstream	A	G	0.360	0.094	*		
rs9491696	6	*RSPO3*	Intron	C	G	0.399	0.149	*		
rs987237	6	*TFAP2B*	Intron	A	G	0.457	0.531	*		
rs16139[a]	7	*NPY*	Missense	T	C	0.006	<0.001			
rs1055144	7	*NFE2L3*	Intergenic	C	T	0.222	0.659	*		
rs2191349	7	*TMEM195*, *DGKB*	Intergenic	T	G	0.434	0.429		*	
rs4607517[a]	7	*GCK*	Intron	G	A	0.479	0.030		*	
rs13266634	8	*SLC30A8*	Missense	C	T	0.256	0.958		*	
rs10968576	9	*LINGO2*	Intron	A	G	0.212	0.228	*		
rs7034200	9	*GLIS3*	Intron	A	C	0.355	0.311		*	
rs10885122	10	*ADRA2A*	Intergenic	G	T	0.169	0.574		*	
rs1800497	11	*ANKK1*	Missense	A	G	0.433	0.344			
rs3817334	11	*MTCH2*	Intron	C	T	0.427	0.516	*		
rs4929949	11	*STK33*	Intron	T	C	0.500	0.345	*		
rs6265	11	*BDNF-AS*	Missense	C	T	0.166	0.860	*		
rs925946	11	*BDNF-AS*	Intron	G	T	0.166	0.767	*		
rs11605924	11	*CRY2*	Intron	A	C	0.444	0.535		*	
rs7944584[a]	11	*MADD*	Intron	A	T	0.127	0.028		*	
rs10830963	11	*MTNR1B*	Intron	C	G	0.221	0.649		*	
rs174550	11	*FADS1*	5 prime UTR	T	C	0.276	0.323			*
rs1443512	12	*HOXC13*	Intergenic	A	C	0.344	0.934	*		

Table 1 continued

SNP	Chr	Reported gene	Functional class	A	a	MAF	HWE (*p*)	Obesity[b]	Gluc/Ins[c]	Lipids[d]
rs718314	12	*ITPR2*	Intron	G	A	0.368	0.095	*		
rs7138803	12	*BCDIN3D*	Intergenic	G	A	0.380	0.506	*		
rs4771122	13	*MTIF3*	Intron	A	G	0.157	0.372	*		
rs10150332	14	*NRXN3*	Intron	T	C	0.225	0.266	*		
rs11847697[a]	14	*PRKD1*	Intron	C	T	0.042	0.572	*		
rs2856929[a]	15	*PKM*	Intron	T	C	0.494	0.004			
rs2241423	15	*MAP2K5*, *LBXCOR1*	Intron	G	A	0.312	0.072	*		
rs12444979[a]	16	*GPRC5B*	Intergenic	T	C	0.050	0.326	*		
rs7498665	16	*NPIPB8*	Missense	G	A	0.488	0.531	*		
rs9939609	16	*FTO*	Intron	T	A	0.084	0.319	*	*	
rs2229616[a]	18	*MC4R*	Missense	C	T	0.088	<0.001			
rs571312	18	*MC4R*	Intergenic	C	A	0.239	0.827	*		
rs11084753	19	*KCTD15*	Intergenic	G	A	0.267	0.234	*		
rs2287019	19	*QPCTL*	Intron	C	T	0.084	0.319	*		
rs3810291	19	*ZC3H4*	3 prime UTR	G	A	0.478	0.431	*		
rs4823006	22	*ZNRF3*	3 prime UTR	A	G	0.453	0.874	*		

SNP single nucleotide polymorphism, *Chr* chromosome, *A* major allele, *a* minor allele, *MAF* minor allele frequency, *HWE* Hardy–Weinberg equilibrium in all controls

[a] SNPs excluded from the analysis

[b] Association with obesity-related traits in GWAS (waist–hip ratio, body mass index, weight, or obesity) [10]

[c] Association with glucose- and insulin-related traits in GWAS (diabetes, glucose, HOMA-IR, HOMA-B, HbA1c, disposition index or acute insulin response to glucose) [10]

[d] Association with lipids in GWAS (triglycerides, HDL-cholesterol, or fatty acids) [10]

additive model; and a 0.05 type I error rate, the statistical power would be 50, 80 and 82%, respectively. Assuming an OR $\geq$2.3 and the same MAF as above, the statistical power would be 82, 98 and 98%, respectively.

Results

The study group comprised 99 pediatric patients with T2D with a mean age of 12.9 years ($\pm$2.6), 83 unrelated pediatric controls of similar age (12.5 $\pm$ 2.9 years), 101 siblings of the patients with a higher average age (16.5 $\pm$ 5.4 years), and 137 adults in their third or fourth decade of life. Demographic, anthropometric and biochemical characteristics of the study participants are summarized in Table 2.

After filtering out SNPs with MAF <0.08 or deviating from HWE frequencies among the controls, fifty-one SNPs remained. Of these SNPs, five showed a statistically significant association with pediatric-onset T2D when compared to unrelated pediatric controls and to adult controls, after adjusting for age, sex and BMI ($p < 0.05$). These five polymorphisms were as follows: *ADORA*/rs903361, *CADM2*/rs13078807, *GNPDA2*/s10938397, *VEGFA*/rs6905288 and *FTO*/rs9939609. Of these polymorphisms, in the case–sibling analysis, only *CADM2*/rs13078807 reached significance, although not after Bonferroni correction (dominant model: OR 2.5, 95% CI 1.2; 4.9, $p = 0.009$). In the combined analysis, only *GNPDA2*/rs10938397 showed a significant association after Bonferroni correction (dominant model: OR 2.2, 95% CI 1.4; 3.7, $p \leq 0.001$) (Table 3).

We also evaluated the association between SNPs and quantitative glucose- and insulin-related traits in unaffected individuals (Table 4). We observed that 16 SNPs were associated with one or more of the following traits: fasting glucose, 2-h glucose, HbA1c, C-peptide, fasting insulin, HOMA-IR, HOMA-B and QUICKI. Two of the five SNPs that were associated with pediatric-onset T2D were also associated with quantitative glucose- and insulin-related traits in participants without diabetes: *CADM2*/rs13078807 with HbA1c ($\beta = 0.25$ per risk allele, $p = 0.032$) and *VEGFA*/rs6905288 with fasting glucose and 2-h glucose ($\beta = 4.59$ per risk allele, $p = 0.034$ and $\beta = 2.07$ per risk allele, $p = 0.012$, respectively). Only the SNPs *SLC2A2*/rs11920090 and *PCSK1*/rs6232 reached statistical significance after Bonferroni correction ($p \leq 0.001$) for associations with fasting insulin, HOMA-IR and QUICKI for *SLC2A2*/rs11920090 and with QUICKI for *PCSK1*/rs6232.

Table 2 Characteristics of study participants

	Cases ($n = 99$)	Pediatric controls ($n = 83$)	Adult controls ($n = 137$)	Siblings ($n = 101$)
Sex [male *n* (%)]	45 (45.4)	45 (54.2)	57 (41.6)	47 (46.5)
Age (years)[a]	12.9 ± 2.6	12.5 ± 2.9	**41 ± 6.5**	**16.5 ± 5.4**
Antropometric measurements[a]				
Weight (kg)	63.7 ± 20.4	**57.3 ± 21.3**	**74.7 ± 16.0**	64.1 ± 20.8
Height (cm)	156.9 ± 13.5	**152.4 ± 14.7**	159.5 ± 8.1	156.9 ± 11.9
BMI (kg/m^2)	25.3 ± 5.3	24.4 ± 6.7	**29.6 ± 5.6**	25.5 ± 6.0
Glucose metabolism[b]				
Glucose (mg/dL)	119.0 (93, 192)	**88.0** (**84**, **95**)	**92.0** (**84**, **98**)	**88.0** (**82**, **94**)
2-h glucose (mg/dL)	–	97.0 (81, 110)	102.0 (91, 125)	96.0 (87, 116)
HbA1c (%)	9.4 (6.4, 12.1)	**5.6** (**5.4**, **5.7**)	**5.8** (**5.5**, **6.0**)	**5.6** (**5.4**, **5.9**)
HbA1c (mmol/mol)	79.2 (46.4, 108.8)	**37.7** (**35.5**, **38.9**)	**39.9** (**36.6**, **42.1**)	**37.7** (**35.5**, **41**)
Insulin (mU/mL)	9.9 (6.2, 15)	7.6 (3.6, 15.6)	**7.0** (**3.7**, **11.9**)	**6.5** (**4.2**, **9.9**)
2-h insulin (mU/mL)	25.1 (18.6, 31.6)	44.7 (23.7, 80.3)	42.4 (25.3, 62.6)	40.0 (22.3, 78)
HOMA-IR	3.2 (1.5, 5.2)	**1.7** (**0.8**, **3.8**)	**1.6** (**0.8**, **2.6**)	**1.3** (**0.9**, **2.2**)
C-peptide (ng/dL)	2.1 (1, 3.4)	2.2 (1.6, 3.9)	**2.5** (**1.8**, **3.1**)	2.0 (1.4, 2.7)
HOMA-B	72.4 (27.4, 144.2)	**103.4** (**62.1**, **187.9**)	78.8 (52.9, 130.0)	**102.3** (**63.6**, **166.8**)
QUICKI	0.3 (0.3, 0.4)	**0.4** (**0.3**, **0.4**)	**0.4** (**0.3**, **0.4**)	**0.4** (**0.3**, **0.4**)

$p < 0.05$ in bold characters compared with cases; – non informative markers

[a] Data are the means ± standard deviations

[b] Data are medians and (25pc, 75pc)

Table 3 SNPs exhibiting significant association with pediatric-onset T2D

Gene chromosome	rs OA/RA	Risk model[c]	Case–pediatric control			Case–adult control			Case–sibling			Combined	
			$n = 99/83$			$n = 99/137$			$n = 99/101$			99/321	
			MAF (%) Ca Co	OR[a] 95% CI	*p*	MAF (%) Ca Co	OR[b] 95% CI	*p*	MAF (%) Ca Co	OR[a] 95% CI	*p*	OR[a] 95% CI	*p*
ADORA1	rs903361	Dom	34.8	2.4	**0.009**	34.8	1.8	**0.033**	33.9	1.0	0.983	1.9	**0.010**
1	A/G		20.8	1.2; 4.7		27.1	1.0; 3.2		35.8	0.6; 1.7		1.2; 3.0	
CADM2	rs13078807	Dom	12.6	2.6	**0.048**	12.6	2.8	**0.012**	13.1	2.5	**0.009**	2.2	**0.009**
3	A/G		5.9	1.1; 6.7		5.7	1.2; 6.3		5.6	1.2; 4.9		1.2; 4.0	
GNPDA2	rs10938397	Dom	41.4	2.7	**0.005**	41.4	2.5	**0.003**	40.1	1.6	0.057	2.2	**<0.001**
4	A/G		23.3	1.4; 5.5		25.9	1.4; 4.6		35.5	1.0; 2.6		1.4; 3.7	
VEGFA	rs6905288	Add	41.1	1.6	**0.032**	41.1	1.6	**0.037**	41.8	1.3	0.255	1.4	**0.044**
6	G/A		29.9	1.0; 2.5		28.7	1.0; 2.5		39.5	0.8; 2.3		1.1; 2.1	
FTO	rs9939609	Add	27.3	2.5	**0.003**	27.3	1.9	**0.032**	14.4	1.3	0.512	1.8	**0.039**
16	T/A		13.9	1.4; 4.6		14.4	1.1; 3.5		11.2	0.6; 2.7		1.0; 3.2	

OA other allele, *RA* risk allele, *Ca* case, *Co* control, *MAF* minor allele frequency, *Dom* dominant, *Add* additive

[a] Adjusted for age, sex

[b] Adjusted for sex and BMI

[c] Risk model with smallest *p* value. $p < 0.05$ in bold characters. Bonferroni correction ($\alpha = 0.05$; k = 51; P = 0.0009); – non informative markers

Discussion

A number of genetic variants have been identified related to T2D. Some associations are heterogeneous across ethnic groups, and most of the studies were done with Europeans. The information on Hispanic and Mexican populations is not abundant, and there is even less information about pediatric-onset T2D. To our knowledge, this is the second report related to pediatric-onset T2D in a Mexican

Table 4 SNPs with significant association to quantitative phenotypes in control subjects without diabetes

SNP	Trait	β	p	95% CI	Previous associations [10]
rs13078807	HbA1c (%)	0.25	0.032	0.02; 0.49	Obesity and BMI
rs7138803	Fasting insulin (mg/dL)	1.57	0.028	0.17; 2.98	Obesity, BMI, weight and waist–hip ratio
	HOMA-IR	0.33	0.046	0.01; 0.66	
rs206936	C-peptide (ng/dL)	−0.35	0.041	−0.68; −0.01	BMI
rs2890652	QUICKI	−0.01	0.027	−0.02; 0.00	BMI
rs1555543	Fasting insulin (mU/mL)	1.54	0.050	0.00; 3.08	BMI
	C-peptide (ng/dL)	0.38	0.044	0.01; 0.74	
rs151475	HOMA-B	−27.70	0.020	−51.16; −4.39	BMI
rs10195252	Fasting glucose (mg/dL)	1.76	0.038	0.10; 3.43	Waist–hip ratio and triglycerides
	QUICKI	−0.01	0.005	−0.02; 0.00	
rs1443512	HOMA-B	−27.03	0.012	−47.97; −6.08	Waist–hip ratio
rs1294421	HOMA-B	−18.90	0.040	−36.96; −0.84	Waist–hip ratio
rs9491696	HOMA-B	−24.17	0.033	−46.34; −2.00	Waist–hip ratio
rs6905288	2-h glucose (mg/dL)	4.59	0.034	0.36; 8.81	Waist–hip ratio and coronary heart disease
	Fasting glucose (mg/dL)	2.07	0.012	0.47; 3.67	
rs10885122	HOMA-B	−38.46	0.014	−69.14; −7.79	Fasting glucose and HOMA-B
rs2191349	Fasting insulin (mU/mL)	−1.89	0.003	−3.14; −0.64	Fasting glucose and HOMA-B
	HOMA-B	−21.75	0.023	−40.50; −3.01	
rs10830963	Fasting glucose (mg/dL)	1.95	0.034	0.14; 3.76	Fasting glucose, HOMA-B, DI, AIRg and T2D
rs11920090	Fasting insulin (mU/mL)	2.76	0.001	1.18; 4.33	Fasting glucose and HOMA-B
	C-peptide (ng/dL)	0.35	0.007	0.10; 0.61	
	HOMA-IR	0.63	0.001	0.26; 0.99	
	QUICKI	−0.02	<0.001	−0.03; −0.01	
rs6232	Fasting glucose (mg/dL)	3.07	0.008	0.82; 5.31	Obesity and BMI
	Fasting insulin (mU/mL)	2.67	0.006	0.76; 4.58	
	C-peptide (ng/dL)	0.57	0.015	0.11; 1.02	
	HOMA-IR	0.63	0.006	0.18; 1.07	
	QUICKI	−0.02	<0.001	−0.03; −0.01	

β (beta coefficient): this refers to the unit change in diabetes-related quantitative traits per added risk allele

DI disposition index, *AIRg* acute insulin response to glucose

population; therefore, the findings of these patients with T2D onset before age 19 are of particular interest.

Our study reveals the existence of the association between five SNPs with the presence of pediatric-onset T2D. All these variants had previously been related to metabolic traits in GWAS. The SNPs *ADORA*/rs903361 [21], *CADM2*/rs13078807 [22, 23], *GNPDA2*/s10938397 [23] and *FTO*/rs9939609 [24–26] had been associated with BMI; *CADM2*/rs13078807 [22, 27] and *GNPDA2*/s10938397 [24, 27] with obesity; and *VEGFA*/rs6905288 with waist–hip ratio [28] and coronary heart disease [29]. Of these variants, only *GNPDA2*/rs10938397 [30, 31] and *FTO*/rs9939609 [32–34] had been associated with T2D in adults previously. Interestingly, our results demonstrate that these SNPs are also associated with pediatric-onset T2D.

Several genotyped variants have been shown to be associated with obesity or obesity-related phenotypes in several previous studies. It is known that obesity is a significant risk factor for T2D, and for this reason, all statistical analyses were adjusted for BMI. There are many obese children who do not develop the disease and some non-obese children who do, indicating that obesity is not the only factor involved in the etiology of pediatric T2D [11]. It has been observed that genetic predisposition to obesity leads to increased risk of type 2 diabetes (obesity-predisposing effect) [35]. However, we identified five SNPs associated with T2D independently of BMI. Although BMI is not a perfect measure of adiposity, our findings suggest that these genetic variants may predispose carriers to develop T2D at lower obesity thresholds. On the other hand, the association of the SNPs independently of BMI could explain why not all individuals with obesity develop T2D.

This study provides confirmation that some variants (*ADRA2A*/rs10885122, *DGKB*/rs2191349, *MTNR1B*/

rs10830963 and *SLC2A2*/rs119200909) are associated with glucose- and insulin-related traits, regardless of age, sex and BMI in participants without diabetes. In addition, we identified another 12 SNPs (*CADM2*/rs13078807, *BCDIN3D*/rs7138803, *RPS10-NUDT3*/rs206936, *LRP1B*/rs2890652, *PTBP2*/rs1555543, *TNNI3 K*/rs151475, *GRB14*/rs10195252, *HOXC13*/rs1443512, *LY86*/rs1294421, *RSPO3*/rs9491696, *VEGFA*/rs6905288 and *PCSK1*/rs6232) previously associated with obesity-related phenotypes that are also associated with quantitative glycemic and insulin traits, independently of BMI. Although only two of the SNPs associated with glucose- and insulin-related traits (*CADM2*/rs13078807 and *VEGFA*/rs6905288) were also associated with pediatric-onset T2D in this study, the other variants could be genetic susceptibility markers of the future risk of T2D in individuals without diabetes. Follow-up studies could corroborate this hypothesis (see Table 4 for associations previously reported from GWAS).

Among the SNPs associated with pediatric-onset T2D and glucose- and insulin-related traits, only the variant *PCSKI*/*rs6232* is located in the coding region. *PCSKI* encodes prohormone convertase 1/3, which functions in the proteolytic activation of polypeptide hormones and neuropeptide precursors. The non-synonymous polymorphism rs6232 (N221D) is associated with obesity risk, and this variant exhibited 30% lower enzymatic activity [36]. Other associated SNPs either are not located within coding regions of genes or do not reside within genes and therefore do not affect protein function (Table 1). It is not clear how all these genes influence insulin-secretory capacity (beta cell growth, survival or function) or modulate adiposity and/or insulin sensitivity. Further studies are needed to identify the biological pathways affected by these risk gene variants. This would allow establishing the mechanisms which make some individuals more susceptible to environmental risk factors and predispose them to develop the disease at a very early age. The inclusion of adults as controls, as we have done in this study, in addition to the comparison against pediatric controls, reduces the risk of incorrect ascertainment since some children assigned to the control group might go on to develop T2D at a later date. On the other hand, it arguable that the effect of a given polymorphism might change with time, being associated with a trait during growth and development, but not at a later stage, or interacting with different risk factors associated with the lifestyle choices made as the individual matures and ages. Even though this scenario cannot be dismissed, it is worth pointing out that the association between the five SNPs was observed when using pediatric or adult controls.

Also, we observed a discordance in the results between case–pediatric control and case–sibling analysis. This could be due to the fact that the genetic similarity of the siblings (given that they share the same parents) may require a larger sample size to identify differences. Other possibility is that siblings in the future may develop the disease; this might result in a classification bias as individuals without diabetes. Genetic testing might become a future tool to identify individuals at risk for early-onset T2D. Although the increased risk of T2D for carriers of the SNPs identified in this study is small, they could be part of a multiple-risk alleles model analysis in future research. The identification of individuals with high genetic susceptibility could reinforce the measures for prevention of environmental risk factors for T2D in population with greater risk.

In some situations, the distinction between MODY and T2D might be difficult to make on a purely clinical basis [15, 16]. In this regard, even though the participants in the current study did not present with clinical features of MODY, the exclusion of this diagnosis was not made through genetic testing of the relevant genes. This might be a limitation of this study, to the extent that some of the participants might have MODY, instead of T2D. At the same time, MODY is a rare disease, affecting a small number of individuals, compared to T2D, which is a common disease. Despite the fact that the frequency of pediatric-onset T2D has increased, there are still few patients who develop T2D prior to age 19, and the sample size is the main limitation of our study. However, even with these low numbers, we observed statistically significant associations with T2D, although the possibility of false-negative results in our sample cannot be excluded. Our moderate sample size is not well powered to detect low-frequency variants or associations with OR < 2.0, and we only could detect common variants or SNPs with strong associations. Therefore, more studies with larger sample sizes are needed to corroborate the associations identified in the present study. Other limitations are the methods of measuring resistance, secretion and insulin sensitivity, which were evaluated through surrogate variables with limitations in their interpretation.

Conclusion

In conclusion, to our knowledge, the present study is the first confirmation that *ADORA*/rs903361, *CADM2*/rs13078807, *GNPDA2*/s10938397, *VEGFA*/rs6905288 and *FTO*/rs9939609 potentially contribute to pediatric-onset T2D risk in the Mexican population. The associations with G*NPDA2*/rs10938397 and *FTO*/rs9939609 have been reported previously with T2D in adults, and the other three variants have only been associated with obesity-related phenotypes. Other SNPs associated with glucose- and insulin-related traits could be related to T2D in a

longitudinal follow-up study. Replication in independent studies and functional follow-up studies will be important for determining the true susceptibility from these variants.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Human and animal rights All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments.

Informed consent Informed consent and assessment was obtained from all participants for being included in the study.

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