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Association of β1 and β3 adrenergic receptors gene polymorphisms with insulin resistance and high lipid profiles related to type 2 diabetes and metabolic syndrome

Ana I. Burguete-García[1], Gabriela A. Martínez-Nava[1], Adán Valladares-Salgado[2], V. H. Bermúdez[1], Bárbara Estrada-Velasco[1], Niels Wacher[3], Jesús Peralta-Romero[2], Jaime Garcia-Mena[4], Esteban Parra and Miguel Cruz[2]

[1]Dirección de Infecciones Crónicas y Cáncer, CISEI. Instituto Nacional de Salud Pública. [2]Unidad de Investigación Médica en Bioquímica, Hospital de Especialidades, Centro Médico Nacional Siglo XXI del IMSS, México. [3]Unidad de Investigación Médica en Epidemiología, Hospital de Especialidades, Centro Médico Nacional Siglo XXI de IMSS, México. [4]Departamento de Genética y Biología Molecular, Cinvestav-IPN Zacatenco, México.

Abstract

Background: **Among the diverse genes associated to type 2 diabetes (T2D), the β-adrenergic receptors are an excellent candidate to study in Mexican population. The objective of this work was to analyze the association of polymorphisms in *ADRB1* (rs1801253) (Arg389Gly) and *ADRB3* (Trp64Arg) genes with T2D and metabolic syndrome (MS).**

Methods: **We studied 445 MS patients, 502 with T2D and 552 healthy controls. Anthropometric features and complete biochemical profile were evaluated, and Arg389Gly and Trp64Arg SNPs were determined by TaqMan assays. Data analysis was adjusted by African, Caucasian and Amerindian ancestral percentage.**

Results: **The variant Arg389Gly of *ADRB1* was statistically associated with an increase of LDL levels ($P < 0.008$), and the variant *ADRB3* Trp64Arg was associated to larger HOMA-IR ($P < 0.018$) and with an increase of insulin levels ($P < 0.001$). A multiple logistic regression analysis was made in three grouping models: For *ADRB3* in the codominant model Trp/Arg genotype, there was an *OR* of 1.53 (1.09–2.13, $P < 0.003$) which was increased up to *OR* 2.99 (1.44–6.22, $P < 0.003$) for the Arg/Arg genotype. Similar risk association was found under the dominant model Trp/Arg-Arg/Arg genotype with *OR* 1.67 (1.21–2.30; $P < 0.002$). In the recessive model (Arg/Arg genotype), there was also a high association OR 2.56 (1.24–5.26, $P < 0.01$).**

Conclusions: **The *ADRB3* Trp64Arg variant is a susceptibility gene polymorphism for T2D and the *ADRB1* Gly389Arg for lipid metabolism disruption. These results show that these variants are potential biomarkers for predicting metabolic alterations and evolution in diabetic and metabolic syndrome patients.**

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Key words: *Polymorphism. Type 2 diabetes. Metabolic syndrome. Adrenergic receptors.*

Correspondence: Ana I. Burguete-García.
E-mail: aburguete@insp.mx

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ASOCIACIÓN DE LOS POLIMORFISMOS GÉNICOS DE LOS RECEPTORES ADRENÉRGICOS β1 Y β3 CON LA RESISTENCIA A LA INSULINA Y LOS PERFILES ELEVADOS DE LÍPIDOS RELACIONADOS CON LA DIABETES TIPO 2 Y EL SÍNDROME METABÓLICO

Resumen

Antecedentes: **Entre los diversos genes asociados a la diabetes tipo 2 (DT2), los receptores β –adrenérgicos– son excelentes candidatos para estudiar en la población mexicana dada la alta prevalencia de estas patologías. El objetivo de este trabajo fue analizar la asociación de polimorfismos en los genes *ADRB1* (rs1801253) (Arg389Gly) y *ADRB3* (Trp64Arg) con DT2 y SM.**

Métodos: **Se estudiaron 445 pacientes con Síndrome Metabólico, 502 con diabetes tipo 2 y 552 controles sanos. Se evaluaron las características antropométricas, perfil bioquímico completo y los polimorfismos Arg389Gly y Trp64Arg SNPs se determinaron mediante ensayos TaqMan. El análisis de datos fue ajustado por porcentaje de ancestralidad.**

Resultados: **Para la variante ADRB1 Arg389Gly se observó una asociación estadísticamente significativa con un aumento de los niveles de LDL ($P < 0,008$), y la variante ADRB3 Trp64Arg se asoció a mayor HOMA- IR ($p < 0,018$) y con un aumento de los niveles de insulina ($P < 0,001$). Mediante modelos de regresión logística múltiple en los tres modelos de heredabilidad se evaluó la asociación de ambos polimorfismos y DT2 y SM, observando un asociación significativa en los 3 modelos solo con DT2, ADRB3 en el modelo codominante Trp/Arg un OR de 1.53 (1.9 a 2.13 , $P < 0.003$) que se incrementó hasta OR 2,99 (1,44 a 6,22 , $P < 0,003$) para el genotipo Arg/Arg . Se encontró bajo el modelo dominante genotipo Trp/Arg- Arg/Arg con OR 1.67 (1.21 a 2.30, $p < 0.002$). En el modelo recesivo (Arg/Arg), también un OR 2.56 (1.24 a 5.26 , $P < 0.01$).**

Conclusiones: **La variante ADRB3 Trp64Arg se asoció significativamente con DT2 y ADRB1 Gly389Arg en alteraciones en el metabolismo de lípidos. Nuestros resultados demuestran que estas variantes son posibles biomarcadores para predecir las alteraciones metabólicas y la evolución en pacientes con síndrome Metabólico y diabetes tipo 2.**

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Palabras clave: *Diabetes. Síndrome metabólico. Receptor adrenérgico.*

Introduction

Type 2 diabetes (T2D) is a complex multifactorial and polygenic metabolic disorder and its pathogenesis is influenced by diverse environmental factors[1]. Diabetes is one of the most prevalent diseases and its complications are one of the leading causes of death worldwide, and also in México[2]. The prevalence of diabetes has dramatically increased in Mexico since the second half of last century[3] and it has recently been estimated that 14.4% of Mexican adults suffer diabetes[2].

Impaired fasting glucose and impaired glucose tolerance are metabolic abnormalities known as prediabetes since they predict later occurrence of T2D. They are both associated with insulin resistance, as well as an increased risk of cardiovascular disease[4]. Metabolic syndrome (MS) on the other hand is a combination of metabolic disorders that increase the risk of cardiovascular disease and T2D. Several criteria exist to define MS[5-7], however until now, there is not a universal criterion to define MS in different populations[8]. MS and impaired glucose tolerance identify nearly 70% of subjects with high T2D risk[8,9]. The prevalence of metabolic syndrome in Mexico is 26.6% or 21.4% if those with diabetes are excluded. Genetic studies have shown a variety of genes associated to the development of MS and T2D; in particular genes that participate in the control of adipose tissue metabolism, lipolysis, thermogenesis, and glucose metabolism in muscle[10].

Attempts to identify genes causing MS or T2D in humans using candidate genes have reported differences in different populations; therefore more work is required to identify the causal variants, to test their role in disease prediction and to ascertain their therapeutic implications[11-13]. The involvement of *ADRB1* and *ADRB2* adrenergic receptors is a remarkable case since this type of G protein-coupled receptors, had been only associated with heart failure[14]. The *ADRB* receptor are activated by the specifically binding of their endogenous ligands, the catecholamines (adrenaline and noradrenaline). *ADRBs* are highly homologous; nevertheless they play clearly distinct roles in cardiac physiology and pathology. For example, to meet the increased metabolic demands of stress or exercise, the sympathetic nervous system stimulates cardiac function through activation of these closely related receptors, and chronic stimulation of *ADRB1*produces myocyte hypertrophy and apoptosis, whereas *ADRB2* signaling promotes cell survival[15].

Some polymorphisms in the *ADRB* genes have been associated with obesity[16]; the Arg389Gly polymorphism of *ADRB1* gene[17] showed differences for the Arg allele frequency among several ethnic groups[17,18]. This variant has been associated with obesity in some populations, but it has not been associated with hypertension and the association with obesity is not consistent among the case-control studies reported[19-21].

The Trp64Arg polymorphism of the *ADRB3* gene was originally reported in Pima Indians with a particular high frequency of 31% for the Arg allele. This variant is apparently present in all studied populations except in individuals of the Nauru Republic. In most cases the Arg64 variant has been associated to overweight, obesity and early onset of T2D[22-25]. Discrepancies in the association of this polymorphism with metabolic risk factors, for instance lipids and insulin resistance, have been reported; however this might be attributed to differences due to confounding variables such as age, ethnicity or low statistical power[18,26,27]. Most authors agree the Arg/Arg and Trp/Arg variants of the *ADRB3* gene have a significant effect in the increase of the relative risk for MS and T2D, associated with weight gain, increase of visceral fat, decrease of insulin sensitivity and glucose control[26,28]. Although this variant is considered closely associated in numerous populations with susceptibility to T2D, however, no data has been reported for specific relationship with insulin resistance and lipid profile. The purpose of this work was to determine the possible association of two important *ADRB* genes polymorphisms, Arg389Gly (*ADRB1*) and Trp64Arg (*ADRB3*) with insulin resistance and lipid profiles related to T2D and MS in a sample of adult population of Mexico City.

Material and methods

Study participants and phenotype definitions

In a case-control design (table I), 502 subjects with T2D (according to the ADA criteria[29]) and 445 with MS[7] were compared to 552 controls aged 35 to 65 years, selected from the Regional Hospital Number 1 (Diabetes Research Unit) and the National Medical Center Blood Bank (controls and those with MS), from the Mexican Institute of Social Security (IMSS). In our study we defined MS individuals as those without family history of diabetes (ADA criteria) and fasting glucose <126 mg/dL (no T2D patients were included in this group). Parents and grandparents of all studied subjects were born in Mexico. The inclusion criteria to select controls were the absence of family history of T2D among parents, brothers, sisters and siblings. 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 included fasting glucose (mg/dL), insulin (pmol/L), insulin sensitivity (HOMA-IR), total cholesterol (mg/dL), LDL (mg/dL), HDL (mg/dL) and triglycerides (mg/dL). These parameters were determined using the ILab350 Clinical Chemistry System (Instrumentation Laboratory, Barcelona Spain). Anthropometric measurements included weight (kg), height (cm), waist to hip ratio (WHR) and

Table I
Characteristics of MS and T2D Mexico City subjects

Parameters	*Controls (552)*	*MS (445)*	*T2D (502)*
Age (years)	43.47 ± 6.6	45.00 ± 7.0*	53.40 ± 7.50*
BMI	27.50 ± 3.6	30.50 ± 4.3*	29.30 ± 4.70*
WHR	0.89 ± 0.58	0.92 ± 0.08*	0.92 ± 0.17*
SBP (mm Hg)	116.30 ± 9.8	126.40 ± 12.9*	118.10 ± 14.20*
DBP (mm Hg)	73.80 ± 7.3	78.20 ± 8.9	75.90 ± 8.70*
Glucose (mg/dL)	88.00 ± 15.7	96.24 ± 12.6	182.60 ± 80.00*
Insulin (pmol/L)	9.40 ± 5.15	12.00 ± 7.8*	14.20 ± 10.10*
HOMA-IR	2.07 ± 1.2	3.00 ± 2.0*	6.30 ± 5.00*
Total cholesterol (mg/dL)	199.90 ± 40.0	206.10 ± 40.3	221.60 ± 65.00*
LDL (mg/dL)	128.00 ± 34.2	127.50 ± 35.0	138.30 ± 38.90*
HDL (mg/dL)	44.50 ± 11.4	37.80 ± 9.8	48.60 ± 15.20*
Triglycerides (mg/dL)	166.90 ± 93.0	257.90 ± 149.9*	236.30 ± 169.60*
Atherogenic index	4.73 ± 1.34	5.71 ± 1.51*	4.76 ± 1.33
Ancestral contribution			
Amerindian	0.64 ± 0.13	0.67 ± 0.11	0.64 ± 0.11
European	0.33 ± 0.12	0.30 ± 0.10	0.33 ± 0.10
African	0.03 ± 0.02	0.03 ± 0.02	0.03 ± 0.025

The results are shown as mean ± standard deviation *$P < 0.05$ (Controls versus T2D or MS). BMI: Body mass index. WHR: waist hip ratio. SBP: systolic blood pressure. DBP: diastolic blood pressure. LDL: low density lipoprotein. HDL: high density lipoprotein.

body mass index (BMI in kg/m^2) using the Body Composition Analyzer BC-418 (TANITA Corporation, Illinois, USA). Systolic and diastolic blood pressures were measured using a sphygmomanometer (America Diagnostic Corp., NY). MS was defined according to the criteria of the AHA/NHLBI (American Heart Association/National Heart, Lung and Blood Institute Scientific Statement)[7]. Atherogenic index was calculated according to the formula: AI = Total cholesterol/HDL-C, a cardiovascular risk was considered as a value ≥ 4.5[30].

Genotyping

Genomic DNA was extracted from a peripheral blood sample using QIAamp kit (Qiagen, Germany), and analyzed by electrophoresis in 0.8% agarose gels stained with ethidium bromide and visualized in a Gel Doc 2000 (BIORAD, California). DNA concentration was determined using a VICTOR3 1420 spectrophotometer (Perkin-Elmer, Germany). The SNPs analyses were made using real time PCR by TaqMan technology (7900HT Applied Biosystems, Foster City, USA), using probes for the *ADRB1*gene Arg389Gly (rs1801253) polymorphism, and the *ADRB3* gene Trp64Arg (rs4994) according to the manufacturer (Applied Biosystems, Foster City, USA).

In order to control for the potential effect of population stratification (*eg*. variation of individual admixture proportions in the samples), we also genotyped a panel of Ancestry Informative Markers (AIMs), which are markers showing large allele frequency differences between European, West African and Native American populations. In the sample of T2D, SM subjects and controls, we genotyped 27 AIMs (rs2814778, rs723822, rs1008984, rs1435090, rs17203, rs768324, rs719776, rs1112828, rs1403454, rs3340, rs2077681, rs1935946, rs1320892, rs1373302, rs2695, rs1980888, rs1327805, rs2207782, rs1487214, rs2078588, rs724729, rs292932, rs1369290, rs386569, rs718092, rs878825 and rs16383). Information on the parental frequencies for these markers in Mexicans is available in a previous report from our group[31]. The AIMs were genotyped using a modified allele-specific PCR method with universal energy transfer-labeled primers by the company Prevention Genetics (Marshfield, Wisconsin, USA).

Statistical analysis

Comparison between groups was made using Kruskal–Wallis test for continuous variables and chi-square (χ^2) test for categorical variables. The allelic and phenotypic frequencies were calculated, and with the allelic frequencies of the control group the Hardy-Weinberg equilibrium was corroborated

We performed a logistic regression analysis to predict the risk of either T2D or MS in relation to the different genotypes in the three main inheritance models: codominant, dominant and recessive; adjusting by age, gender, BMI and individual ancestry. Odds ratio (*OR*) were estimated to assess the strength of the association.

To examine the likelihood that the results were false-positive findings, false-positive report probabilities (FPRP) were calculated using the methods described by Wacholder et al.[31]. We set 0.5 as the FPRP cut-off for a noteworthy value. The expected odds ratios (*ORs*) were based on reported ORs from previous stu-

dies[18,24,25,27,28,33]. Taking the previous literature recommendations between these polymorphisms, we set the prior probability of an association between each SNP and T2D and MS at 0.1-0.01. A prior probability of 0.1 represents a moderate to high prior probability of association and has been used in studies involving candidate genes/SNPs with prior evidence of association with disease[29].

Finally, we performed multiple linear regression analysis to examine the differences and the impact of these variants on insulin resistance and lipid profiles variables, (related to T2D and MS) including fasting insulin, HOMA-IR and LDL levels; all the models where adjusted by BMI, age, disease status and gender. A bootstrap bias-corrected confidence intervals and *p*-value was performed with 1473 Jacknife replications, and 10,000 bootstrap replications. Also we evaluated the potential interaction model between the two SNP's, but we didn't find a significative interaction (data not shown).

All Statistical analysis was performed with the Statistical Package STATA v9.1 software (Incorporation, Chicago).

Power Analysis

We used the power program v.3.00 (US National Institutes of Health (NIH), National Cancer Institute (NCI)) and Stata v9.1 software (Incorporation, Chicago) to estimate the statistical power of the study using the allele frequency reported in dbSNP database.

Power calculation

	T2D (CASES: 502, CONTROLS: 552)		SM (CASES:445, CONTROLS: 552)	
OR	***ADRB1*** **(rs1801253) (Arg389Gly**	***ADRB3*** **(rs4994) (trp64Arg)**	***ADRB1*** **(rs1801253) (Arg389Gly)**	***ADRB3*** **(rs4994) (trp64Arg)**
1.5	0.69	0.86	0.63	0.83
2	0.98	0.99	0.97	0.99
2.5	1	1	1	1

Results

Clinical data

General information and ethnic admixture of participants in the study is shown in table I. Mean age of controls was lower than the mean age of those with MS or with T2D. Patients with T2D were characterized by hyperglycemia, hyperinsulinemia and insulin resistance, while those with MS showed obesity, hypertension, hyperinsulinemia, low HDL levels, hypertriglyceridemia and a high atherogenic index.

Genotype and allele frequencies for the Arg389Gly variant of *ADRB1* gene were in Hardy-Weinberg equilibrium ($P > 0.05$). For the *ADRB1* gene, the MAF (minor allele frequency) was similar in the three groups (controls 13.8%, MS 11.52% and T2D 11.25%). The *ADRB3* gene was in Hardy-Wenberg equilibrium ($p < 0.05$), the MAF showed a higher frequency in the T2D group (26.49%), followed by the observed in the MS group (23.48%), compared with the frequency obtained for the control group (21.20%).

Risk analysis

Table II shows a multiple logistic regression analysis adjusted by age, gender, BMI and individual ancestry in three inheritance models: codominant, dominant and recessive. For *ADRB3* Trp/Arg genotype in the codominant model, there was an *OR* of 1.53 (1.09–2.13, $P < 0.003$) which was increased up to2.99 (1.44–6.22, $P < 0.003$) for the Arg/Arg genotype. Similar risk association was found under the dominant model Trp/Arg-Arg/Arg genotypes with an *OR* of 1.67 (1.21–2.30; $P < 0.002$). In the recessive model (Arg/Arg genotype), there was also a high association (*OR* =2.56, 95%CI=1.24–5.26, $P < 0.01$).

Based on a moderate to high prior probability of 0.1 to an expected *OR* of 3, the FPRP for an association between *ADRB3* (rs4994) and T2D was 0.026. The FPRP ranged from 0.026 for a prior probability of 0.1 and an *OR* of 3, to 0.88 for a prior probability of 0.01 and an *OR* of 1.5 (see supplementary material 1).

Finally, to explore the possible mechanisms of these variants we tested their effects in the quantitative traits for insulin resistance and lipid profile measured by insulin level, glucose, HOMA-IR, LDL, HDL, triglycerides and cholesterol The variant Arg389Gly of *ADRB1* was statistically associated with an increase of LDL levels ($P < 0.008$), and the variant *ADRB3* Trp64Arg was associated to higher HOMA-IR ($P < 0.018$) and to an increase of insulin levels ($P < 0.001$) (table III). After resampling 1,473 Jacknife and 10,000 bootstrap replications, bias-corrected confidence intervals and *p*-value were significant (table III). An association analysis of these two SNPs with quantitative traits such as body mass index, fasting glucose, blood HDL cholesterol, fasting blood triglycerides, systolic blood pressure, diastolic blood pressure, waist-hip ratio was also performed using a linear regression to compare the equality of means across genotypes. This analysis was adjusted by gender, age and disease status; however, we did not find any suggestion of association for the quantitative traits assessed (see supplementary material 2).

Discussion

In this study we verified the association of Arg389Gly *ADRB1* and Trp64Arg *ADRB3* polymorphisms with the phenotype of adult population of Me-

Table II
ADRB1 and ADRB3 association with MS and T2D (n = 997)

ADRB1 (rs1801253)	*Genotype (Arg389Gly)*	*Controls*	*MS*	*OR (95% CI)*[§]	*p*[§]	*OR (95% CI)*[&]	*p*[&]	*T2D*	*OR (95% CI)*[§]	*p*[§]	*OR (95% CI)*[&]	*p*[&]
Codominant	Arg/Arg	408 (73.9%)	340 (76.4%)	1	0.64	1	0.60	398 (79.4%)	1	0.29	1	0.74
	Arg/Gly	136 (24.6%)	99 (22.2%)	0.90 (0.65–1.23)		0.90(0.63- 1.27)		94 (18.8%)	0.72 (0.48–1.09)		0.81(0.55,1.18)	
	Gly/Gly	8 (1.4%)	6 (1.4%)	0.66 (0.21–2.07)		1.23(0.28-5.34)		9 (1.8%)	0.79 (0.22–2.84)		1.26 (0.30, 5.15)	
Dominant	Arg/Arg	408 (73.9%)	340 (76.4%)	1	0.43	1	0.50	398 (79.4%)	1	0.12	1	0.33
	Arg/Gly–Gly/Gly	144 (26.1%)	105 (23.6%)	0.88 (0.65–1.20)		0.90(0.65-1.21)		103 (20.6%)	0.73 (0.49–1.08)		0.83(0.57, 1.20)	
Recessive	Arg/Arg–Arg/Gly	544 (98.5%)	439 (98.7%)	1	0.50	1	0.67	492 (98.2%)	1	0.80	1	0.70
	Gly/Gly	8 (1.4%)	6 (1.4%)	0.68 (0.22–2.12)		0.78(0.24-2.46)		9 (1.8%)	0.85 (0.24–3.04)		1.32(0.32,5.40)	
ADRB3	Genotype											
(rs4994)	(Trp64Arg)	Controls	MS	OR (95% CI)	P	OR (95% CI)[&]	P[&]	T2D	OR (95% CI)	P	OR (95% CI)[&]	P[&]
Codominant	Trp/Trp	336 (60.9%)	261 (58.6%)	1	0.38	1	0.44	276 (55.1%)	1	0.001	1	0.003
	Trp/Arg	198 (35.9%)	159 (35.7%)	1.12 (0.84–1.49)		1.31 (0.82-1.55)		184 (36.7%)	1.42 (1.00–2.01)		1.53(1.09, 2.13)	
	Arg/Arg	18 (3.3%)	25 (5.6%)	1.53(0.79–2.97)		1.58(0.75-3.33)		41 (8.2%)	3.37 (1.62–6.99)		2.99(1.44, 6.22)	
Dominant	Trp/Trp	336 (60.9%)	251 (58.6%)	1	0.29	1	0.30	276 (55.1%)	1	0.005	1	0.002
	Trp/Arg–Arg/Arg	216 (39.1%)	184 (41.4%)	1.16 (0.88–1.53)		1.17(0.86-1.59)		225 (44.9%)	1.60 (1.15–2.24)		1.67(1.21, 2.30)	
Recessive	Trp/Trp–Trp/Arg	534 (96.7%)	420 (94.4%)	1	0.24	1	0.27	460 (91.8%)	1	0.002	1	0.01
	Arg/Arg	18 (3.3%)	25 (5.6%)	1.47 (0.77–2.83)		1.51(0.72-3.14)		41 (8.2%)	2.93 (1.44–5.99)		2.56 (1.24, 5.26)	

[§]Adjusted by age, gender and BMI, [&]Adjusted by age, gender, BMI and ancestral percentage of African, Caucasian and Amerindian.

Table III
Polymorphism effect over quantitative phenotypes, adjusted by BMI, gender, age and disease status

	LDL	*Genotype*	*Beta (95% CI)*	*P*	*P (95% CI)&*
ADRB1 (rs1801253)	Codominant	Gly/Gly	Reference	–	
		Arg/Gly	20.50 (5.35, 35.6)	0.008	0.004 (6.34, 34.62)
		Arg/Arg	15.62 (0.72, 30.0)	0.040	0.026 (1.89, 29.16)
	Dominant	Arg/Gly–Gly/Gly	16.64 (1.86, 31.42)	0.027	0.015 (3.22, 30.07)
	Recessive	Gly/Gly–Arg/Gly	Reference	–	
		Arg/Arg	–3.62 (–7.95, 0.70)	0.101	0.123 (–8.23, 0.98)
		R2: 0.04			
	HOMA-IR	*Genotype*	*Beta (95% CI)*	*P*	*P (95% CI)&*
	Codominant	Gly/Gly	Reference	–	
		Arg/Gly	0.56 (–0.78, 1.90)	0.413	0.197 (–0.29, 1.41)
		Arg/Arg	0.68 (–0.55, 2.07)	0.255	0.068 (–0.057, 1.58)
	Dominant	Arg/Gly–Gly/Gly	0.71 (–0.59, 2.02)	0.283	0.086 (–0.11, 1.53)
	Recessive	Gly/Gly-Arg/Gly	Reference	–	
		Arg/Arg	0.23 (–0.14, 0.62)	0.224	0.17 (–0.01, 0.57)
		R2: 0.27			
	Insulin level	*Genotype*	*Beta (95% CI)*	*P*	*P (95% CI)&*
	Codominant	Gly/Gly	Reference	–	
		Arg/Gly	–0.66 (–3.92, 2.59)	0.690	0.651 (–3.53, 2.21)
		Arg/Arg	0.12 (–3.05, 3.30)	0.937	0.941 (–3.23, 3.49)
	Dominant	Arg/Gly–Gly/Gly	–0.05 (–3.22, 3.12)	0.976	0.972 (–2.84, 2.74)
	Recessive	Gly/Gly–Arg/Gly	Reference	–	
		Arg/Arg	0.74 (–0.18, 1.77)	0.115	0.093 (–0.13, 1.62)
		R2: 0.11			
	LDL	*Genotype*	*Beta (95% CI)*	*P*	*P (95% CI)&*
ADRB3 (rs4994)	Codominant	Trp/Trp	Reference	–	
		Trp/Arg	–5.14 (–9.03, –1.26)	0.009	0.010 (–9.02, –1.28)
		Arg/Arg	–2.59 (–10.56, 5.57)	0.533	0.54 (–10.96, 5.76)
	Dominant	Trp/Arg–Arg/Arg	–4.81 (–8.53, –1.08)	0.011	0.010 (–8.46, –1.15)
	Recessive	Trp/Trp–Trp/Arg	Reference	–	
		Arg/Arg	–0.62 (–8.66, 7.42)	0.880	0.882 (–8.79, 7.55)
		R2: 0.04			
	HOMA-IR	*Genotype*	*Beta (95% CI)*	*P*	*P (95% CI)&*
	Codominant	Trp/Trp	Reference	–	
		Trp/Arg	0.42 (0.07, 0.75)	0.018	0.022 (0.06, 0.77)
		Arg/Arg	0.26 (–0.45, 0.98)	0.473	0.473 (–0.048, 1.00)
	Dominant	Trp/Arg–Arg/Arg	0.39 (0.07, 0.72)	0.020	0.021 (0.06, 0.73)
	Recessive	Trp/Trp–Trp/Arg	Reference	–	
		Arg/Arg	0.10 (–0.60, 0.81)	0.775	0.783 (–0.63, 0.84)
		R2: 0.27			
	Insulin level	*Genotype*	*Beta (95% CI)*	*P*	*P (95% CI)&*
	Codominant	Trp/Trp	Reference	–	
		Trp/Arg	1.43 (0.60, 2.26)	0.001	0.001 (0.55, 2.32)
		Arg/Arg	0.70 (–1.03, 2.44)	0.426	0.427 (–1.03, 2.44)
	Dominant	Trp/Arg–Arg/Arg	1.34 (0.54, 2.13)	0.001	0.001 (0.52, 2.16)
	Recessive	Trp/Trp–Trp/Arg	Reference	–	
		Arg/Arg	0.15 (–1.56, 1.87)	0.859	0.859 (–1.55, 1.86)
		R2: 0.12			

LDL: low density lipoprotein , *Multiple linear regression adjusted by BMI, gender, age and disease status, & bootstrap bias-corrected confidence intervals and p-value

xico City affected of MS or T2D. The results of this study show that the Trp64Arg of *ADRB3* gene is a biomarker for T2D, and the Arg389Gly variant of *ADRB1* is statistically associated with an increase in LDL levels ($P < 0.008$).

Others reports have demonstrated that the Trp64Arg variant is associated to an early onset of T2D and obesity[23,24,33]. Our results are consistent with this notion; we proved that the Arg64 genotype of Trp64Arg polymorphism is associated with an increased risk of T2D in Mexicans, with a dose dependent allele effect, being higher in the homozygous carriers for the Arg64 allele (2.99 *CI* 95%; 1.44–6.22, $P < 0.003$) (table II). Furthermore, the Trp/Arg heterozygous genotype was associated with lower insulin secretion (*CI* 95%; 1.56, 0.71–2.40, $P < 0.001$) (table III), and a dose dependent allele effect was observed with insulin resistance, and this association was particularly high in homozygous individuals to the Arg64 allele. This data agrees with a previous *in vitro* experimental report where the RIN-M5F cell line transfected with the variant of *ADRB3* gene (Arg64) showed abnormal insulin secretion[34]. Couple with this, it has been reported[35] that individuals homozygous for the Arg64 allele, exhibited low insulin secretion levels in response to glucose, in comparison to individuals homozygous for the Trp64 allele. These observations suggest that obese individuals with T2D have a genetic predisposition to functional abnormalities in the pancreatic β-cells[36]. A recent model proposed that subjects with Arg64 allele are exposed to an increased risk to develop T2D through insulin resistance at the target organs (with larger effect in fatty tissue), due to insulin secretion deficiency, characterized by defective glucose detection[33].

Our results for the Arg/Arg genotype of Trp64Arg in Mexicans, indicates that the frequency for this allele (7.29%) is lower than that determined in Pima Indians (9.6%), but larger than the observed in a Japanese population (1.4.%)[33], showing that ethnic differences influence the genotypic distribution of this variant. In Pima and Japanese population, the Arg64 variant has been associated with early onset of T2D, low metabolic rate, insulin resistance and abdominal obesity[33,37]. Our data suggests that the Arg64 allele of *ADRB3* gene can be considered an important risk factor for T2D in our population.

Likewise, the frequency for Arg389 allele in healthy subjects (79.28%) was lower to the frequency previously reported for mestizos (85.30%), differing also with what was reported in Hispanic (67.2%) and American (72.3%) populations. Like the Trp64Arg polymorphism of the ADRB3 gene, this variant also shows ethnic differences for the genotypic distribution[18]. *In vitro* studies suggest that Glycine to Arginine substitution in position 389 brings an improvement in the receptor function[17]. At functional level it has been reported that the stimulation of the 1 adrenergic receptor causes a decrease of the circulating levels of leptin and that there is a relationship between weight loss and reduction of the β1 adrenergic receptor expression. Supporting our results, it has been previously reported an association of Arg389 allele with an increase in BMI and fat accumulation in Caucasian women[19].

On the other hand, the Arg389Gly polymorphism of *ADRB1* gene did not increase the risk for T2D in our study. Although the study is not focused on hypertension, we found that homozygous individuals for Arg389 have a tendency to exhibit elevated levels of diastolic pressure (data not shown). It is possible that the apparent non association with hypertension must be due to the experimental design that did not target hypertension or cardiovascular disease in the population. Also, it has been observed the Arg389 variant can potentially be a drug-genetic target for hypertension, since it has been demonstrated that homozygous individuals for Arg389 allele are more responsive to treatment with β-blocking drugs[20].

Taken these data together, our data suggest that for the Mexico City population, the Trp64Arg polymorphism of *ADRB3* is a marker for T2D. On the other hand, the analysis of the quantitative phenotype/genotype traits showed the *ADRB3* Trp64Arg was associated to higher HOMA-IR ($P < 0.018$) and insulin levels ($P < 0.001$); and the *ADRB1* Gly389 allele was associated with an increased LDL levels ($P < 0.009$) (table III). We conclude that the *ADRB3* Trp64Arg variant is a susceptibility gene for T2D and *ADRB1* Gly389Arg *for* lipid metabolism disruption. These results make these variants potential biomarkers for predicting metabolic alterations and evolution in diabetic and metabolic syndrome patients.

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