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Association of Gly972Arg polymorphism of *IRS1* gene with type 2 diabetes mellitus in lean participants of a national health survey in Mexico: a candidate gene study

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Abstract

Type 2 diabetes mellitus (T2D) is a main public health problem in the Mexican population. It is characterized by insulin resistance in peripheral tissues and a relative deficiency in the pancreatic β-cell functions. Diverse single nucleotide polymorphisms (SNPs) of the *IRS1* gene have been associated with insulin resistance and T2D risk. The aim of this study was to identify the association between known IRS1 polymorphisms (Pro512Ala, Asn1137Asp, Gly972Arg, and Arg158Pro) in a sample of diabetic patients compared with healthy controls selected from Mexico's 2000 National Health Survey, both with normal body mass index (BMI). We identified 444 diabetes cases that were age matched with the same number of controls. Genotypic and allelic frequencies were evaluated, and conditional logistic regression was used to evaluate the association between the SNPs and diabetes risk. Of the 4 SNPs studied, only Gly972Arg showed significant differences between cases and controls, with allele frequency of 2.6% in controls as compared with 7.9% in cases. Subjects with at least 1 copy of the Gly972Arg polymorphism of the *IRS1* gene showed a greater risk for diabetes, with a crude odds ratio of 3.26 (95% confidence interval, 2.00-5.33); after adjusting for BMI, age, family history of T2D, and sex, the odds ratio was 2.91 (95% confidence interval, 1.73-4.90). Our results suggest the participation of Gly972Arg polymorphism of *IRS1* in the genetic susceptibility to TD2 in Mexican population. The restriction of including only participants with normal BMI might increase the power to detect genetic determinants of T2D.
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1. Background

Type 2 diabetes mellitus (T2D) is a phenotypic and genetically heterogeneous chronic disease [1] that represents 90% to 95% of all diabetes types; given its magnitude, it has become an increasingly important public health problem worldwide, occurring in ever-younger individuals [2]. In México, the National Health Survey 2000 (ENSA 2000) showed a T2D prevalence of 7.5% in individuals 20 years and older [3].

Type 2 diabetes mellitus is a multifactorial disease that derives from the coexistence of genetic and environmental factors, as well as particular behaviors that contribute to the development of the disease. It is characterized by insulin resistance in peripheral tissues, not compensated by a relative deficiency in the insulin synthesis and secretion of pancreatic β-cells [4,5]. At the experimental level, pathogenic diabetes models suggest that hyperglycemia is the result of insulin resistance and of β-cells being exhausted [6]. Studies performed in normoglycemic subjects also suggest

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that insulin resistance in skeletal muscle is the earliest defect in the development of T2D [7,8].

Studies carried out to identify genetic and nongenetic components participating in homeostatic regulation of glucose and in T2D physiopathology have identified insulin resistance as a postreceptor defect that ultimately affects translocation of the glucose transporter GLUT4 toward the cell surface [9,10]. The transduction of insulin signals is mediated by a series of phosphorylation cascades linked to the initial activation of the tyrosine kinase receptor of insulin and its action on the substrates of the insulin receptors (insulin receptor substrate IRS1, IRS-2, IRS-3, and IRS-4) [11]. Tyrosine phosphorylation of IRS1 and its binding to phosphatidylinositol 3-kinase are critical events in the insulin signaling cascade leading to insulin-stimulated glucose transport. [12]. The importance of IRS1 in insulin signaling has been confirmed in studies showing that this gene plays a very important role not only in peripheral insulin sensitivity, but also in the regulation of insulin secretion by pancreatic β-cells [12,13]. In addition, IRS1 knockout mice adipocytes showed considerable decrease in glucose transport and in the translocation of GLUT4 to the plasma membrane as a response to insulin [14]. Insulin receptor substrate–1, whose gene is located in chromosome 2q36, has 21 sites for tyrosine kinase phosphorylation, which are responsible for most of its enzymatic function.

Several polymorphisms have been reported in coding and noncoding regions of the IRS1 gene. Among those, the Gly972Arg polymorphism has been found to be related to an imbalance of insulin action and T2D risk [15,16]. In a meta-analysis of studies looking at the association of the IRS1 Gly972Arg polymorphism with T2D, Jellema et al [17] in 2003 assessed articles reporting such an association in 27 populations and found a significant association between the Gly972Arg polymorphism of the IRS1 gene and T2D, with an odds ratio (OR) of 1.25 (95% confidence interval [CI], 1.05-1.48) in carriers of the polymorphism with respect to noncarriers. However, despite the body of evidence supporting the functionality of this single nucleotide polymorphism (SNP), subsequent to this publication, at least 2 large studies failed to replicate this association [18,19].

Based on our previous observation suggesting a greater genetic predisposition among lean diabetics [20], in the present analysis, we aimed to evaluate the association of the Gly972Arg polymorphism and other polymorphic variants on the IRS1 gene with T2D in a representative sample of the Mexican population with body mass index (BMI) less than 25 kg/m^2.

2. Methods

2.1. Study population

The data and specimen source was the Mexican ENSA 2000 conducted by Mexico's Ministry of Health and the National Institute of Public Health. This was a nationally representative survey using multistage stratified cluster sampling, where the households were the primary sampling units. Participants were randomly selected within each household. A detailed description of the methods has been published elsewhere [21].

From the entire database of the ENSA 2000 survey, we defined as the eligible population for the selection of unrelated cases and controls participants 35 years and older who accepted to donate a blood sample and who had a measured BMI less than 25 kg/m^2 at the time of the survey. Anthropometric measurements were performed. Height was measured for each participant in the survey with standardized stadiometers (SECA ADEX Products, Mexico City, Mexico); and weight, with standardized Solar Scale (Tanita, Arlington Heights, IL). Both were measured to the nearest 5 mm and 0.1 kg, respectively, with the subject in light clothing without shoes. Body mass index was calculated as kilograms per meters squared. [21]. Blood pressure (BP) was measured in the right arm by the same research nurse, after sitting for at least 5 minutes, using a standard aneroid sphygmomanometer (ADEX Products). The Korotkoff sound V was taken as the diastolic BP. *Hypertension* was defined as a systolic BP of 140 mm Hg and/or diastolic BP of 90 mm Hg or more. Furthermore, we did not find statistical differences between cases and controls (data shown in Table 1).

In this subset of the original database, we selected a total of 444 diabetes cases that were matched by age categories (5 years) to a random selection of controls with BMI not exceeding 25 kg/m^2.

We considered a T2D case if, during the survey, the participants self-reported a previous medical diagnosis with antidiabetic therapy. Controls were nondiabetic subjects

Table 1
General characteristics of the study population

Characteristics	Cases (444)	Controls (444)	*P* value
Age (y)			
Mean (SD)	62 (11.21)	62 (11.16)	.901[a]
Age at time of diagnosis (ys)			
Median (min-max)	50 (35-88)	NA	NA
Years with T2D median (min-max)	10 (0-40)	NA	NA
Family history of T2D (%)			
Yes	36	13	.001[b]
No	64	87	
Country region (%)			
North	26	20	.135[b]
Center	38	41	
South	36	39	
BMI (kg/m^2)			
Median (min-max)	23.38 (14.48-24.99)	22.84 (14.46-24.99)	.001[a]
Sex (%)			
Male	34	48	.001[b]
Female	66	52	

NA indicates not available.
[a] Mann-Whitney test.
[b] χ^2 test.

without a medical self-reported diagnosis and with capillary casual (independent of last eating time) glycemia less than 120 mg/dL. The use and analysis of the DNA Bank were approved by the Institutional Review Board of the National Institute of Public Health.

2.2. SNPs selection

Among validated SNPs (by frequency or utilization in the HapMap project) within the IRS1 gene, we selected SNPs that were more likely to be causal because they either resulted in a nonsynonymous change in the amino acid sequence or were located in the 3′ untranslated region (UTR) and 5′ UTR. We excluded SNPs without any evidence of a heterozygosis greater than 0.01. Based on this strategy, using the software from the Ensembl project (http://www.ensembl.org/Homo_sapiens), we selected 4 nonsynonymous SNPs: Pro512Ala (rs1801276), Asn1137Asp (rs3731594), Gly972Arg (rs1801278), and Arg158Pro (rs1801108). None of the SNPs located in the UTRs fulfilled the previous selection criteria.

2.2.1. DNA extraction and SNP genotyping

Using peripheral blood samples, genomic DNA extraction was performed by a semiautomated method with the ABI PRISM 6100 Nucleic Acid PrepStation (Applied Biosystems, Foster City, CA). Purity of the samples was evaluated by spectrophotometry and the DNA integrity in electrophoresis in agarose gels at 0.8% [22]. Allelic discrimination was done based on the 5′-nuclease assay, using the polymerase chain reaction technique, combined with fluorogenic TaqMan assay, using automated Applied Biosystems 7900HT Fast Real-Time equipment. All assays were done in duplicate and were analyzed and determined by graphic visualization using the Sequencing Detection System (SD1.1.1, Applied Biosystems).

The concordance between duplicate genotypes was greater than 90%; and for quality control of the genotyping, we used a call rate of 0.99 for cases and controls. When the call rate was less than 0.99, the DNA was reextracted; and new genotyping was done (only 5 samples required this procedure).

2.3. Statistical analysis

We compared relevant variables between cases and controls using χ^2 or Mann-Whitney U test for categorical and continuous variables, respectively. Hardy-Weinberg equilibrium was assessed for each SNP.

Given the low minimum allele frequency of 3 of the 4 genotyped SNPs, we evaluated exclusively the dominant model for these SNPs by grouping the heterozygous and the homozygous for the rare variant in one category. For Arg158Pro SNP, in addition to the dominant model, we were able to evaluate other genetic models including the codominant (3 categories), the allele additive, and the recessive model.

Using a bivariate analysis with conditional logistic regression (by age categories), ORs were obtained with their respective 95% CI for each one of the possible genotypic variants. The reference variant was the homozygous of the most common variant. Potential predictors of T2D risk were included in the multivariate model (sex, BMI, family hereditary background). The SNP-SNP and SNP-environment interactions were tested by adding multiplicative terms in the multivariate logistic models.

To examine the likelihood that our results were false-positive findings, the false-positive report probability (FPRP) was calculated using the methods described by Wacholder et al [23]. We set .5 as the FPRP cutoff for a noteworthy value. The expected ORs were based on the average of reported ORs from previous studies [17]. Given the previous literature suggesting associations between this polymorphism, we set the prior probability of an association between each SNP and T2D at .1 to .01. A prior probability of .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 [23].

All analyses were done using the version 9.1 STATA statistical package (College Station, TX). On the other hand, the linkage disequilibrium (LD) was evaluated using the *R* and Haploview software programs.

3. Results

The characteristics of the study population are described in Table 1. Significant differences were observed between cases and controls for the variables concerning family history of T2D, BMI, and sex ($P < .05$); these variables were considered in the multiple model as adjustment variables.

Table 2
Allelic and genotypic frequencies and Hardy-Weinberg equilibrium

	Genotypic frequency (%)		Allelic frequency (%)		HWE[a]
	Cases (444)	Controls (444)	Cases (444)	Controls (444)	*P* value
Gly972Arg (rs1801278)					
GG	84.7	94.8	G 92.1	G 97.4	.99
GA	14.9	5.2	A 7.9	A 2.6	
AA	0.4	0			
Pro512Ala (rs1801276)					
CC	79.5	79.5	C 89.3	C 89.1	.63
CG	19.6	19.1	G 10.7	G 10.9	
GG	0.9	1.4			
Asn1137Asp (rs3731594)					
GG	97.1	95.9	G 98.3	G 98.0	.99
GA	2.5	4.1	A 1.7	A 2.0	
AA	0.4	0			
Arg158Pro (rs1801108)					
GG	62.6	59.7	G 79.1	G 77.9	.27
GC	32.9	36.5	C 20.9	C 22.1	
CC	4.5	3.8			

[a] Hardy-Weinberg equilibrium *P* value χ^2 test in controls.

A descriptive analysis of selected polymorphisms was carried out (Table 2), establishing genotypic frequencies for the Pro512Ala (rs1801276), Asn1137Asp (rs3731594), Gly972Arg (rs1801278), and Arg158Pro (rs1801108) variants of IRS1 gene in the sample of individuals with T2D and in controls. A greater frequency was observed for the G/A genotype of the Gly972Arg polymorphism among cases (14.9%) than controls (5.2%). We did not find significant deviations from Hardy-Weinberg equilibrium among controls in any of the genotyped SNPs.

There were no significant differences of the distribution frequencies of the polymorphism and their haplotypes between the T2D group and nondiabetic control group ($P > .05$). (Figs. 1 and 2).

Table 3 shows the effect of the 4 polymorphisms and the risk of T2D with ORs estimated through conditional logistic regression. We observed that only the Gly972Arg polymorphism had a significant association with an OR of 3.26 (95% CI, 2.0-5.3) for the dominant model. The other 3 SNPs showed no significant differences in any of the genetic models tested, including the recessive model for the Arg158Pr SNP (not shown), for which the crude OR was 1.17 (95% CI, 0.60-2.26). The multiple model adjusted for sex, BMI, and family history of T2D did not change considerably the conclusion in any of the results. Specifically for the Gly972Arg polymorphism, the association was still significant, with an OR of 2.80 (95% CI, 1.2, 4.8) in the heterozygous and an OR of 2.91 in the dominant model. We did not observe a significant association for the other polymorphic variants of the IRS1 gene. All possible 2-way interactions between SNPs and between SNPs and relevant variables (sex, family history of T2D) were tested, and we did not find a statistical significance in any of them (data not shown). However, a stratified analysis by BMI suggested a stronger association between the Gly972Arg and T2D among participants with a BMI less than 23.1 kg/m^2 (the median value of the entire study sample) than among participants with a BMI of at least 23.1 kg/m^2, with ORs of 5.2 (95% CI, 2.2-12.3) and 2.38 (95% CI ,1.2-4.7) respectively, for the multivariate adjusted dominant model.

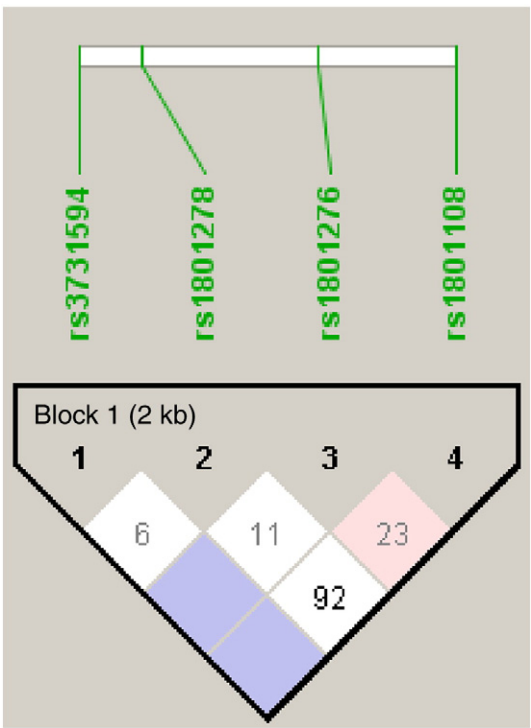

Fig. 1. Controls' LD.

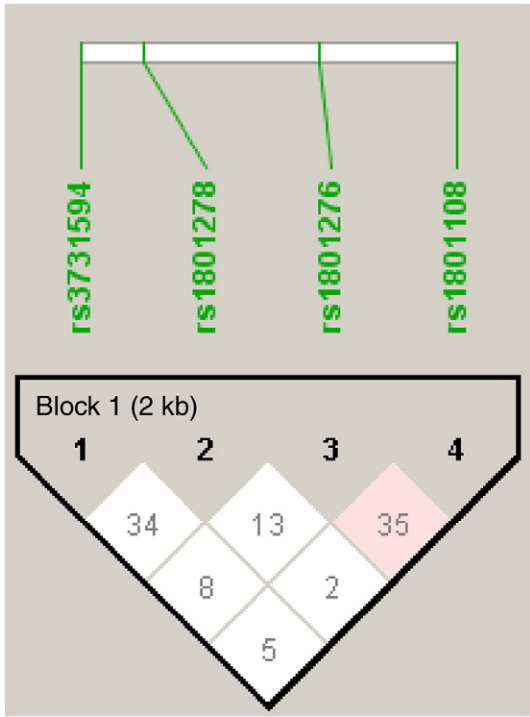

Fig. 2. Cases' LD.

4. Discussion

The main result of our study shows that, among lean individuals, carriers of polymorphism Gly972Arg of the IRS1 gene are at 3 times greater odds of having T2D, as compared with noncarriers. This association with T2D exists independently of potentially associated environmental factors like BMI, family history of diabetes, and sex. This observation suggests a possible relationship of polymorphism Gly972Arg in the pathogenesis of T2D. The other 3 tested SNPs on this gene were not associated with the presence of T2D. The SNP-SNP and SNP-environment interactions were not significant.

As in our results, Gly972Arg has been found with greater frequency in patients with T2D in many other studies and confirmed in a meta-analysis of 27 independent studies (3408 cases and 5419 controls) that showed the influence of

Table 3
Association of variants of IRS-1 gene and risk of T2D (conditional logistic regression)

Genotype	Cases	Controls	ORc	95% CI	*P* value	ORa	95% CI	*P* value
Gly972Arg								
GG	376	421	1			1		
GA	66	23	3.17	1.93-5.18	<.0001	2.80	1.66-4.73	<.0001
AA[a]	2	0	-	-	-	-	-	
GA + AA[b]	68	23	3.26	2.00-5.33	<.0001	2.91	1.73-4.90	<.0001
G	818	865	1					
A	70	23	3.22	1.99-5.20	<.0001			
Pro512Ala								
CC	353	353	1			1		
CG[a]	87	85	1.024	0.73-1.43	.87	0.95	0.64-1.40	.57
GG	4	6	-	-				
CG + GG[b]	91	91	1.00	0.72-1.38	1.00	0.88	0.60-1.30	.95
C	793	791	1					
G	95	97	0.977	0.72-1.32	.83			
Asn1137Asp								
GG	431	426	1			1		
GA	11	18	0.604	0.28-1.29	.19	0.51	0.21-1.23	.12
AA[a]	2	0	-	-	-	-	-	
GA + AA[b]	13	18	0.714	0.34-1.47	.36	0.61	0.27-1.4	.31
G	873	870	1					
A	15	18	0.830	0.42-1.66	.47			
Arg158Pro								
GG	278	265	1			1		
GC	146	162	0.85	0.65-1.14	.29	0.93	0.68-1.30	.38
CC	20	17	1.21	0.62-2.37	.74	1.53	0.62-3.77	
GC + CC[b]	166	179	0.884	0.67-1.16	1.00	0.91	0.66-1.25	.32
G	702	692	1					
C	186	196	1.069	0.85-1.34	.40			

ORc indicates nonadjusted; ORa, adjusted by sex, BMI, and family history of T2D.
[a] Exact confidence levels cannot be calculated because there are boxes with zero value or value less than 5.
[b] Dominant model.

this polymorphism on increased risk for T2D in carriers [17], with a summary OR of 1.25 (95% CI, 1.05-1.48). However, more recently, Florez et al [18], in a single study with 4279 cases and 3532 controls, failed to confirm the association of Gly972Arg with T2D risk despite an estimated power of more than 95% to detect an OR of 1.25. Another large study with 971 T2D cases and 1257 controls, enriched for family history and early onset of T2D, showed similarly null results for this association [19]. The publication of these 2 large well-powered studies has raised reservations about the validity or estimation of this association. Differences between populations could explain the heterogeneity of a true effect of this polymorphism on diabetes risk.

One of the potential explanations of the difference between our results and the 2 large independent studies with null results is that they included almost exlusively participants of Caucasian origin without any representation of Mexican origin. However, the Caucasian origin was also overrepresented in the metanalysis and showed the strongest significant association. Mexicans are only represented in 1 of the 27 studies included in the metanalysis [24]. In that study, Celi et al [24] included 58 Mexican Americans (31 T2D cases and 27 unrelated controls) and found a 4.3% frequency of the Arg allele of the Gly972Arg SNP (similar to our finding of 3% in the control populations), without any differences between cases and controls. Furthermore, Sanchez-Corona et al [25], in a study of 163 individuals with a high prevalence of obesity and dyslipidemia in Yucatan, Mexico, found no association of the Gly972Arg polymorphism with diabetes-related metabolic abnormalities. Similarly, Flores-Martinez et al [26], in a study of 73 Mexican subjects, found no association between Gly972Arg and T2D. These results, which are different from ours, could be due in part to the lack of statistical power due to small sample size. However, they suggest that the Mexican origin does not explain the differences between our finding and the 2 large well-powered studies. It should be further noted that the IRS1 polymorphism under study (or any good proxy for it, ie, a proxy with a LD >0.5) has not been confirmed in any genomewide association (GWA) study [27-31]. Furthermore, GWA studies will have to confirm the lack of association in the Mexican population, although some of them have done it in Mexican American populations, because the Mexican population may differ from European populations in mutational and demographic histories. On the other hand, it is important to mention that these GWA studies have not studied specifically the polymorphisms of the *IRS1* gene.

A more likely reason to explain why we found the strongest association of the Gly972Arg SNP and T2D risk can be supported by our design that restricted the study

population to subjects with BMI less than 25 kg/m^2. This restriction can be importantly enriching the genetic component of T2D in general and probably the effect of the IRS variation in particular. Although they were not statistically significant, the differences of the ORs we found in the stratified analysis by BMI support this notion. Moreover, differences in the ORs depending on the mean BMI, reported by Jellema et al [17] in the meta-analysis study, are consistent with our analysis, in that a stronger association was found among individuals with BMI less than 27 kg/m^2 (OR, 1.23; 95% CI, 0.75-2.01) than among individuals with BMI greater than 27 kg/m^2 (OR, 0.76; 95% CI, 0.46-1.25). However, the limited number of studies from which they were able to retrieve information on BMI (only 10 of 27 studies) limits the conclusion on this potential interaction, and further analysis is needed to explore this possibility.

Whether this potential effect modification by BMI has a biological meaning is something we are unable to explore in our analysis given the lack of intermediate biomarkers. Some [32,33] reports have shown that the effect of the Gly972Arg polymorphism on worsening insulin sensitivity is significantly stronger among obese individuals, which is against our findings. However, other studies have failed to demonstrate this significant interaction [34]; and others have suggested that insulin secretion rather than insulin sensitivity is the causal pathway by which this polymorphism increases T2D risk [35]. Moreover, even if this polymorphic variant has a stronger effect on insulin resistance among obese in contrast to lean individuals, it does not rule out the possibility of an interaction in the opposite way for the risk for T2D, as we show in this analysis. The relative contribution of this polymorphism to the risk of developing a binary outcome (T2D) can be qualitatively different than its contribution on the absolute difference of the continuous biomarker that, in theory, would explain the association, especially when the biomarker-disease risk association reaches a plateau where increments of biomarker do not necessarily translate into increasing risk. Given that obesity is the strongest risk factor for T2D risk, it is possible that its effect on diabetes risk masks a minor effect of the gene variation.

As suggested by others [36], haplotype block variation between populations can also explain the heterogeneity of the Gly972Arg-T2D association, assuming that this SNP is only a marker that has a strong LD with the truly causal variation or with the haplotype block where it is located. Given that our strategy to select the 4 tested SNPs was based on their potential functionality (nonsynonymous SNPs and in UTR regions) rather than on their potentiality to tag specific haplotype blocks, we were unable to evaluate this possibility. However, in contrast to what happens with most SNP association studies, there is a growing body of evidence on the mechanism by which the Gly972Arg polymorphism has an effect on IRS1 function and insulin signaling. Knockout models have shown that this polymorphism inhibits insulin signaling dependent on phosphatidylinositol 3-kinase in tissues that are sensitive to insulin, such as muscle and pancreatic β-cells. This causes multiple defects, including the translocation of the glucose transporter [37]. In addition to insulin resistance in peripheral tissues, diverse studies have shown that insulin secretion is lower in pancreatic β-cells that express the Gly972Arg polymorphism compared with carriers with the wild-type IRS1 variant, which suggests that this polymorphism decreases β-cells' ability to compensate insulin resistance [15,38]. Clinically, this translates into decreased concentration of postprandial plasma insulin and C peptide, which are present in states of hyperglycemia [35]. Recent reports also showed that this polymorphism has a direct effect on the survival of pancreatic islets, which confers susceptibility to a greater apoptosis of pancreatic β-cells [39] and thus diabetes risk. All these evidences of the causal role of specifically this Gly972Arg polymorphism make it unlikely that its effect on the association can be explained purely by its LD with other potentially causal variation of the gene.

Besides heterogeneity of a true effect, lack of consistency between studies can be due to spurious results. One of the reasons that can explain false-positive results is confounding by population stratification. Our study population comes from a national survey from different regions of the country. Having a homogenous distribution of the population and of the polymorphisms studied across different regions of the country (data available upon request) minimizes the possibility of confounding by population stratification. In case this effect is present, the strength of association of our results is unlikely to be totally due to it. However, we cannot totally reject that at least part of the association may be explained by population stratification; evaluation using family-based studies or ancestry-informative markers would avoid this potential confounder effect.

Finally, the greatest difficulty in identifying responsible genes associated with T2D in our study is defining the phenotype because the sample of cases and controls comes from a national cross-sectional survey with only self-reported clinical information and capillary blood samples, most of them taken without fasting. The case definition was not ideal; however, our definition is strengthened by the fact that all cases self-reported the medical diagnosis and use of hypoglycemic medication. Regarding the controls, we tried to minimize the misclassification by including only participants with capillary casual glucose less than 120 mg/dL. However, if misclassification exists in our case-control definition, there is no reason to think that it is related to the genotype of the individual (nondifferential misclassification); thus, if any bias exists, this will tend to move the estimate toward the null.

To assess the robustness of our findings, we determined the FPRP suggested by Wacholder et al [23]. Because it requires prior assignment of the probability that the polymorphism under study will be associated with the phenotype and although this may be subjective, it does force the use of small P values when the likelihood of an

association is small. The FPRP value we obtained was compatible with the stringent level set by Wacholder et al [23]; because this approach was only recently proposed, there are no comparative data and further analyses and studies of this polymorphism are required (see supplementary material).

In conclusion, having only considered subjects with a BMI less than 25 kg/m^2 provides strong evidence of the importance of the genetic effect of Gly972Arg on diabetes risk. Although its contribution to the overall risk in the general population could be minimal, this evidence supports the line of research seeking to clarify the role of IRS1 in lean patients with diabetes. Further studies of this genetic effect are needed to evaluate its potential interaction with other factors—especially with genetic variation, risk factor as obesity—that participate in the same metabolic pathway.

5. Competing interests

The authors declare no competing interests.

6. Authors' contributions

BGAI: made substantial contributions to conception, design, acquisition of data, analyses, interpretation of data, and drafting of manuscript. She carried out the molecular genetics study and coordinated all the development of the research.

CLM: participated in analyses and interpretation of the results and in drafting and revising the manuscript.

VME: contributions to conception, design, acquisition of data, ethical issues and institutional review board clearance, and drafting and revising the manuscript.

WCS: contributed to conception and design.

RLR: made substantial contributions to conception, design, interpretation of results, and drafting of the manuscript.

HAM: contributions to conception, design, and acquisition of data.

CB: carried out the molecular genetics study.

GRE: carried out the molecular genetics study.

MMV: made substantial contributions to conception, design, acquisition of data, analyses, interpretation of data, manuscript drafting, and revising the manuscript and coordinated all the development of the research.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.metabol.2009.07.007.

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