Clinical Medicine Reviews in Vascular Health

Libertas Academica
FREEDOM TO RESEARCH

CMR
Clinical Medicine Reviews

ORIGINAL RESEARCH

Polymorphism Study of the Insulin Receptor Substrate IRS1 and IRS2 Genes Associated with Type 2 Diabetes in Ethnic Groups of Djerba Island

T. Baroudi Ouederni[1], J. Sanchez-Corona[2], A. Fadiel[3], T. Johnson-Scalise[6], A. Beeghly-Fadiel[7], S. Flores Martinez[2], H. Ben Maiz[4], H. Kammoun Abid[4], H. Aounallah Skhiri[5], A. Benammar-Elgaaied[1] and A. Hamza[1]*

[1]Laboratory of Genetics, Immunology and Human Pathologies, Faculty of Sciences of Tunis, El Manar University, 2092 Tunis, Tunisia. *Present Address: Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, Kentucky, USA. [2]División de Medecina Molecular, Centro de Investigación Biomédica de Occidente, Instituto Mexicano Del Seguro Social, Guadalajara, Jalisco, México. [3]Department of Obstetrics and Gynecology, New York University School of Medicine, New York, NY 10016, USA. [4]Charles Nicolle Hospital, Internal Medicine A, 1060 Tunis, Tunisia. [5]National Institute of Public Health and Preventive Medicine Tunis, Tunisia. [6]Department of Obstetrics and Gynecology, Meharry Medical College, Nashville, Tennessee. [7]Vanderbilt Epidemiology Center, Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville, Tennessee.
Corresponding author email: ahamz3@email.uky.edu

Abstract

Aim: We aimed to evaluate the influence of the Gly972 Arg and Ala513Pro variants of the insulin receptor substrate1 gene (IRS1) and the Gly1057 Asp variant in IRS on type 2 diabetes mellitus (T2DM) in Arab and Berber men and women from Djerba Island, Tunisia.
Methods: Genotypes, allelic and genotypic frequencies were studied. The amplified products were analyzed by Restriction Fragment Length Polymorphism (RFLP) methods by comparing T2DM with healthy controls from the same ethnicity.
Results: No differences in genotype or allelic frequencies were found between T2DM and healthy controls in either Arab or Berber ethnic groups.
Conclusion: The Gly972 Arg and Ala513Pro variants in IRS1 and Gly1057 Asp in IRS2 polymorphisms are not associated with T2DM in the Arab or Berber populations of Djerba Island

Keywords: T2DM, IRS1, IRS2, Gly972Arg, Ala513Pro, Gly1057Asp

Clinical Medicine Reviews in Vascular Health 2010:2 185–190

This article is available from http://www.la-press.com.

Introduction

Type 2 diabetes mellitus (T2DM) usually results from a combination of defects in insulin action and insulin secretion.[1] Normally, in response to elevation of plasma glucose, insulin is secreted by β cells in the islets of Langerhans, which then promotes energy uptake by myocytes, adipocytes and hepatocytes, metabolism, and storage. The development of insulin resistance in target tissues precedes T2DM,[2] but the molecular mechanisms underlying T2DM's initiation and progression remain unclear although the combination of genetic and environmental factors may contribute.[3]

The insulin receptor substrate (IRS) proteins are critical to signal transduction in insulin target tissues.[4] The binding of insulin to its receptor induces the phosphorylation of the cytosolic substrates IRS1 and IRS 2.[5] Nevertheless, the response of IRS1 and IRS2 to tyrosine protein kinases depends upon the binding specificity and affinity of the tyrosine phosphorylation sites within the IRS, which can be altered by mutated amino acid polymorphisms within the phosphotyrosine binding (PTB) domain.[6] IRS1 and IRS2 have been considered candidate genes for T2DM. The insulin receptor substrate 1 (IRS1) mediates the signal from the insulin receptor tyrosine-kinase that initiates insulin action in sensitive tissue.[7] Almind[8] describes two single base-pair mutations in diabetic patients at codons 513 $GCC^{Ala} \rightarrow CCC^{Pro}$ and 972 $GGG^{Gly} \rightarrow AGG^{Arg}$ of the IRS1 gene. Although those mutations were also found in normoglycaemic individuals, their combined prevalence was significantly higher in diabetic patients. IRS-1 gene is associated with the development of insulin resistance and T2DM in some populations but not in others.[9] Taken together, these data raise the possibility that Arg972 polymorphism of IRS1 might contribute to insulin resistance in T2DM. In humans, a number of polymorphisms have been identified in the IRS2 gene. Among those, the amino acid substitution Gly1057 Asp codon 1057 $GGC^{Gly} \rightarrow GAC^{Asp}$ was found in various populations with a prevalence sufficiently high to modulate a population's risk of T2DM. Disruption of IRS-2 was shown to cause T2DM in mice.[10] In contrast, three recent studies have shown a lack of association between IRS-2 polymorphisms and T2DM, thus arguing against a major role of polymorphisms in the IRS-2 gene in the etiology of both common and early onset autosomal dominant T2DM.[11]

Djerba is an island situated in the south-eastern section of Tunisia where several ethnic groups, i.e. Arabs and Berbers, have cohabited for centuries. Religious and cultural differences have prevented the interblending of these two groups. To evaluate the genetic differences between the two ethnic groups (Arabs and Berbers), we have analyzed the polymorphism at codons 513 and 972 of the IRS-l gene polymorphisms and Gly1057 Asp polymorphism of the IRS2 gene in these well defined populations with a high prevalence of T2DM.[12]

Materials and Methods

Subjects

A total of 272 Djerbian subjects (men and women), including 162 unrelated patients with T2DM, and 110 unrelated control subjects were analyzed in this study. These subjects belong to two closely related villages, i.e. Hoummet Essouk and Middoun for the Arab ethnic group and Sedwikch and Guellala for the Berber ethnic group. All members of the prospective populations had resided in the community during at least 10 years.

The patients were recruited from the Departments of Endocrinology at Djerba hospitals. Informed consent was obtained from all participants, and the study was approved by the Ethical Committee of the University of Medicine. The normoglycemic controls were recruited from the same community villages as the patients.

Weight and height were measured on barefooted and lightly clothed subjects. Body mass index (BMI; kg/m^2) was calculated and obesity was defined as BMI $\geq$ 30 kg/m^2. Diabetes mellitus was defined as hyperglycemia requiring antidiabetic drugs or testing blood glucose over 7.0 mmol/l. Hypertension was defined as systolic blood pressure (SBP) $\geq$140 mm Hg and/or diastolic blood pressure (DBP) $\geq$90 mm Hg. A daily consumption of more than five cigarettes was considered a smoking habit.

DNA analysis and PCR Restriction fragmented length polymorphism (RFLP)

Genomic DNA was extracted from blood. PCR primers designed to amplify PCR were used to amplify two regions of the flanking codon 972 and codon 513

of IRS1. Participant's genomes were analyzed for the presence of the IRS1 972 by PCR-RFLP method. The sequences of the primers for PCR are listed as: (1) The primers for codon 513: Forward primer, 5′GCG GTG AGG AGG AGC TAA GC 3′, and reverse primer 3′ GGG CAG GGT CAGGAG TCA CCG 5′; (2) The primers for Codon 972: Forward primer, 5′ CTT CTG TCA GGT GTC CAT CC3′, and reverse primer 3′ CGA TGC ACC TGTGGA GCG GT 5′. PCR was performed using 10 µl primers with 0.3 ng of genomic DNA. The assay conditions were: 0.1% Triton X-100, and 0.2 mmol/L of the four deoxynucleoside triphosphates (Promega, Madison, Wisconsin, USA) 5U/µl of Go Taq and 100 ng/µl of each primer. The selected samples were subjected to 35 cycles of amplification: denaturation at 94 °C for one minute, annealing at 60 °C for one minute, and extension at 72 °C for two minutes. The resulting products were 268 bp and 263 bp for codon 513 and 972, respectively. The PCR products were digested with restriction enzyme(s) followed by 6% polyacrylamid gel electrophoresis: 1) For the PCR products of codon 972 digested with *Bst N1*, the resulting wild-type samples contained three bands sized 23, 81 and 159 bp and the mutant samples contained four bands sized 23, 81, 108 and 51 bp. We noted five bands sized 23, 81, 159, 108 and 51 bp for Gly972/Arg972 heterozygotes and four bands sized 23, 81, 108 and 51 bp for Arg972 homozygotes. 2) For the PCR products of codon 513 digested with *Dra III*, the resulting wild-type samples contained one band sized 268 bp, and the mutant samples contained two bands sized 168 bp and 100 bp; three bands sized 268 bp, 168 bp and 100 bp were noted for Ala513/Pro513 heterozygotes and two bands sized 168 bp and 100 bp for Pro513 homozygotes.

To study IRS2 polymorphism we used these primers: The forward primer was 5′-GTCCCCGTCGTCGTCTCT-3′ and reverse primer was 5′CTCGACTCCCGACACCTG-3′. The reaction contained template DNA, buffer solution, four types of dNTPs, primer and taq enzyme. PCR included an initial denaturation at 94 °C for 5 minutes, followed by 30 cycles of denaturation at 94 °C for 50 seconds, annealing at 58 °C for 50 seconds, elongation at 72 °C for 50 seconds, and final elongation at 72 °C for 7 minutes. PCR products were confirmed by electrophoresis on 6% polyacrylamid gel and digested overnight with restriction enzyme HhaI at 37 °C and stained on polyacrylamid gel. The digested products were stained with silver nitrate. The resulting wild-type samples contained one band sized 188 bp, and the mutant samples contained one band sized 188 bp. Three bands sized 188 bp, 153 bp and 35 bp were noted for heterozygote and two bands sized 153 bp and 35bp for Gly1057 homozygotes.

Statistical analysis

Data were analysed by using the Statistical Program Social Sciences (SPSS 10.0.7C for Windows). Results were presented as mean values ± standard deviation (SD). Comparisons between T2DM patients and normal control group were performed using Student's *t*-test. A value of $P < 0.05$ was considered statistically significant.

Results

172 patients were finally included in the diagnosis of T2DM from the two ethnic groups, Arabs and Berbers compared to their respective ethnic cohort controls. The average age for T2DM Arab subjects was 57.96 ± 10.96 years compared to the Arab control population with an average age of 54.13 ± 12.53 years, while for T2DM Berber subjects the average age was 59.20 ± 11.85 years, compared to the Berber control population's average age of average age 52.70 ± 13.44 years. The range in age of both ethnic groups and their controls was 30 to 75 years old. Both ethnic groups had nearly equal body weights and BMIs when compared to their ethnically-matched control populations. Arab T2DMs had a similar average body weight (73.03 ± 12.79 kg) and equally matched average BMI (27.08 ± 5.15) compared to their ethnically-matched control group (average weight: 75.51 ± 14.15 kg, average BMI: 27.83 ± 4.91). The Berbers T2DM's weight and BMI (average weight of 71.55 ± 5.20 kg; average BMI: 28.68 ± 5.2) was almost the same as their ethnically-matched controls (average weight: 72.30 ± 14.27 kg, average BMI: 29.20 ± 5.08). Thus, all participants were equally matched in phenotype. Other clinical feature distributions of the groups are shown in Table 1. The prevalence of hypertension (HTA) was higher in T2DM Arab patients (compared to Arab controls) but in the Berber groups,

Table 1. Clinical characteristic of patients with T2DM and controls.

Characteristics	Arab group			Berber group		
	T2DM	Controls	*P*	T2DM	Controls	*P*
N (women/men)	102 (44/58)	70 (31/39)	NS	60 (34/26)	40(28/12)	NS
Age at examination (years)	57.96 ± 10.96	54.13 ± 12.53	<0.05	59.20 ± 11.85	52.70 ± 13.44	<0.05
Age of diagnosis (years)	48.64 ± 11.29	–	<0.001	49.35 ± 10.87	–	<0.001
Duration of the disease (years)	8.46 ± 6.36	–	<0.001	9.48 ± 6.88	–	<0.001
Weight (Kg)	73.03 ± 12.79	75.51 ± 14.15	NS	71.55 ± 11.89	72.30 ± 14.27	NS
BMI (Kg/m2)	27.08 ± 5.15	27.83 ± 4.91	NS	28.68 ± 5.20	29.20 ± 5.08	NS
Low education level (%)	63 (61.8%)	45 (64.3%)	NS	50 (83.3%)	36 (90.0%)	NS
Low or medium SEL (%)	80 (78.4%)	50 (71.4%)	NS	50 (83.3%)	36 (90.0%)	NS
Low physical activity	73 ± 71.6	44 ± 62.9	NS	46 ± 76.7	16 ± 40.0	<0.05
Tobacco (%)	19 (18.6%)	11(15.7%)	NS	5 (8.3%)	3 (7.5%)	NS
Alcohol (%)	6 (5.9%)	3 (4.3%)	NS	3 (5.0%)	4 (10.0%)	NS
HTA (%)	56 (54.9%)	57 (81.4%)	<0.05	24 (40.0%)	35 (87.5%)	<0.05
Systolic (mmHg)	13.79 ± 1.74	12.91 ± 1.13	NS	14.30 ± 2.13	13.15 ± 1.77	NS
Diastolic (mmHg)	8.07 ± 0.76	7.96 ± 0.79	<0.05	8.38 ± 1.48	8.13 ± 0.72	<0.05

Data are expressed as means ± SD for some and as percentages (%) in other variables.
Abbreviations: BMI, body mass index; T2DM, HTA, hypertension; SEL, socioeconomic level; *P* values were obtained by Student's *t*-test.

there were fewer T2DM patients with HTA compared to the Berber control group.

We have genotyped the polymorphisms Gly1057 Asp variant of the IRS2 gene and Ala 513 Pro and Gly972 Arg variants of IRS1 gene in 172 patients with T2DM and 100 control subjects. To assess for any association of Gly1057 Asp and Ala 513 Pro and Gly972 Arg variants of IRS1 polymorphisms with T2DM our ethnic groups, genotypic distribution of these polymorphisms were analysed under the dominant and the additive models. These two variants are not associated with T2DM in either ethnic group.

Under both dominant and additive models there were no significant differences in allelic or genotypic distribution of these variants between healthy and diabetic patients in Arab and Berber ethnic groups. The genotypes frequencies were in agreement with those predicted by the Hardy Weinberg equilibrium in diabetic and control groups. Genotypic frequencies differed significantly only in the Berber group ($P < 0.05$), indicating that the GA genotype of Gly972 Arg acts as a protective genotype against T2DM in Berber ethnic groups with an OR = 0.037; 95% CI (0.14–1.02).

Table 2 shows the genotype and allele frequencies of the Gly1057 Asp variant in the study subjects. There was no significant difference in the frequency of the 'A' allele between the controls and T2DM subjects. Owing to the complex inheritance pattern of T2DM, different genetic models that could be compatible with the data were tested. The genotype frequencies were in agreement with those predicted by the Hardy Weinberg equilibrium in T2DM and control groups. The frequency of the GG, GA and AA genotypes were similar in GG genotype for the Arab T2DM group (68.63%) versus the Berber T2DM group (61.66%), in GA genotype for the Arab T2DM group (27.45%) versus the Berber T2DM (38.34%) and in AA genotype for the Arab T2DM group (3.92%) versus the Berber T2DM group (0%). The frequencies of "A" allele were 17.65% in Arabs T2DM patients and 19.17% in Berber T2DM patients. There was no significant association in the distribution neither in the genotype nor in allelic frequencies in these two ethnic groups of Djerba Island.

Discussion

Insulin receptor substrate IRS2 plays an important role in insulin signalling. Together with IRS1, it mediates most insulin effects, especially those associated with somatic growth and carbohydrate metabolism.[13] Since IRS proteins are critical for signal transduction in

Table 2. Case control association analyses for 972 and 513 Codons of IRS1 gene and 1057 Codon of IRS2.

	Arabs control (n = 70 (%))	Arabs T2DM (n = 102 (%))	Berbers control (n = 40 (%))	Berbers T2DM (n = 60 (%))	*P* value	Odds ratio (95% CI)
Codon 972						
Genotype GG	48 (68.57)	69 (67.65)	25 (62.5)	48 (80)	NS	
GA	22 (31.43)	33 (32.35)	15 (37.5)*	11 (18.34)	<0.05*	OR = 0.037; (0.14–1.02)
AA	0	0	0	1 (1.66)	NS	
Allele G	118 (84.28)	172 (83.83)	65 (81.25)	107 (89.16)	NS	
A	22 (15.72)	33 (16.17)	15 (18.75)	13 (10.84)	NS	
Codon 513					NS	
Genotype GG	69 (98.57)	98 (96.08)	38 (95)	58 (96.66)	NS	
GC	1 (1.43)	4 (3.92)	2 (5)	2 (3.34)	NS	
CC	0	4 (0)	0	0	NS	
Allele G	139 (99.28)	200 (98.04)	78 (97.5)	118 (98.33)	NS	
C	1 (0.72)	12 (1.96)	2 (2.5)	2 (1.66)		
Codon 1057					NS	
Genotype GG	46 (65.72)	70 (68.63)	21 (52.5)	37 (61.66)	NS	
GA	19 (27.14)	28 (27.45)	18 (45)	23 (38.34)	NS	
AA	5 (7.14)	4 (3.92)	1 (2.5)	0	NS	
Allele G	111 (79.28)	168 (82.36)	60 (75)	97 (80.84)	NS	
A	29 (20.72)	36 (17.64)	20 (25)	23 (19.16)		

Notes: **P*-value was <0.05 as significant; OR, odds ratios (approximating relative risk) were calculated with the effects of dominant, recessive, and additive.
Abbreviation: CI, confidence Interval.

insulin target tissues,[4] the IRS1 Gly972 Arg together with Ala 513 Pro and IRS2 Gly1057 Asp polymorphisms have been studied in many populations. Mutations in genes encoding proteins involved in insulin signal transduction pathways could result in insulin resistance and T2DM. Although several mutations in the insulin receptor gene have been described in subjects with rare genetic syndromes of extreme insulin resistance,[14] the actual role of mutations of the insulin receptor gene in the pathogenesis of the mild insulin resistance observed in subjects with typical T2DM is not yet clear. Characterization of IRS l as a critical component of the insulin signal transduction pathway has spurred great interest in its gene as a candidate for mutations in T2DM. Indeed, a report of polymorphism in the IRS l gene appears to be associated with T2DM in a Danish Caucasian population.[15] However, these findings could not be substantiated in a French Caucasian population.[16] The impact of this disease was assessed in two different ethnic groups who have lived in the same environment, under similar conditions, albeit in two different ethnic cultures, i.e. Berber Tunisians and Arab Tunisians. To our knowledge, this is the first study of T2DM among Berber and Arab groups. The major finding of our study is that in Tunisian ethnic groups, the Gly1057 Asp genotype of IRS-2 is not susceptible to T2DM.

Our conclusion is in agreement with other studies[17,18] showing that a polymorphic marker in the IRS2 gene was not linked to T2DM in Ashkenazi Jewish and Italian Caucasian families excluding the IRS2 gene as a major predisposing gene for this disease. Indeed polymorphisms in the regions encoding codons 513 and 972 of IRSl, although present in some European Caucasians, is not responsible for T2DM in Djerbian studied groups. In conclusion, this case-control study indicated that frequencies of the IRS polymorphisms were not significantly different between the healthy controls and T2DM groups.

Acknowledgments

We are grateful to all members of Djerba island hospital for their participation in the study, and the collection of blood samples. We also thank all members of the Centro de Investigación Biomédica de Occidente, Instituto Mexicano Del Seguro Social, Guadalajara, Jalisco in México for their considerable help in the genetic study

Disclosure

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

References

1. de Fronzo RA. Pathogenesis of type 2 diabetes: metabolic and molecular implications for identifying diabetes genes. *Diabetes Rev*. 1997;5:177–269.
2. Shulman GI. Cellular mechanisms of insulin resistance. *J Clin Invest*. 2000;106:171–6.
3. Saltiel AR. New perspectives into the molecular pathogenesis and treatment of type 2 diabetes. *Cell*. 2001;104:517–29.
4. Sesti G, Federici M, Hribal ML, et al. Defects of the insulin receptor substrate (IRS) system in human metabolic disorders. *Faseb J*. 2001;15:2099–111.
5. Ogihara T, Isobe T, Ichimura T, et al. 14-3-3 protein binds to insulin receptor substrate-1, one of the binding sites of which is in the phosphotyrosine binding domain. *J Biol Chem*. 1997;272:25267–74.
6. Garcia P, Shoelson SE, George ST, et al. Phosphorylation of synthetic peptides containing Tyr-Met-X-Met motifs by nonreceptor tyrosine kinases in vitro. *J Biol Chem*. 1993;268:25146–51.
7. Sun XJ, Rothenberg P, Kahn CR, et al. Structure of the insulin receptor substrate IRS1 defines a unique signal transduction protein. *Nature*. 1991; 352:73–7.
8. Almind K, Inoue G, Pedersen O, et al. A common amino acid polymorphism in Insulin receptor substrate-1 causes impaired insulin signalling. *J Clin Invest*. 1996;97:2569–75.
9. Hager J, Zouali H, Velho G, et al. Insulin receptor substrate (IRS-l) gene polymorphisms in French NIDDM families. *Lancet*. 1993;342:1430.
10. Withers DJ, Gutierrez JS, Towery H, et al. Disruption of IRS-2 causes type 2 diabetes in mice. *Nature*. 1998;391:900–4.
11. Bektas A, Warram JH, White MF, et al. Exclusion of insulin receptor substrate 2 (IRS-2) as a major locus for early-onset autosomal dominant type 2 diabetes. *Diabetes*. 1999;48:640–2.
12. Ennafaa H, Amor MB, Yacoubi-Loueslati B, et al. Alu polymorphisms in Jerba Island population (Tunisia): comparative study in Arab and Berber groups. *Ann Hum Biol*. 2006;33(5–6):634–40.
13. White MF. IRS proteins and the common path to diabetes. *Am J Physiol Endocrinol Metab*. 2002;283:E413–22.
14. Taylor SI, Cama A, Accili D, et al. Mutations in the insulin receptor gene. *Endocr Rev*. 1992;13:566–95.
15. Hager J, Zouali H, Velho G, et al. Insulin receptor substrate (IRSl) gene polymorphisms in French NIDDM families. *Lancet*. 1993;342:1430.
16. Lillioja S, Mott DM, Spraul M, et al. Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus: prospective studies of Pima Indians. *N Engl J Med*. 1993;329:1988–92.
17. Kalidas K, Wasson J, Glaser B, et al. Mapping of the human insulin receptor substrate-2 gene, identification of a linked polymorphic marker and linkage analysis in families with type 2 diabetes: no evidence for a major susceptibility role. *Diabetologia*. 1998;41:1389–91.
18. D'Alfonso R, Marini MA, Frittitta L, et al. Polymorphisms of the insulin receptor substrate-2 in patients with type 2 diabetes. *J Clin Endocrinol Metab*. 2003;88(1):317–22.