Lack of association between the insulin receptor substrates-1 Gly972Arg polymorphism and type-2 diabetes mellitus among Saudis from Eastern Saudi Arabia

Hawra A. Alsalman, MSc (Student), *Yahia A. Kaabi,* PhD.

ABSTRACT

الأهداف: للتحقيق في العلاقة بين تعدد الأشكال IRS1 Gly972Arg ومرض السكري من النوع الثاني(T2DM) في السعوديين من سكان المنطقة الشرقية.

الطريقة: أجريت هذه الدراسة في الفترة ما بين مايو وديسمبر 2014م في مستشفى الملك فهد التعليمي التابع لجامعة الدمام بالخبر، المملكة العربية السعودية. شارك في هذه الدراسة 143 متبرع (الفئة العمرية 73-35 عاماً) مقسمين إلى: 74 أصحاء و 69 مرضى بالسكري من النوع الثاني. تم استخلاص المادة الوراثية الـDNA من عينات دم المشاركين. ثم تم فحص وجود الطفرة الجينية Gly972Arg في عينات الـDNA باستخدام تقنية PCR-RFLP.

النتائج: وجدنا أن نسبة وجود هذه الطفرة في الأصحاء 10.8% وفي مرضى السكري 8.7%. نسبة الأرجحية بلغت 1.04 مع فترات ثقة تتراوح من 0.28 الى 3.95 والقيمة الإحصائية *p* تساوي 0.94.

الخاتمة: فشلنا في العثور على أي ارتباط بين IRS1 Gly972Arg تعدد الأشكال وT2DM.

Objectives: To investigate the association between the insulin receptor substrate-1 (*IRS1*) Gly972Arg polymorphism and type-2 diabetes mellitus (T2DM) among Saudis from Eastern Saudi Arabia.

Methods: This study was conducted between May and December 2014 at King Fahad Hospital of the University, Al-Khobar, Kingdom of Saudi Arabia. In a case-control study design, a total of 143 subjects (age range: 35-73 years) comprising 74 healthy controls and 69 patients with T2DM were examined. Blood samples were collected from subjects and subjected to genomic DNA extraction and chemical analysis. The *IRS1* Gly972Arg polymorphism was then genotyped using the standard polymerase chain reaction-restriction fragment length polymorphism technique.

Results: Eight out of 74 (10.8%) of the control group carried at least one copy of the mutated allele. The frequency (8.7%) of the *IRS1* variant was also found in the diabetic group. Logistic regression analysis showed an adjusted odds ratio of 1.04, 95% confidence interval 0.28 - 3.95, and a *p*-value of 0.94.

Conclusion: We failed to find any association between the *IRS1* Gly972Arg polymorphism and T2DM.

Saudi Med J 2015; Vol. 36 (12): 1420-1424
doi: 10.15537/smj.2015.12.12904

From the Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, University of Dammam, Dammam, Kingdom of Saudi Arabia.

Received 13th July 2015. Accepted 21st October 2015.

Address correspondence and reprint request to: Dr. Yahia A. Kaabi, Assistant Professor (Clinical Chemistry), Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, University of Dammam, Dammam, Kingdom of Saudi Arabia. E-mail: yakaabi@uod.edu.sa

Diabetes mellitus (DM) is a major medical and public health concern worldwide. In 2014, it has been estimated that there were 382 million sufferers of diabetes worldwide.[1] The prevalence of diabetes in Saudi Arabia is 23.9%, which is among the highest in the gulf area and the world.[1,2] Type-2 diabetes mellitus (T2DM) represents approximately 90-95% of all diabetes cases.[3] Type-2 diabetes mellitus is characterized by high blood glucose due to the inability of body cells to respond to insulin, a phenomenon known as insulin resistance.[4] The genetic factors behind the insulin resistance are not well-understood and are under intensive research. Insulin is a metabolically important peptide hormone secreted from ß-cells of the pancreas in response to high blood glucose. Insulin signaling starts with binding of the insulin molecule to its cognate membrane insulin receptor (IR). This binding triggers conformational

changes in the juxtamembrane intracellular region of the receptor causing autophosphorylation of certain tyrosine residues. The activated receptor then phosphorylates and activates a family of intracellular effector proteins known as insulin receptor substrates 1 to 4 (IRS-1 to 4).[5] These activated proteins then interact with the regulatory subunit (p85) of the enzyme phosphatidylinositol (PI) 3-kinase causing glucose transporter-4 (Glut-4) translocation to the cell membrane to increase glucose uptake.[6] In this signaling pathway, *IRS1* seems to play a major role as suggested by *IRS1* knockout mice studies.[7] Additionally, several polymorphisms in the *IRS1* gene were associated with insulin resistance, obesity, and T2DM.[8,9] The single nucleotide polymorphism (SNP) (rs1801278) in the *IRS1* gene causing an amino acid substitution where glycine (GGG) is replaced by arginine (AGG) at codon 972 was first described by Almind et al.[10] It is the most widely studied candidate of the *IRS1* gene variants by testing its association with insulin resistance and T2DM.[11] However, the association of this polymorphism with the development of T2DM has not been consistent around the world. In a study focusing on Mexican participants, Burguete-Garcia et al[8] found that the *IRS1* Gly972Arg variant was significantly associated with T2DM. Another study that also focused on Mexican population similarly found a significant association between Gly972Arg variant and genetic susceptibility to T2DM.[12] A study on Indian population from Hyderabad also reported significant association with T2DM.[13] A meta-analysis study conducted in 2009 of 30 studies had shown that there was no significant association between the *IRS1* Gly972Arg variant and T2DM.[11] In addition, previous studies also reported no association between the *IRS1* Gly972Arg polymorphism and T2DM.[14-18] There is no report on the frequency of this polymorphism and its association with T2DM in the Eastern Province of Saudi Arabia. Thus, the aim of the current work is to examine the association between the *IRS1* Gly972Arg polymorphism with T2DM among Saudis.

Methods. The literature review search methods included using PubMed, Google Scholar, and Summon Web Scale Discovery databases to search for the following words: *IRS1*, diabetes AND IRS, insulin receptor substrate 1, *IRS1* polymorphism, Gly972Arg, and G972R. Using these words, we generated a recent study on polymorphism. In addition, all studies mentioned in the meta-analysis article were collected and critically read.[11]

An informed consent was collected from all subjects involved in this study (IRB-2014-04-284) for institutional local ethics approval granted by the University of Dammam, Saudi Arabia. The study was conducted in accordance with the Declaration of Helsinki. The study design is a case-control study. Volunteer subjects were recruited from the outpatient clinic at King Fahad Hospital of the University (KFHU), University of Dammam, Al-Khobar, and Eastern Province, Saudi Arabia between May and December 2014. A total of 143 subjects from both genders, with an average age of 47.2 years (range 35-73 years), participated in this study. They were divided as 74 healthy non-diabetic control group and 69 subjects with T2DM. Demographic data such as age, gender, height, weight, body mass index (BMI) and family history of diabetes were determined from all subjects.

Two blood samples were drawn from each subject after an overnight fast in 7 ml of ethylenediaminetetraacetic acid (EDTA) and plain tubes. The serum from plain tubes was recovered and sent for routine clinical chemistry laboratory for blood glucose measurement. The EDTA whole blood was used for subsequent DNA extraction analysis. The genomic DNA was extracted from 200 µl of the whole blood leukocytes using DNA extraction kit (GE Healthcare Life Sciences Ltd, Buckinghamshire, UK). The extracted DNA concentration ranged from 10-25 ng/µl. The DNA purity was checked by OD260:OD280 ratio of 1.8 ± 1. All DNA samples were then stored at -20 °C until the genetic analysis was performed. The *IRS1* Gly972Arg polymorphism in all subjects was genotyped using the standard polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. First, the DNA area in *IRS1* gene flanking the polymorphism was amplified by PCR. The PCR reaction was carried out at a final volume of 25 µl as: 1µl of sample DNA, 1 µl of each primer (forward; 5' GCT TTC CAC AGC TCA CCT TC 3', reverse; 5' TTT AAA TTT AAA TTT AAA TTT AAA TTT AAA TTT GGT AGG CCT GCA AAT GCT A 3'), and 9.5 µl of sterile nucleic acid free H_2O. The above mixture was then mixed with 12.5 µl of 2x PCR master mix (Promega; Wisconsin, USA). The reverse primer was designed to contain additional 33 unbound (TTTAAA) nucleotide repeats to increase the PCR product size to 321 base pair (bp)

Disclosure. Authors have no conflict of interest, and this research was not supported or funded by any pharmaceutical company. This research is funded by the Deanship of Scientific Research in the University of Dammam, Damman, Saudi Arabia.

and further enhance difference in sizes between digested DNA fragments later. The initial PCR thermal profile was denaturation (94°C, 4 minutes) followed by 35 repeated cycles of denaturation (94°C, 30 seconds), annealing (58°C, 30 seconds), elongation (72°C, 30 seconds), and final extension (72°C, 10 minutes). The amplified PCR product (231 bp) was digested with the XmaI restriction endonuclease according to following reaction mixture: 5 µl of the PCR product, 2 µl of bovine serum albumin, 2 µl of 10x digestion buffer, 10 µl of sterile nucleic acid free H_2O, and 1 µl of the restriction enzyme. The digestion mixture was incubated for 2 hours at 37°C and run on 2.5% agarose gel stained with ethidium bromide. As the *IRS1* Gly972Arg allele polymorphism disrupts a recognition site for the XmaI enzyme, the presence of 2 DNA fragments at 169 and 62 bp indicated normal non-mutated alleles. Heterozygous genotypes were indicated by the presence of 3 bands at 231, 169, 62 bp while the homozygous mutated genotype was indicated by the presence of one band at 231 bp.

Data from both control and diabetic groups were presented as mean±standard deviation (SD) or percentages for non-continuous variables. The statistical significance was measured by simple student t-test, or Fisher's exact test if both variables were dichotomous. Relationship between the *IRS1* Gly972Arg polymorphism and T2DM was evaluated using the logistic regression testing by calculating odds ratios in addition to 95% confidence intervals (CI) and a p-value based on Fisher's exact test to indicate the statistical significance. To avoid differences that may result from confounding factors such as age, gender, or BMI, data were stratified into categories by gender (male/female), age (>40/<40 years old), or BMI (>30 kg/m^2 and <30 kg/m^2), and re-analyzed separately. The odds ratios, 95% CI, and p-values were then pooled to one common adjusted value using Mantel-Haenszel method. Statistical analyses were carried out using GraphPad prism 6 software (GraphPad Software Inc., La Jolla, CA, USA). A p-value of <0.05 was considered statistically significant.

Results. The baseline characteristics of the study population are summarized in **Table 1**. To evaluate the familial effect on the *IRS1* genotypes in 143 total population of the study, the observed genotype frequencies were compared to the expected frequencies. Data showed similar values with a Chi-squared p=0.31 indicating that all genotypes reached the Hardy-Weinberg equilibrium (**Table 2**).

The overall frequency of the *IRS1* Gly972Arg variant in the total study population was 9.8% with only one subject carrying the homozygous mutant genotype (Arg/Arg) (**Table 3**). Considering control and diabetic groups separately, frequencies were 8.7% and 10.8%. Allelic frequencies in total population were 94.8% for Gly allele and 5.2% for Arg allele. To evaluate the association between the presence of the Arg allele and T2DM, logistic regression analysis was conducted using the Gly/Gly genotype as a non-exposed reference (**Table 3**). Adjusted odds ratios were 1.06 (95% CI: 0.3 - 4.0) for the Gly/Arg variant carriers and 1.04 (95% CI: 0.28 - 3.95) for subjects that carry either Gly/Arg or Arg/Arg variants. Therefore, there was no statistically significant association between the presence of the Arg allele and the occurrence of T2DM. Analysis based on allelic frequencies showed an odds

Table 2 - Consistency with Hardy-Weinberg equilibrium.

Genotype	Observed	Expected	x^2	*P*-value
Gly/Gly	129	128.4	1.04	0.31*
Gly/Arg	13	14.2		
Arg/Arg	1	0.4		

*with 1 degree of freedom

Table 1 - Baseline characteristic of the control and type-2 diabetes mellitus (T2DM) subjects.

Variables	Control (n=74)	T2DM (n=69)	*P*-value
Gender (male/female)	25/49	36/33	0.029*
Age (years) (mean±SD)	43.2 ± 9.3	51.6 ± 9.0	<0.0001
Height (cm) (mean±SD)	160.9 ± 7.9	161.5 ± 8.3	0.654
Weight (kg) (mean±SD)	80.2 ± 16.4	83.0 ± 17.5	0.275
Body mass index (kg/m^2) (mean±SD)	30.9 ± 5.9	31.9 ± 6.1	0.327
Waist circumference (cm) (mean±SD)	99.7 ± 13.4	105.5 ± 13.1	0.002
Fasting glucose (mg/dL) (mean±SD)	97.1 ± 9.6	177.4 ± 73.0	<0.0001
Treatment (oral/insulin) n (%)	-	47 (68.1)/22 (31.9)	-
Family history of T2DM n (%)	49 (66.2)	56 (81.2)	0.058*

*Fisher's exact test.

Table 3 - Genotype and allele distribution of the insulin receptor substrate-1 Gly972Arg polymorphism in type-2 diabetic patients and healthy control group.

Genotype and allele distribution	Total (n=143) n (%)	Control (n=74) n (%)	T2DM (n=69) n (%)	OR* (95% CI) *p*-value	OR† (95% CI) *p*-value
Gly/Gly	129 (90.2)	66 (89.2)	63 (91.3)	Reference	Reference
Gly/Arg	13 (9.1)	7 (9.5)	6 (8.7)	0.79 (0.3 - 2.4) 0.78	1.06 (0.3 - 4.0) 0.99
Arg/Arg	1 (0.7)	1 (1.3)	0 (0)	NA	NA
X/Arg‡	14 (9.8)	8 (10.8)	6 (8.7)	0.78 (0.26 - 2.39) 0.67	1.04 (0.28 - 3.95) 0.94
Gly	271 (94.8)	139 (93.9)	132 (95.7)	Reference	Reference
Arg	15 (5.2)	9 (6.1)	6 (4.3)	0.70 (0.2 - 2.0) 0.60	0.83 (0.2 - 2.8) 0.76

*Crude odds ratio and 95% confidence intervals (CI), †age and body mass index adjusted odds ratio and 95% CI, ‡X can be Gly or Arg, NA - not applicable, T2DM - type 2 diabetes mellitus

ratio of 0.83 (95% CI: 0.2 - 2.8) for the Arg allele to develop T2DM.

Discussion. Insulin receptor substrate-1 is an essential component in the insulin signaling pathway. The common polymorphism in the *IRS1* gene Gly972Arg has been shown to affect the appropriate function of the *IRS1* protein.[19] Structurally, this polymorphism is positioned between 2 potential sites of tyrosine phosphorylation (Tyr941 and Tyr989) in *IRS1* that are necessary for binding to the p85 subunit of the effector molecule PI3K during insulin signaling.[20,21] Accordingly, this mutation has been shown to alter the *IRS1* interaction with tyrosine protein kinases, decrease the PI3K activation and contribute to insulin resistance.[9,19,22,23] In this case-control study, we intended to determine any relationship between the *IRS1* Gly972Arg polymorphism and T2DM among Saudi Arabian population in the Eastern Province. The results showed that 10.8% of the control group carried at least one copy of the mutated Arg allele variant, while most of the subjects were normal. Similar distribution was also found in the diabetic group and the logistic regression analysis revealed no association between the *IRS1* Gly972Arg polymorphism and T2DM. Our finding might contradict with some published reports that suggested an association, including a recently published study based in the middle area of the country.[24] However, the association between the *IRS1* Gly972Arg variant with T2DM has been controversial throughout the literature and the lack of association has been also reported in multiple studies.[14-18] For example, in a published meta-analysis study reviewing more than 30 studies and involving 12,076 diabetic cases and 11,285 controls found no statistically significant association between this polymorphism and T2DM.[11] Therefore, the *IRS1* Gly972Arg polymorphism might not be a major element of the genetic architecture necessary for development of T2DM. However, if present, this polymorphism might contribute to a more complicated and difficult to control form of T2DM. It has been linked to impaired glucose tolerance,[25] poor glycemic control,[9] decreased glomerular filtration rate,[26] increased levels of triglyceride,[26] failure of oral treatment[27] impaired insulin secretion from the ß-cell of the pancreas,[20] and beta cells apoptosis.[28] The limitations of this study include the small sample size and lack of a variable that could measure insulin resistance.

In conclusion, our findings did not support the association between the *IRS1* Gly972Arg polymorphism and T2DM among Saudis from the Eastern Province. Therefore, the presence or absence of this polymorphism may not be a main determinant for the development of T2DM. However, further research with larger sample size may be required to establish an accurate relationship.

References

1. International Diabetes Federation. IDF Diabetes Atlas. [Update 2014; cited 2015 March]. Available from: https://www.idf.org/diabetesatlas
2. Al-Baghli NA, Al-Ghamdi AJ, Al-Turki KA, Al Elq AH, El-Zubaier AG, Bahnassy A. Prevalence of diabetes mellitus and impaired fasting glucose levels in the Eastern Province of Saudi Arabia: results of a screening campaign. ***Singapore Med J*** 2010; 51: 923-930.
3. Association AD. Diagnosis and Classification of Diabetes Mellitus. ***Diabetes Care*** 2013; 36 (Suppl 1): S67-S74.
4. Nowotny B, Zahiragic L, Krog D, Nowotny PJ, Herder C, Carstensen M, et al. Mechanisms underlying the onset of oral lipid-induced skeletal muscle insulin resistance in humans. ***Diabetes*** 2013; 62: 2240-2248.
5. Tanti JF, Jager J. Cellular mechanisms of insulin resistance: role of stress-regulated serine kinases and insulin receptor substrates (IRS) serine phosphorylation. ***Curr Opin Pharmacol*** 2009; 9: 753-762.

6. Jezewski AJ, Larson JJ, Wysocki B, Davis PH, Wysocki T. A novel method for simulating insulin mediated GLUT4 translocation. ***Biotechnol Bioeng*** 2014; 111: 2454-2465.
7. Tamemoto H, Kadowaki T, Tobe K, Yagi T, Sakura H, Hayakawa T, et al. Insulin resistance and growth retardation in mice lacking insulin receptor substrate-1. ***Nature*** 1994; 372: 182-186.
8. Burguete-Garcia AI, Cruz-Lopez M, Madrid-Marina V, Lopez-Ridaura R, Hernandez-Avila M, Cortina B, et al. 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. ***Metabolism*** 2010; 59: 38-45.
9. Huri HZ, Makmor-Bakry M, Hashim R, Mustafa N, Wan Ngah WZ. Optimisation of glycaemic control during episodes of severe/acute hyperglycaemia in patients with type 2 diabetes mellitus. ***Int J Clin Pharm*** 2012; 34: 863-870.
10. Almind K, Bjorbaek C, Vestergaard H, Hansen T, Echwald S, Pedersen O. Aminoacid polymorphisms of insulin receptor substrate-1 in non-insulin-dependent diabetes mellitus. ***Lancet*** 1993; 342: 828-832.
11. Morini E, Prudente S, Succurro E, Chandalia M, Zhang YY, Mammarella S, et al. IRS1 G972R polymorphism and type 2 diabetes: a paradigm for the difficult ascertainment of the contribution to disease susceptibility of 'low-frequency-low-risk' variants. ***Diabetologia*** 2009; 52: 1852-1857.
12. Martinez-Gomez LE, Cruz M, Martinez-Nava GA, Madrid-Marina V, Parra E, Garcia-Mena J, et al. A replication study of the IRS1, CAPN10, TCF7L2, and PPARG gene polymorphisms associated with type 2 diabetes in two different populations of Mexico. ***Ann Hum Genet*** 2011; 75: 612-620.
13. Kommoju UJ, Maruda J, Kadarkarai Samy S, Irgam K, Kotla JP, Reddy BM. Association of IRS1, CAPN10, and PPARG gene polymorphisms with type 2 diabetes mellitus in the high-risk population of Hyderabad, India. ***J Diabetes*** 2014; 6: 564-573.
14. Arikoglu H, Aksoy Hepdogru M, Erkoc Kaya D, Asik A, Ipekci SH, Iscioglu F. IRS1 gene polymorphisms Gly972Arg and Ala513Pro are not associated with insulin resistance and type 2 diabetes risk in non-obese Turkish population. ***Meta Gene*** 2014; 2: 579-585.
15. Bodhini D, Radha V, Mohan V. Association Study of IRS1 Gene Polymorphisms with Type 2 Diabetes in South Indians. ***Diabetes Technol Ther*** 2011; 13: 767-772.
16. Feng X, Tucker KL, Parnell LD, Shen J, Lee YC, Ordovas JM, et al. Insulin receptor substrate 1 (IRS1) variants confer risk of diabetes in the Boston Puerto Rican Health Study. ***Asia Pac J Clin Nutr*** 2013; 22: 150-159.
17. Vergotine Z, Kengne AP, Erasmus RT, Matsha TE. No evidence for association of insulin receptor substrate-1 Gly972Arg variant with type 2 diabetes mellitus in a mixed-ancestry population of South Africa. ***S Afr Med J*** 2014; 104: 420-423.
18. Yiannakouris N, Cooper JA, Shah S, et al. IRS1 gene variants, dysglycaemic metabolic changes and type-2 diabetes risk. ***Nutrition, Metabolism, and Cardiovascular Diseases.*** 2012; 22: 1024-1030.
19. Yoshimura R, Araki E, Ura S, Todaka M, Tsuruzoe K, Furukawa N, et al. Impact of natural IRS-1 mutations on insulin signals: mutations of IRS-1 in the PTB domain and near SH2 protein binding sites result in impaired function at different steps of IRS-1 signaling. ***Diabetes*** 1997; 46: 929-936.
20. Almind K, Inoue G, Pedersen O, Kahn CR. A common amino acid polymorphism in insulin receptor substrate-1 causes impaired insulin signaling. Evidence from transfection studies. ***J Clin Invest*** 1996; 97: 2569-2575.
21. McGettrick AJ, Feener EP, Kahn CR. Human insulin receptor substrate-1 (IRS-1) polymorphism G972R causes IRS-1 to associate with the insulin receptor and inhibit receptor autophosphorylation. ***J Biol Chem*** 2005; 280: 6441-6446.
22. Dziwura J, Binczak-Kuleta A, Miazgowski T, Ziemak J, Widecka K. The associations between G972R polymorphism of the IRS-1 gene, insulin resistance, salt sensitivity and non-dipper hypertension. ***Hypertens Res*** 2011; 34: 1082-1086.
23. Boura-Halfon S, Zick Y. Phosphorylation of IRS proteins, insulin action, and insulin resistance. ***Am J Physiol Endocrinol Metab*** 2009; 296: E581-E591.
24. Alharbi KK, Khan IA, Munshi A, Alharbi FK, Al-Sheikh Y, Alnbaheen MS. Association of the genetic variants of insulin receptor substrate 1 (IRS-1) with type 2 diabetes mellitus in a Saudi population. ***Endocrine*** 2014; 47: 472-477.
25. Yamada K, Yuan X, Ishiyama S, Shoji S, Kohno S, Koyama K, et al. Codon 972 polymorphism of the insulin receptor substrate-1 gene in impaired glucose tolerance and late-onset NIDDM. ***Diabetes Care*** 1998; 21: 753-756.
26. Thameem F, Puppala S, Schneider J, Bhandari B, Arya R, Arar NH, et al. The Gly(972)Arg Variant of Human IRS1 Gene Is Associated With Variation in Glomerular Filtration Rate Likely Through Impaired Insulin Receptor Signaling. ***Diabetes*** 2012; 61: 2385-2393.
27. Prudente S, Morini E, Lucchesi D, Lamacchia O, Bailetti D, Mercuri L, et al. IRS1 G972R missense polymorphism is associated with failure to oral antidiabetes drugs in white patients with type 2 diabetes from Italy. ***Diabetes*** 2014; 63: 3135-3140.
28. Federici M, Hribal ML, Ranalli M, Marselli L, Porzio O, Lauro D, et al. The common Arg972 polymorphism in insulin receptor substrate-1 causes apoptosis of human pancreatic islets. ***FASEB J*** 2001; 15: 22-24.