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ASSOCIATION OF IRS1 GLY971ARG GENE POLYMORPHISM WITH INSULIN RESISTANCE IN IRANIAN NEWLY DIAGNOSED DIABETIC ADULTS

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Abstract

Context. Insulin receptor substrate-1 (IRS-1) has an important role in insulin signaling and the common Gly971Arg polymorphism is related to type 2 diabetes (T2D). IRS-1 Gly971Arg polymorphism can modify tyrosine phosphorylation at a specific site of IRS-1 and may have a critical role in the development of insulin resistance (IR).

Objective. The purpose of this study was to investigate the association between this polymorphism and IR in Iranian patients with newly-diagnosed type 2 diabetes.

Design. The study was conducted on 114 individuals with newly-diagnosed T2D and 118 healthy matched controls, aged 20-80 years. Fasting blood glucose and insulin were measured by the enzymatic method and enzyme-linked immunosorbent assay, respectively. Insulin-resistance was calculated by homeostasis model assessment estimated-insulin resistance (HOMA-IR). The gene polymorphism was examined by polymerase chain reaction-restriction fragment length polymorphism.

Results. There are significant differences between IRS1 Gly971Arg polymorphism and studied individuals ($P<0.0001$). The findings showed that the risk of developing T2D in individuals who had R-alleles was 3.74 folds higher than those without R-alleles. However, IRS1 Gly971Arg polymorphism was not associated with high HOMA-IR, high BMI and familial history of diabetes.

Conclusions. Even though there was not a significant relationship between IRS-1 G971R polymorphism with insulin resistance and high BMI, this polymorphism was correlated to newly-diagnosed diabetic patients. Thus, the evaluation of IRS-1 G971R polymorphism may be helpful for predicting T2D new cases.

Key words: type 2 diabetes, insulin resistance, IRS-1, gene polymorphism.

INTRODUCTION

Type 2 diabetes (T2D) as an epidemic metabolic disease in the 21st century is characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Deficient insulin action arises from inadequate insulin secretion and/or reduced target tissue responses to insulin at one or more points in the complex intracellular pathways (1). Insulin resistance (IR), as a condition with diminished target cell response to normal levels of insulin, plays a central role in the development of T2D (1).

Insulin binding to its receptor triggers the activation of multiple signaling molecules. Basically, the autophosphorylation of the tyrosine kinase domain on the cytoplasmic surface of the receptor is induced by the interaction between insulin and its receptor; then, phosphorylation of cytosolic insulin receptor substrates (IRS) is continued for downstream stimulation of Phosphatidylinositol 3-kinase (PI3K), phosphotyrosine phosphatase 2 and Growth factor receptor-bound protein 2 (GRB2); so that the Akt and mitogen-activated protein (MAP) kinase signaling pathways are activated (2). Structurally, there is an N-terminal pleckstrin homology (PH) domain near to a phosphotyrosine binding (PTB) domain and a variable-length C-terminal tail which contains multiple tyrosine residues in IRS proteins. Hence, there is a series of phosphorylation sites at the C-terminus of the proteins which can regulate the downstream effector molecules (3, 4). IRS-1 binding to signal proteins can link the receptor kinase to several cellular functions that are regulated by insulin. Studies have shown that the IRS-1 can participate in glucose production, glucose clearance, and insulin secretion (5, 6).

Different studies have shown that the single nucleotide polymorphism Gly972Arg (G972R) in IRS1 gene is associated with a reduction in PI3K activity and subsequent development of insulin

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resistance (7, 8). Moreover, it has been demonstrated that the Gly972Arg polymorphism is located near the Tyr-Met-X-Met (YMXM) motifs and is related to hyperinsulinemia, insulin resistance and fatty acid composition in myocytes (9).

So far, several studies have reported that IRS1 gene polymorphisms located in 2q36-37 region, especially Gly972Arg substitution, can be associated with insulin resistance and T2D in different populations (10 – 13). However, others could not show the positive correlation between IRS1 Gly972Arg polymorphism and T2D (14 – 16).

Although most studies have mentioned this amino acid substitution at the position 972 of IRS1, however, our bioinformatics study showed that the real substitution occurs at amino acid 971 (17).

According to contradictory reports in the different populations, this study aimed at investigating the association of IRS-1 Gly971Arg (rs1801278) gene polymorphism with insulin resistance in the population with newly diagnosed T2D.

MATERIALS AND METHODS

Study population

In this case-control study, 232 Iranian individuals in Bojnurd city, North Khorasan province, aged 20–80 years, comprised 114 individuals with type 2 diabetes, who were newly diagnosed, and 118 healthy subjects matched for age and gender were enrolled. Informed written consent was obtained from each individual before admitting into the clinical facility. The study protocol was approved by the Ethical Committee of the North Khorasan University of Medical Sciences (ethics code: IR.nkums.REC.1395.90).

According to clinical investigations and American Diabetes Association criteria (1) by an endocrinologist and clinical biochemist, 114 patients were selected as case group with type 2 diabetes mellitus who had fasting blood glucose (FBG) more than 7 mmol/L which is confirmed twice at different times, and 118 healthy individuals with normal levels of FBG and without any complication were proposed as the control group. The structural questionnaire including some demographic contents and history of drug use, especially anti-diabetic, lipid-lowering, and antihypertensive drugs, were obtained from participants. The exclusion criteria from the study were history of hyperglycemia and any other diseases, and also taking any anti-inflammatory, anti-hypertensive, anti-diabetic, and lipid-lowering drugs during a month before sampling.

Laboratory and Molecular Diagnosis

As we published recently (18), the fasting blood glucose (FBG), lipid profile include triglyceride (TG), total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C) and low-density lipoprotein-cholesterol (LDL-C) were measured by enzymatic methods (Pars Azmun and Pishtaz Teb, Iran) using biochemistry autoanalyzer (Dirui, China). Fasting serum insulin levels were also measured by enzyme-linked immune absorbent assay (ELISA) method (Demeditec Diagnostics GmbH, Germany) using a plate reader (BioTek, USA). The formula of homeostasis model assessment-estimated insulin resistance (HOMA-IR), which is developed by Matthews *et al.* (19), was used for insulin resistance assessment (20). It is calculated multiplying fasting serum insulin by fasting blood glucose, then dividing by the constant 22.5 (21). Individuals with a HOMA-IR value higher than 2.0 were considered to be resistant to insulin (18, 22).

For analysis of IRS-1 gene polymorphism, genomic DNA was extracted from blood samples, which were collected in EDTA-coated vacuum tubes, using the column method according to the protocol of the manufacturer (Genet Bio, Korea). In order to amplify 285 bp for determining the polymorphism SNP G971R in the IRS1 gene, the polymerase chain reactions (PCR) were carried out using the oligonucleotide primers F: 5'-AGTCTGGCTACTTGTCTGGC-3' and R: 5'-TCTGACGGGGACAACTCAT-3'. PCR amplifications were performed in a 20 μL volume containing genomic DNA, 2× PCR premix (Genet Bio, Korea) and 0.5 mM of each primer. PCR reactions were carried out in a thermocycler (Veriti, Applied Biosystems, USA) as follows: an initial denaturation at 95°C for 5 minutes was followed by 40 cycles of denaturation at 95°C for 45 seconds, annealing at 57.2°C for 45 seconds, elongation at 72°C for 45 seconds, and final extension at 72°C for 5 minutes. After that, the restriction fragment length polymorphism (RFLP) analyses of the target SNP Gly971Arg were achieved using the restriction enzyme SmaI (ThermoFisher Scientific, USA) according to the manufacturers' instruction. Within the IRS-1 gene, the polymorphic codon AGG or CGG encoding Arg eliminates by SmaI, whereas the codon GGG encoding Gly is recognized by the enzyme. The resultant restriction fragments were ascertained on a 2.5% agarose gel and visualized by gel documentation (UVITEC, England).

Statistical analysis

The comparisons between the study groups were performed using the Student t-test. In addition, the allele

frequencies and genotypes distribution were analyzed using the Pearson Chi-square test. The evaluation of allele frequencies in the study groups was performed using odds ratio (OR). All analyses were performed in SPSS software (version 18) and the P value lower than 0.05 was considered statistically significant. The power of design for this study was estimated at around 85 %.

RESULTS

Demographic and laboratory analyses

General characteristic and laboratory profile of participants are shown in (Table 1). Familial history of diabetes, hypertension and BMI were greater in patients with type 2 diabetes (P <0.0001, P <0.01 and P <0.01, respectively). The fasting blood glucose average in the case and control groups was 8.33 ± 3.49 mmol/L and 4.93 ± 0.5 mmol/L, respectively (P <0.0001), but insulin levels were not significant between groups (P = 0.859). According to HOMA-IR threshold in Iranian population (22), in the present study 81.6% of diabetic patients had high levels of HOMA index (P<0.0001; OR = 3.74, CI 95 %).

Distribution of IRS-1 Gly971Arg polymorphism

For genotyping of G971R polymorphism, on the basis on Hardy–Weinberg Equilibrium law and according to the bi-allelic region inside of the IRS-1 gene, digested fragments (197 and 88 bp) as normal homozygotes for the G allele (genotype GG), undigested fragment (285 bp) as homozygous for the R allele (genotype RR) and both digested and undigested fragments (285, 197 and 88 bp) were detected as heterozygous (genotype GR) (Fig. 1). Genotype frequencies of heterozygosity for the G/R alleles in the case and control groups were 64.9 % and 26.3 %, respectively; furthermore, the frequency of two allele polymorphism (RR) in the case group was 5.3 % (P<0.0001; OR = 4.0, CI 95% 2.86 – 5.14) (Fig. 2). In order to determine the relationship between G971R SNP and insulin resistance in the study groups, 19.25% of individuals who had elevated HOMA index and also 6.05 % of individuals with normal HOMA index were positive for R allele; however, it was not observed a significant difference between insulin resistance and IRS-1 G971R polymorphism (P=0.164) (Table 2). Moreover, there were not associations between the IRS-

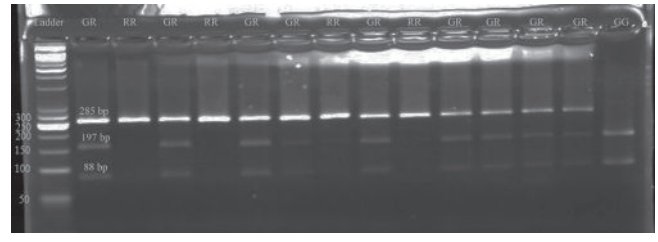

Figure 1. The electrophoretic pattern for the determination of IRS-1 Gly971Arg polymorphism. IRS-1 G971R genotypes include GG (normal), GR (polymorphism in one allele), RR (polymorphism in two alleles).

Table 1. General characteristics, laboratory profiles of diabetes screening tests and lipid panel from participating individuals

Parameters †	Case (n=114)	Control (n=118)	P value
Age (years)	49.8 ± 11.6	49.5 ± 12	0.857
Gender (female %)	71 %	70.3 %	1.051
Familial history of diabetes (%)	70.1 %	45.8 %	0.0001
Hypertension (%)	43 %	21.2 %	0.0001
Smoking (%)	21.9 %	20.3 %	0.872
BMI	27.85 ± 3.97	25.73 ± 3.69	0.0001
FBG (mmol/L) ‡	8.33 ± 3.49	4.93 ± 0.5	0.0001
Insulin (µIU/mL) ‡	12.06 ± 7.26	12.29 ± 9.74	0.859
HOMA-IR ‡	4.51 ± 3.32	2.82 ± 2.63	0.0001
TG (mg/dL) §	181.55 ± 39.56	124.68 ± 28.40	0.0001
Total cholesterol (mg/dL) §	179.18 ± 45.27	162.91 ± 30.22	0.212
LDL (mg/dL) §	95.62 ± 29.33	89.58 ± 26.08	0.099
HDL (mg/dL) §	39.87 ± 10.39	39.47 ± 12.17	0.787
TC/HDL ratio §	4.66 ± 1.34	4.42 ± 1.18	0.142
LDL/HDL ratio §	2.48 ± 0.82	2.47 ± 0.90	0.884
IRS-1 Gly971Arg polymorphism (allele %)	70.2%	26.3%	0.0001

† Data are expressed as arithmetic mean ± SD.

‡ Diabetes screening tests reference values: Fasting Blood Glucose (FBG) Normal: 3.9 – 5.5, Prediabetic: 5.6 – 6.9, Diabetes ≥ 7; Insulin: 2 – 25; Homeostatic Model Assessment of Insulin Resistance (HOMA-IR): Normal: < 2.0;

§ Lipid panel reference values: Triglyceride (TG): Normal: < 150, Borderline: 150 – 199, High: 200 – 499, Very high: > 500; Total cholesterol (TC): Normal: < 200, Borderline: 200 – 239, High: > 240; Low density lipoprotein (LDL): Optimal: < 100, Near optimal: 100 – 129, Borderline: 130 – 159, High: 160 – 189, Very high: > 190; High density lipoprotein (HDL): Low: <40, Normal: 40 – 60, High: >60; Total cholesterol/High density lipoprotein ratio (TC/HDL): Low risk < 5, Average risk < 5 – 7, High risk > 7, Very high risk > 11; Low density lipoprotein/ High density lipoprotein ratio (LDL/HDL): Low risk: <3.4, Average risk: 3.4 – 5, High risk > 5.

1 G971R polymorphism with BMI (P=0.094) and family history of diabetes (P=0.311); although, compared with 9.5% of participants without familial history, 15.8% of participants with the familial history of diabetes had R-allele. In order to genotype association with lipid profile, there was a positive correlation between G971R polymorphism with TG, TC, and TC/HDL ratio (P values 0.004, 0.0001 and 0.030, respectively); however, there was no any relationship between G971R polymorphism with LDL, HDL and LDL/HDL ratio (P values were 0.129, 0.314 and 0.220, respectively).

DISCUSSION

So far, different studies have shown that multiple risk genes play a critical role in the biological functions of insulin, include secretion, interaction with its receptor and insulin intracellular signaling. One of these genes is Insulin receptor substrate-1 (IRS-1) which is known as a central molecule in insulin signaling (13). IRS-1, as a linker protein between the insulin receptor and downstream molecules in different signaling pathways, mediates the metabolic and growth-promoting effects of insulin in target tissues (23). Some studies have shown that the common IRS-1 Gly972Arg polymorphism can be associated to decreased insulin responses in skeletal muscle cells, increased apoptosis and impaired insulin secretion in pancreatic beta-cells (23 – 26), but other studies could not confirm these results (27 – 30). Moreover, the content of insulin in mature secretory granules in the human islets with Arg972 IRS-1 is reduced (31).

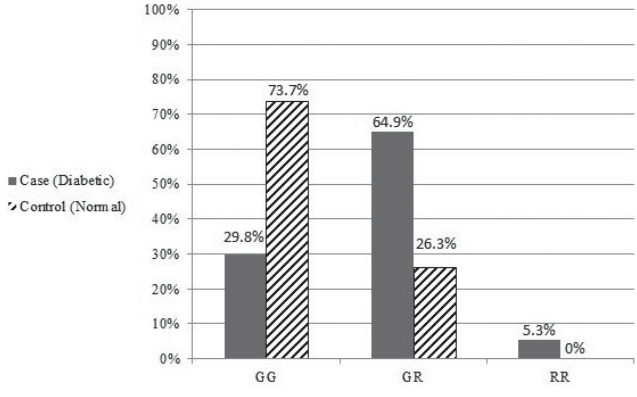

Figure 2. Genotype distribution of IRS-1 Gly971Arg gene polymorphism in study groups. Genotypes include GG (normal), GR (polymorphism in one allele), RR (polymorphism in two alleles).

The relationship between the IRS-1 Gly972Arg polymorphism and T2D has been inconclusive, especially in European studies (32–35). However, several investigations have suggested that the susceptibility to T2D in individuals with Arg972 IRS-1 polymorphism is higher than in those with normal alleles (27, 32, 36-38). Moreover, several studies suggested that the IRS-1 variants and their effects on IR and T2D may differ by ethnicity (23), degree of obesity (24, 25), and disease phenotype (12). In carriers of the Arg972 variant the average odds ratio for T2D was reported 1.25 (95% CI, 1.05–1.48) in a meta-analysis study (12). In a large population-based study including Caucasian Dutch, aged 40-70 years, the IRS-1 Gly972Arg polymorphism was examined in symptomatic (treated) and also newly diagnosed cases of T2D and the association between the IRS-1 genotypes and the degrees of obesity was determined. It has been shown that carriers of the R allele with newly detected (OR: 0.49) or treated T2D (OR: 0.71) did not have a higher prevalence of type 2 diabetes. Moreover, the R variant was not associated with hypertension, high BMI, waist circumference, plasma HDL-C and total cholesterol (16). Similarly, we did not observe a positive relationship between IRS-1 971/Arg variant and BMI, hypertension, LDL-C and HDL-C, but our findings have shown that there was a significant relationship between R allele and newly diagnosed T2D, triglycerides and total cholesterol concentrations. It has been reported that carriers of the R allele with hypertension had significantly higher levels of plasma glucose, insulin, triglycerides and HOMA-IR index than patients with normal genotype (39). Moreover, the association of IRS-1 rs1801278 variant (or G972R polymorphism) with T2D has been shown in a Pakistani population (40).

Some studies have reported that T2D patients had a greater degree of insulin resistance, carrying the R allele of G972R IRS-1 variant (24, 41), but other investigations could not observe similar findings (28, 42). In the current study, 19.25% of individuals with high HOMA index were positive in R allele; however, there was no significant association between R variant and insulin resistance, statistically.

A meta-analysis study has concluded that there

Table 2. Association between IRS-1 Gly971Arg gene polymorphism and HOMA-IR in the study population

	***IRS-1* Gly971Arg polymorphism Genotype and allele data (%)**					
HOMA-IR	GG	GR	RR	G allele	R allele	P value
HOMA-IR Normal (<2.0)	20.7 %	11.1 %	0.5%	26.25 %	6.05 %	
HOMA-IR High (>2.0)	31.4 %	34.1 %	2.2%	48.45 %	19.25 %	0.164

was a significant association between IRS-1 G971R polymorphism and the risk of T2D in a recessive (AA *vs*. GA + GG, P = 0.043) and codominant models (AA *vs*. GG, P = 0.007). Thus, it has been suggested that this polymorphism may participate in T2D risk in the Asian and Caucasian populations (13). The present study was performed on a population who lived in North Khorasan, the province in the northeast of Iran with various ethnicities such as Turkmen, Kurdish and Kurmanji. Our results have shown that there was a correlation between IRS-1 Arg variant and T2D. Despite no significant relationship between this polymorphism with increased values of insulin resistance index, there was a significant percentage of people with a higher HOMA index and 971/Arg variant (approximately three to four folds higher than those with normal HOMA-IR). It seems that the relationship between 971/Arg IRS-1 variant and insulin resistance might be statistically significant if the larger sample size was used.

A study in Turkish population showed that there is no association between Gly972Arg and Ala513Pro polymorphisms of IRS-1 gene with T2D. Both polymorphisms were not associated with fasting plasma levels of glucose, insulin, HbA1c, c-peptide, and HOMA-IR index, although these phenotypes have significant differences between study groups including diabetic patients and normal individuals. Moreover, it was used AluI restriction enzyme for determining the G972R polymorphism in Turkish study (15). However, bioinformatics study shows that the AluI, which is recognized AG↓CT restriction site, cannot recognize the IRS-1 Gly971Arg polymorphic region with CCC(A/C) GG sequence. Indeed, the best choice for detection of this sequence is SmaI restriction enzyme, which detects CCC↓GGG as a normal sequence and digests it. On the other hand, the Gly971Arg gene polymorphism that varies Gly (with GGG codon) to Arg (with AGG or CGG codon) is not detectable by SmaI. Thus, the reported results by above study (15) need more bioinformatics investigation and confirmation.

In conclusion, the obtained results showed that IRS-1 971R variant is associated with T2D, significantly. Moreover, the Gly/Arg and Arg/Arg genotypes in individuals with high HOMA-IR were three times and four times higher than individuals with normal HOMA-IR, respectively. However, the statistically significant differences between IRS-1 polymorphism and insulin resistance index were not obtained. It may occur because of the overlap of HOMA-IR values between healthy control group and newly diagnosed diabetic group. In addition, we did not find any significant relationship between IRS-1 971R variant and hypertension, BMI and familial history of diabetes. Altogether, the IRS-1 G971R polymorphism can be a suitable candidate for genetics investigation of Iranian individuals with newly-diagnosed T2D. We have suggested that the association of other variants of IRS-1 with insulin resistance and newly diagnosed diabetic patients should be investigated in a larger population to implement screening genotyping tests. Further studies are warranted to clarify the roles of the genetic variants of the IRS-1 gene in developing insulin resistance and possible mechanisms responsible for individual's susceptibility to type 2 diabetes.

Conflict of interest

The authors declare that they have no conflict of interest.

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