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ORIGINAL ARTICLE

Association of the genetic variants of insulin receptor substrate 1 (IRS-1) with type 2 diabetes mellitus in a Saudi population

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Abstract Type 2 diabetes mellitus (T2DM) is a chronic degenerative disease, phenotypically and genetically heterogeneous, characterized by high levels of glucose and metabolic complications. Insulin receptor substrate 1 (*IRS-1*) plays a key role in the insulin-stimulated signal transduction pathway. A glycine-to-arginine substitution at codon 972 (G972R) (rs1801278) in the *IRS-1* gene has been associated with impaired insulin action. Another SNP rs2943641 in the *IRS-1* gene has been found to be associated with T2DM and insulin resistance in genome-wide association studies. The aim of the present study was to evaluate whether rs1801278 and rs2943641 are associated with increased risk of T2DM in the Saudi population. The study included 376 T2DM cases and 380 healthy controls. Genomic DNA was isolated using a commercially available kit supplied by Norgen Biotech Corp. Genotyping was performed by PCR and RFLP analysis. There was a significant difference in the genotypic distribution as well as allelic frequency between the T2DM cases and controls in case of both the polymorphisms for rs1801278 (1.752, 95 % CI 1.002–3.121; $p = 0.04$), and for rs2943641 (OR = 1.482, 95 % CI 1.176–1.867; $p = 0.001$). In conclusion, both the (rs1801278 and rs2943641) polymorphisms are associated with T2DM in the Saudi population.

Keywords Type 2 diabetes mellitus (T2DM) · Insulin receptor substrate 1 (*IRS-1*) gene · Insulin resistance (IR) · Saudi population

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Introduction

Type 2 diabetes mellitus (T2DM) is characterized by insulin resistance (IR) in insulin-target tissues and impaired insulin secretion from pancreatic β-cells [1]. The clinical expression of the disease can be prevented by nutritional and lifestyle amendments [2]. Twin studies and family studies have suggested that IR has a genetic component. Many candidate gene variants including insulin receptor substrate 1 (*IRS-1*) gene have been proposed to contribute significantly to the risk of T2DM [3]. The variants of *IRS-1* gene have been related to IR, obesity, and T2DM [4]. *IRS-1,* a member of the *IRS* protein substrate family, is considered to play an important role in the insulin-signaling pathway [5]. *IRS-1* encodes (*IRS*)-1, which is the endogenous substrate of the insulin receptor in the insulin-signaling pathway and is ubiquitously expressed in insulin-sensitive tissues [6].

The *IRS-1* protein is a cytoplasm molecule expressed in many insulin-sensitive tissues, which has an important

role in regulating the cellular effect of insulin. After the binding of insulin to its receptor, the β subunit of the receptor is activated (tyrosine kinase activity), leading to phosphorylation of specific tyrosine residues on *IRS-1*. When phosphorylated, *IRS-1* binds to a series of cellular signal proteins, including phosphatidylinositol 3 kinase [4]. Genetic analysis of the *IRS-1* gene (rs1801278) has shown that a glycine-to-arginine substitution at codon 972 (Gly972Arg) significantly impairs *IRS-1* function and is associated with IR, lipid abnormalities [7], and T2DM [8]. Gly972Arg variant has also been reported to be associated with increased risk of T2DM, albeit inconsistently, in association studies conducted in European Caucasian populations [7]. However, a meta-analysis of 32 studies, including 12,076 cases and 11,285 controls, did not show the rs1801278 variant to be significantly associated with T2DM [9]. In the present study, we evaluated the association of two genetic variants (rs1801278 and rs2943641) of *IRS-1* gene with T2DM in a Saudi population.

Materials and methodology

Ethics

Ethical approval for the study was obtained from the Ethics Committee, King Saud University, Riyadh, Kingdom of Saudi Arabia. Written informed consent was obtained from each patient.

Sample collection

Five milliliter of venous blood was collected; 3 mL of the serum sample was used for the biochemical analysis, and 2 mL of the EDTA sample was used for the molecular analysis.

Study population

A total of 756 individuals were included in this study. The subjects were from the capital city of Riyadh, Kingdom of Saudi Arabia. Of these, 376 were unrelated patients with T2DM who visited the primary health care outpatient and poly clinics in Riyadh. The study excluded all subjects having history of other metabolic disorders apart from T2DM. Healthy individuals ($n = 380$), who had normal glucose levels, formed the control group.

T2DM patients with fasting plasma glucose of >7.0 mmol/L were included in the study. Those with history of ketoacidosis or exocrine pancreatic disease, or with other metabolic disorders, were excluded.

Anthropometric and biochemical measurements

All the subjects were examined in the morning after an overnight fast for 10–12 h. Height, weight, waist circumference, hip circumference, and blood pressure were measured as described previously [6]. BMI was calculated as weight/height 2 (kg/m^2). Subjects with BMI ≥30 kg/m^2 were categorized as obese group. Blood pressure of the subjects was measured in a sitting position taking the mean of the two readings 30 min apart. Hypertension was defined as mean systolic blood pressure of 140 mmHg and/or a diastolic blood pressure of 90 mmHg. Blood samples were collected to measure fasting plasma glucose, fasting serum insulin, and blood lipids, including total cholesterol, total triglyceride, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). Plasma glucose levels were measured by the glucose oxidase–peroxidase method. Fasting serum insulin levels were measured using a radioimmunoassay. Insulin resistance index [Homeostasis Model Assessment-Insulin Resistance (HOMA-IR)] was calculated as fasting insulin (mU/L) × fasting plasma glucose (mmol/L)/22.5, and β-cell function (HOMA-β) was calculated as fasting insulin × 20/(fasting plasma glucose—3.5), as described previously [10].

Molecular analysis

Genomic DNA was extracted from peripheral blood leukocytes using Norgen DNA extraction kit (Norgen Biotek corp, Canada). DNA samples were stored at −80 °C. Molecular analysis was performed at the facility of the Department of clinical laboratory sciences, College of Applied Medical Sciences, King Saud University, Riyadh, and Kingdom of Saudi Arabia.

Genotyping

Genotyping for the rs1801278 and rs2943641 was performed by polymerase chain reaction (PCR) using a thermal cycler (Applied Biosystem, USA). A total volume of 20-μL reaction mixture containing 2 μL of each primer (10 pmol), 7 μL of sterile water, and 10 μL of 2× master mix which includes $MgCl_2$, 10× Taq buffer, 10 unit of Taq DNA polymerase (Norgen Biotek corp, Canada), and the 1 μL template DNA was used for amplification of the regions bearing G972A and C988T polymorphisms. Primers were synthesized by Bioserve Biotechnology (Hyderabad, India) for PCR analysis (Table 1).

DNA was denatured at 95 °C for 5 min, amplified by 35 cycles of 95 °C for 30 s, 61 °C for 30 s for rs1801278 polymorphism (50 °C for 30 s rs2943641 polymorphism in *IRS-1* gene), 72 °C for 45 s, and the final extension with 72 °C for 5 min.

Table 1 List of primers and restriction enzymes used in this study

rs no	Amino acid substitution	Nucleotide change	Forward primer	Reverse primer	PCR product	Annealing temp (°C)	Enzyme
rs1801278	Gly972Arg	C > **T**	CTTCTGTCAGGTGTCCATCC	TGGCGAGGTGTCCACGTAGC	261 bp	61	*Bst*NI
rs2943641	–	C > **T**	ATGGCACCAATTGGGTAAGA	AACTAGGGGAATATCAGGGCTAA	187 bp	50	HpyAv

PCR products were digested for 2 h with *Bst*NI ($CC^{\downarrow}WGG$) (New England Biolabs, USA) at 37 °C (2.5 μL of distilled water with 10 units of enzyme for 15 μL PCR product and 2 μL buffer in a final volume of 20 μL), for rs1801278 and electrophoresed on ethidium bromide added 2 % agarose gel. Three genotypes could be determined after electrophoresis: Gly972 homozygotes (159/81/23 bp band), Arg972 (108/81/51/23 bp band), and Gly972Arg (both bands) that was a heterozygous. The augmented PCR products for rs2943641 were digested for 2 h with HpyAv ($CC^{\downarrow}TCC\ [N]_6$) (New England Biolabs, USA) at 60 °C (2.5 μL of distilled water with 10 units of enzyme for 15-μL PCR product and 2 μL buffer in a final volume of 20 μL), and electrophoresed on ethidium bromide added 3.5 % agarose gel. Three genotypes could be determined after electrophoresis: genotype CC (160/27 bp band), genotype TT (187 bp band), and genotype CT (both the bands).

Statistical analysis

Data are expressed as mean ± SD. Student's *t* test was used to analyze unpaired data. Qualitative data were compared by the chi-square test. Allele frequencies were estimated by the gene-counting method, and the chi-square test was used to identify departures from Hardy–Weinberg equilibrium. The genotype distribution of rs1801278 and rs2943641 polymorphisms was compared between T2DM subjects and controls by the chi-square test. Statistical significance was examined by two-sided tests, and statistical analysis was performed with SPSS version 19.0 software. Yates correction was performed with openepi software. A p value of <0.05 was considered to be statistically significant.

Results

Clinical characteristics

Clinical and the anthropometric data of the subjects have been given in Table 2. The results show that T2DM subjects were significantly older than controls. The anthropometric measurements including weight, height, BMI, hypertension, SBP, DBP, hip circumference were significantly higher in T2DM patients compared with control subjects ($p < 0.05$). T2DM subjects had higher levels of fasting glucose, insulin, and HOMA-IR as well as triglycerides, LDL-C, systolic BP and but not waist and circumference ($p < 0.05$). However, there was no significant difference in DBP as well as waist circumference between the two groups.

Genotype distribution

The genotypic distribution of Gly972Arg polymorphism of *IRS-1* gene has been given in Table 3. We observed a significant difference in the frequencies of CC and CT genotypes of rs1801278 between patients and controls. The frequencies of these genotypes in T2DM patients were 90.9 and 9.1 %, respectively. The frequencies of C and T alleles were 0.95 and 0.05 in patients and 0.97 and 0.03 in controls. TT genotype was completely absent in both the groups. The minor allele frequencies of controls and T2DM were 0.03 and 0.05, respectively ($p = 0.04$). There were significant differences in the frequencies of the genotype distributions of *IRS-1* Gly972Arg between the control group and T2DM group [for CT Vs CC; $p = 0.04$; odds ratio = 1.789 (95 % CI 1.01–3.17)] (Table 3).

In the T2DM, the frequencies of rs2943641 CC, CT, and TT genotypes were 53.5, 33.2, and 13.3 %, respectively. The percentage of T allele was 0.30 %, and C allele was 0.70 % in T2DM cases. In normal subjects, the distribution of CC, CT, and TT genotypes was 65, 25.3, and 9.7 %, respectively. The allele frequency of control subjects of C and T allele was 0.78 and 0.22, respectively. The genotypic distribution of rs2943641 polymorphism and allele frequency of patients and controls has been given in Table 2. We found significant difference in the allele and the genotypic distribution between patients and controls [for T Vs C; $p = 0.001$; odds ratio = 1.482 (95 % CI 1.176–1.867); and CC vs CT+TT; $p = 0.015$; odds ratio = 1.473 (95 % CI 1.075–2.02)] (Table 3). In this study, the power and sample size calculation were found to be 58 %. We have adjusted the potential confounders with both the polymorphisms, and we found significance difference; the details are tabulated in Table 4. We have calculated the Bonferroni correction. For genotypes, $p = 0.0225$ after the correction. Therefore, both the variant genotypes of both the SNPs are significantly associated with the disease.

Discussion

T2DM is a complex, polygenic disorder. The prevalence of T2DM risk loci has grown rapidly. Several genetic polymorphisms have already been implicated in the pathogenesis of IR as well T2DM [11]. The *IRS-1* gene has been considered to be a candidate gene for metabolic diseases such as T2DM and obesity. The presence of genetic variation of the *IRS-1* gene has been reported to be associated with the development of IR [2]. The *IRS-1* gene located on chromosome 2q36 is the substrate of the insulin receptor tyrosine kinase, which participates in insulin signaling. The protein is expressed in a variety of insulin responsive cells and tissues. Binding of insulin to its receptor induces phosphorylation of the cytosolic substrates *IRS-1* and *IRS-2*. Stimulation of *IRS-1* is a key initial step in the insulin-signaling pathway, and the functional studies of variants in the *IRS-1* gene showed impaired insulin signaling through the PI3-kinase pathway [12] and impaired insulin secretion [13].

Table 2 Demographic characteristics of the study population

N	T2DM 376	Controls 380	*p* value
Age (years)	50.6 ± 10.3	46.2 ± 7.6	<0.001
Sex: male/female	225 (59.8 %)/ 151(40.2 %)	202 (53.2 %)/178 (46.8 %)	0.003
Body mass index (kg/m^2)	29.51 ± 5.9	29.19 ± 5.5	0.18
Hypertension (%)	6.5 %	8.9 %	0.001
Waist (cm)	94.3 ± 22.3	91.2 ± 20.26	0.05
Hip (cm)	104.8 ± 21.4	94.4 ± 7.8	<0.001
FBS (mmol/L)	9.8 ± 5.2	5.2 ± 0.60	<0.001
Triglycerides (mmol/L)	2.2 ± 1.6	1.6 ± 0.86	<0.001
Cholesterol (mmol/L)	5.6 ± 1.2	5.05 ± 0.97	<0.001
HDL-cholesterol (mmol/L)	0.93 ± 0.75	0.64 ± 0.23	<0.001
LDL-cholesterol (mmol/L)	3.8 ± 1.07	3.6 ± 0.85	<0.001
Glucose (mmol/L)	9.4 ± 1.5	8.7 ± 1.82	<0.001
Insulin (μU/mL)	16.2 ± 2.2	12.3 ± 1.7	<0.001
Homa-IR	7.1 ± 2.4	2.8 ± 1.7	<0.001
Family history	376 (100 %)	200 (52.6 %)	<0.001

Table 3 Allele and genotype distribution of IRS-1 (rs1801278 and rs2943641) gene

Genotype and allele	T2DM cases ($n = 376$) *N* (%)	Controls ($n = 380$) *N* (%)	*p* value[a]
rs1801278			
CC	342 (90.9)	360 (94.7)	0.04
CT	34 (9.1)	20 (5.3)	
TT	0.0	0.0	
C	718 (0.95)	740 (0.97)	0.04
T	34 (0.05)	20 (0.03)	
rs2943641			
CC	201 (53.5)	247 (65.0)	0.005
CT	125 (33.2)	96 (25.3)	
TT	50 (13.3)	37 (9.7)	
C	527 (0.70)	590 (0.78)	0.001
T	225 (0.30)	170 (0.22)	

[a] Chi-square *p* value

Table 4 Statistical analysis adjust by potential confounders

	Odds ratio (95 % CI)[a]	*p* value	Odds ratio (95 % CI)[b]	*p* value
rs1801278				
CC	1.0		1.0	
CT	2.0 (1.2, 3.5)	0.01	2.2 (1.2, 4.0)	0.01
TT	–	–	–	–
CT+TT	2.0 (1.2, 3.5)	0.01	2.2 (1.2, 4.0)	0.01
C	1.0	–	–	–
T	1.7 (1.0, 3.1)	0.04	–	–
rs2943641				
CC	1.0		1.0	
CT	1.6 (1.2, 2.2)	0.005	1.6 (1.1, 2.2)	0.01
TT	1.6 (1.04, 2.6)	0.03	1.9 (1.1, 3.1)	0.01
CT+TT	1.6 (1.2, 2.2)	0.001	1.7 (1.2, 2.3)	0.002
C	1.0	–	–	–
T	1.5 (1.2, 1.9)	0.001	–	–

[a] Crude odds ratio (95 % CI)

[b] Odds ratio (95 % CI) adjusted for age, gender, and BMI

In the present study, we evaluated the association of two common and broadly studied polymorphisms, rs1801278 and rs2943641 of the *IRS-1* gene with T2DM risk in a Saudi population. To the best of our knowledge, this is the first study evaluating this association in a Saudi population. The results of our study revealed that the variant allele of the (Arg972) of rs2943641 of the *IRS-1* gene is associated significantly with T2DM in the Saudi population.

Several studies have associated G972A SNP in the *IRS-1* gene (rs1801278) with T2DM [7, 11]. This nucleotide alteration at codon 972 results in an amino acid change, glycine to arginine.

Burguete-Garcia et al. [14] have evaluated *IRS-1* gene polymorphism with T2DM in the Mexican population. In Burguete-Garcia et al. [14] studies, 4 SNPs were selected, based on the previous observations and a greater genetic predisposition among lean diabetics. Four nonsynonymous SNPS (rs1801276 Pro512Ala; rs3731594 Asn1137Asp; 1801278 Gly927Arg; and rs1801108 Arg158Pro) were selected 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. Of the 4 SNPs studied, only Gly972Arg showed significant difference between cases and controls, with allele frequency of 2.6 % in controls as compared with 7.9 % in cases. The other 3 SNPs did not show any significant difference in the T2DM cases in comparison with controls [14]. Transfection studies with insulin receptor cells indicated that the Gly972Arg polymorphism of the *IRS-1* gene has the major cytoplasmic substrate in impaired insulin-stimulated signaling [15]. A relationship between this polymorphism with the risk of T2DM is consequently biologically credible [7]. Another SNP evaluated in this study (rs2943641) was found to be associated with T2DM in a French population by genome-wide association studies (GWAS). In the present study, the mutant allele T (SNP in *IRS-1*) was found to be in a higher frequency in T2DM patients than in controls in the study population. The allele frequency, dominant, and co-dominant model showed a significant association with the disease ($p < 0.05$). Other previous studies have also shown an association of this polymorphism with T2DM [16, 17].

Unlike formerly reported T2DM risk loci, which were predominantly companion with impaired β-cell function, the C allele of rs2943641 has been associated with insulin resistance and hyperinsulinemia in the French population involving 14,358 individuals. In a recent study by Ericson et al. [17] employing 15,227 women and 9,614 men aged 45–74 years without established diabetes, dietary data were collected from these subjects with a modified diet history method. During 12 years of follow-up, 1,567 incident T2DM cases were identified. Genotyping for the rs2943641 was performed in the T2DM subjects, and the results concluded that T allele was significantly associated with the lower incidence of T2DM ($p = 0.003$). *IRS-1* (rs2943641) gene interacts with carbohydrate and fat intakes on incident T2DM in a sex-specific fashion. A protective association between the rs2943641 T allele and T2DM was restricted to women with low carbohydrate intake and to men with low fat intake [16, 17].

In the present study, we found significant differences in the distribution of genotypes of rs1801278 and rs2943641 SNPs among controls and T2DM subjects. The constant efforts in cataloging the genotypes of different ethnic groups will further contribute to our understanding of how genetic risk of T2DM is distributed.

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Conflict of interest All the authors declare that there is no conflict of interest.

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