Effect of genetic polymorphisms on the development of secondary failure to sulfonylurea in Egyptian patients with type 2 diabetes

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Abstract:

Objective: This study investigated the possibility that genetic factors, such as polymorphism of K inward rectifier subunit (Kir6.2), E23K, and Arg⁹⁷² polymorphism of insulin receptor substrate-1 (IRS-1), may predispose patients to sulfonylurea failure.

Methods: A total of 100 unrelated Egyptian patients with type 2 diabetes were recruited. They were divided into two equal groups: group I consisted of patients with secondary failure to sulfonylurea (hemoglobin A_{1c} ≥ 8% despite sulfonylurea therapy) while group II consisted of patients whose condition was controlled with oral therapy.

Results: Of all the patients, 45% and 14% were carriers of the K allele and Arg⁹⁷² variants respectively. The frequency of the K allele was 34% among patients with diabetes that was controlled with oral therapy and 56% among patients with secondary failure to sulfonylurea. The frequency of the Arg⁹⁷² IRS-1 variant was 6% among patients with diabetes controlled with oral therapy and 22% among patients with secondary failure.

Conclusion: The E23K variant of the Kir6.2 gene and Arg⁹⁷² IRS-1 variants are associated with increased risk for secondary failure to sulfonylurea.

Keywords: Arg⁹⁷² IRS-1 variant, E23K polymorphism, IRS-1, *KCNJ11*, Kir6.2, sulfonylurea failure

Introduction

Type 2 diabetes is a heterogeneous disease characterized by disturbances in both insulin secretion and peripheral tissue sensitivity to insulin action and expressed in different degrees in particular patients [Borissova *et al.* 1991].

The different major classes of oral antihyperglycemic agents in current use can be divided into those that increase insulin secretion as sulfonylureas, those that decrease insulin resistance as metformin and thiazolidinediones, and those that modify the rate of glucose entry from the gastrointestinal tract as α -glucosidase inhibitors [Kimmel *et al.* 2005].

Sulfonylureas stimulate insulin release from pancreatic β -cells by first binding to the high-affinity sulfonylurea receptor (SUR1), which together with the potassium pore-forming inward-rectifier

(Kir6.2) subunits make up the pancreatic β -cell ATP-sensitive potassium (K_{ATP}) channel. This interaction closes the K⁺ channel, which inhibits potassium efflux and depolarizes the plasma membrane, leading to an opening of voltage-gated calcium channels. Calcium influx, and a corresponding increase in intracellular calcium levels, causes release of insulin from the β -cells [Distefano and Watanabe, 2010].

Treatment with sulfonylureas is initially successful in type 2 diabetes. However, it is often associated with high secondary failure rate (hemoglobin A_{1c} ≥ 8%) with the highest recommended dose in patients who initially respond to sulfonylurea therapy and consequently switch to other treatment progressively as sulfonylurea fails [Nichols *et al.* 2007]. The underlying mechanisms are still undefined. Poor compliance with diet, gain in body weight, deterioration of insulin

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sensitivity, cell exhaustion, conditions requiring the use of a diabetogenic drug, and the presence of anti-islet cell and glutamic acid decarboxylase (GAD) antibodies have been suggested as causative factors. An increased frequency of human leukocyte antigens (HLA21 and HLADRI) may also play a part. The progressive decline of pancreatic β -cell function has been demonstrated to be the stronger predictor of the insulin-requiring stage in type 2 diabetes [Sesti *et al.* 2006; Borissova *et al.* 1991].

Genetic factors are clearly important for the main pathogenic mechanisms leading to common type 2 diabetes, β -cell dysfunction, and impaired insulin action. Among plausible candidate genetic variants that could affect insulin secretion are the E23K polymorphism of the Kir6.2 gene (*KCNJ11*, on chromosome 11), and polymorphism in insulin receptor substrate-1 (IRS-1) causing a Gly972Arg change (Arg⁹⁷²) [Gloyn *et al.* 2005; Sigal *et al.* 1996].

Mice lacking the Kir6.2 gene are characterized by impaired glucose- and tolbutamide-induced insulin secretion. Gain-of-function mutations in Kir6.2 produces permanent neonatal diabetes, whereas loss-of-function polymorphisms in *KCNJ11* have been associated with familial hyperinsulinemic hypoglycemia of infancy [Gloyn *et al.* 2004; Miki *et al.* 1998; Nestorowicz *et al.* 1997].

The common E23K polymorphism, in which substitution in codon number 23 of glutamic acid (E) resulted in the codon of lysine (K) amino acid, has been most extensively studied in the classical form of type 2 diabetes, and has been identified and found to be a major diabetogene in different populations [Hani *et al.* 1997; Lindner *et al.* 1997; Inoue *et al.* 1996; Iwasaki *et al.* 1996]. Some studies demonstrate that the E23K polymorphism increases in intrinsic open probability of K_{ATP} channels, with a consequent reduction in ATP sensitivity, and enhanced activation by Mg nucleotides and long-chain acyl-CoAs [Reidel *et al.* 2003; Schwanstecher *et al.* 2003].

A study of 525 white patients with type 2 diabetes found a higher frequency of the K allele in patients who failed sulfonylurea therapy compared with those who did not (66.8% *versus* 58.0%, respectively) [Sesti *et al.* 2006].

IRS-1 functions as one of the key signaling molecules in the insulin receptor signaling pathway. After autophosphorylation of the insulin receptor, the receptor kinase is activated and phosphorylates IRS-1 and other intracellular substrates. This signaling molecule then acts as a docking protein for multiple Src homology-2 domain (SH2)-containing proteins, including phosphatidylinositol (PI) 3-kinase. Consequently, this leads to activation of several enzymes that may be responsible for mediating insulin action within the target cell. Molecular scanning of the IRS-1 gene in normal individuals and patients with type 2 diabetes has revealed several polymorphisms resulting in amino acid substitutions, the most common of which is a Gly $\rightarrow$ Arg substitution at codon 972 (Arg⁹⁷²) [Strohmer *et al.* 2005; Xu *et al.* 2000].

A meta-analysis of 27 studies including 3408 patients with diabetes and 5419 controls revealed that carriers of the Arg⁹⁷² IRS-1 variant have an increased risk for type 2 diabetes [Jellema *et al.* 2003]. Transfection studies have demonstrated that the Gly972Arg change has a functional consequence causing impairment in IRS-1-associated PI 3-kinase activity owing to their defective interaction [Hribal *et al.* 2000; Porzio *et al.* 1999]. Several investigations have been performed, usually suggesting that the Arg972 IRS-1 polymorphism could predispose people to type 2 diabetes in certain ethnic groups, such as Finnish, Danish, French and Italian [Bezerra *et al.* 2004].

Early investigations in pancreatic β -cell lines and human pancreatic islets of Langerhans found that the genotype at the Gly972Arg polymorphism of *IRS1* affected the level of insulin secretion in response to sulfonylureas. The clinical impact of this polymorphism was also investigated in white patients with type 2 diabetes who were treated with sulfonylurea agents. In these patients, the genotype frequency of the variant allele (Arg972) was almost twice as high in patients who experienced secondary sulfonylurea failure compared with those with controlled glycemia [Sesti *et al.* 2004].

To test the intriguing hypothesis that patients with diabetes carrying E23K, and Arg⁹⁷² polymorphisms are at increased risk for secondary failure to sulfonylurea, the prevalence of the E23K and Arg⁹⁷² variants were examined in

Egyptian patients with type 2 diabetes with and without secondary failure to sulfonylurea.

Material and methods

Patient population

The study was carried out at the Specialized Internal Medicine Hospital, Mansoura University. The study included 100 unrelated Egyptian patients with type 2 diabetes with onset of the condition after the age of 35 years, and absence of the GAD antibody which may be associated with the development of type 1 diabetes. Patients were nonsmokers and their age ranged from 40 to 60 years. Diabetes was diagnosed in accordance with the World Health Organization 1999 criteria. Patients were excluded if they had chronic gastrointestinal diseases associated with malabsorption, chronic pancreatitis, history of any malignant disease, history of alcohol or drug abuse, liver or kidney failure, or clinical problems potentially causing hyperglycemia, including infection, thyroid disease, surgery, and treatments known to modify glucose metabolism. They were divided into two groups (50 patients each): group I included patients with secondary failure to sulfonylurea. Patients with secondary failure are defined as those with uncontrolled hyperglycemia (hemoglobin A_{1c} $\geq 8\%$) despite sulfonylurea therapy. Group II, the control group, consisted of patients whose condition was controlled with sulfonylurea therapy. The two groups were matched according to mean age, mean body mass index (BMI), duration of therapy and sex distribution. Informed consent was obtained from all patients. This study was approved by the Tanta University ethics committee.

Biochemical assays

Blood samples were taken from patients after overnight fasting in order to measure fasting serum levels of glucose enzymatically (Spinreact, Santa Coloma, Sant Esteve De Bas, Spain) [Kaplan, 1984], total cholesterol, triglycerides and high-density lipoprotein cholesterol (Pointe Scientific, Inc., Canton, Ohio, USA) [Fossati and Prencipe, 1982; Lopes-Virella *et al.* 1977; Allain *et al.* 1974]. Hemoglobin A_{1c} concentrations were analyzed by the cation-exchange resin method (Hospitex Diagnostics, Sesto Fiorentino, Italy) [Trivelli, 1971]. Insulin was also analyzed by the Immulite 1000 kit (Siemen Medical Solution Diagnostics, Los Angeles, California, USA) [Sapin *et al.* 1998].

DNA genotyping

The E23K of Kir6.2 and G972R of IRS-1 polymorphisms were genotyped using the polymerase chain reaction-restricted fragment length polymorphism (PCR-RFLP) method. Genomic DNA was isolated from 200 μ l whole blood samples using the Qiagen Extract kit (Qiagen, Hilden, Germany).

Kir6.2 genotyping. The DNA of the Kir6.2 gene was amplified by the PCR technique using a forward primer (5'-GAATACGTCCTGACAC GCCT-3') and a reverse primer (5'-GCCA GCTGCACAGGA AGGACAT-3') (Operon, Ebersberg, Germany), which flanked the region containing the Kir6.2 gene (product size, 218 bp). PCR was performed using a 25 μ l volume containing 5 μ l genomic DNA, 1.5 μ l of each primer, and 17 μ l of the Taq PCR master mix kit (Qiagen). PCR conditions were denaturation at 95°C for 3 min, 35 cycles of denaturation at 95°C for 1 min., annealing at 60°C for 1 min, elongation at 72°C for 1 min, and a final elongation step at 72°C for 9 min.

Ten microliters of PCR product was digested with 2 μ l of 10 U/ μ l *Ban*II enzyme (Promega, Madison, Wisconsin, USA) at 37°C overnight. The digested probes were subjected to electrophoresis on 1% agarose (Sigma Chemical Co., St Louis, Missouri, USA) with 3% Nusieve agarose (Sigma Chemical Co.) and stained with ethidium bromide (Sigma Chemical Co.) for visualization. The gel was run at 150 V for 1 h [Ezenwaka *et al.* 2005].

IRS-1 genotyping. Two oligonucleotide primers: forward 5'-CTTCTGTCAGGTGTC CATCC-3' and reverse 5'-TGGCGAGGTGTCCACG TAGC-3' (Operon) were used for amplification of DNA which was carried out on 3 μ l genomic DNA with a final volume of 25 μ l. The assay consisted of 15 μ l of Taq PCR master mix kit (Qiagen), and 2 μ l of the two oligonucleotide primers. A cycling program using a GeneAmp 9600 thermal cycler (Progene, Tecne, Cambridge, UK) was as follows: initial denaturation at 95°C for 5 min, followed by 37 cycles of denaturation at 95°C for 45 s, annealing at 58°C for 45 s, elongation at 72°C for 45 s, and a final elongation step at 72°C for 5 min. Restriction enzyme digestion was carried out in 30 μ l reactions containing 25 μ l PCR product, 3 μ l of 10 $\times$ reaction buffer, 2 μ l of the restriction enzyme *Mva*I. The reactions were incubated at 37°C overnight before electrophoresis. Electrophoresis was carried out on a 3%

agarose gel and visualized after staining with ethidium bromide [Pavlova *et al.* 2004; Federici *et al.* 2003].

Statistical analysis

Continuous data were shown as means $\pm$ standard deviation (SD) and logarithmic transformation was used for data not normally distributed. Comparisons between genotypes were made using the unpaired Student's *t*-test (E/E *versus* E/K + K/K and G/G *versus* G/R + R/R). Fisher's exact test was used to test for differences in genotype frequencies with 95% confidence interval. The values at $p < 0.05$ were chosen as statistically significant. All analyses were performed using the Statistical Package of the Social Sciences (SPSS) software program.

Results

The clinical characteristics of the study participants stratified according to therapy and genotypes are reported in Table 1. Of all of the patients, 45% were carriers of the K allele. The genotype frequency of the K allele (E/K heterozygous + K/K homozygous) was 34% among patients with diabetes whose condition was controlled with sulfonylurea and 56% among patients with secondary failure to sulfonylurea (Figure 1). Thus, the E23K variant was associated with secondary failure to sulfonylurea with

carriers of the K allele having a relative risk of 1.65 (95% CI 1.04–2.6, $p = 0.04$) compared with those who were E23E homozygous (Table 2).

The study also investigated whether the Arg⁹⁷² IRS-1 variant was associated with increased risk for secondary failure to sulfonylurea in the same patients. Of all the patients, 14% were heterozygous for the Arg⁹⁷² IRS-1 variant, no one was homozygous, and the remainder (86%) were homozygous for the wild-type allele. The genotype frequency of the Arg⁹⁷² IRS-1 variant was 6% among patients with diabetes that was controlled with oral therapy and 22% among patients with secondary failure to sulfonylurea (Figure 1). Thus, the Arg⁹⁷² IRS-1 variant was associated with secondary failure to sulfonylurea, with carriers having a relative risk of 1.75 (95% CI 1.08–12.4, $p = 0.041$). By comparing the Arg⁹⁷² variant and the wild-type allele in both groups, the study found that the Arg⁹⁷² variant was associated with an increase in BMI ($p = 0.009$, 0.03, respectively), and so the Arg⁹⁷² polymorphism of IRS-1 may be associated with obesity (Table 3). Moreover, fasting insulin levels and insulin resistance, estimated as homeostasis model assessment of insulin resistance (HOMA-IR) index, were significantly higher in heterozygous carriers for the Arg⁹⁷² IRS-1 variant compared with wild-type allele carriers in both studied groups ($p = 0.009$ *versus* 0.01 and $p = 0.03$ *versus*

Table 1. Anthropometric and biochemical characteristics of the study population.

	Patients with secondary failure	Patients without secondary failure	<i>p</i>
<i>n</i> (men/women)	24/26	26/24	1
Age (years)	51.5 $\pm$ 2.9	52.1 $\pm$ 4.0	0.4
Duration of therapy with oral agents (years)	6.2 $\pm$ 1.6	6.4 $\pm$ 1.99	0.8
BMI (kg/m ²)	30.5 $\pm$ 1.6	30.0 $\pm$ 1.2	0.06
Hemoglobin A1c (%)	9.5 $\pm$ 1.4	7.5 $\pm$ 0.5	0.000*
Fasting insulin (μ U/ml)	10.3 $\pm$ 0.7	7.5 $\pm$ 0.5	0.000*
Fasting glucose (mmol/liter)	11.3 $\pm$ 0.8	8.7 $\pm$ 0.5	0.000*
HOMA-IR	4.7 $\pm$ 0.6	2.7 $\pm$ 0.4	0.000*
HOMA- β cell	23.9 $\pm$ 1.8	28.7 $\pm$ 1.5	0.000*
QUICKI	0.305 $\pm$ 0.005	0.325 $\pm$ 0.005	0.000*
Total cholesterol (mmol/liter)	5.05 $\pm$ 0.31	4.99 $\pm$ 0.3	0.4
Triglycerides (mg/dl)	1.9 $\pm$ 1.2	1.98 $\pm$ 0.12	0.06
HDL cholesterol (mmol/liter)	1.1 $\pm$ 0.06	1.2 $\pm$ 0.05	0.06
LDL cholesterol (mmol/liter)	3.6 $\pm$ 0.32	3.5 $\pm$ 0.3	0.07

Data are mean $\pm$ standard deviation.

* $p < 0.05$: significant difference.

BMI, body mass index; HDL, high-density lipoprotein; HOMA-IR and HOMA- β cell, homeostasis model assessment of insulin resistance and of β -cell function respectively; LDL, low-density lipoprotein. QUICKI, quantitative insulin check index.

Figure 1. The genotype frequencies of the E23K and Arg⁹⁷² insulin receptor substrate-1 (IRS-1) variants among patients with secondary failure to sulfonylurea (group I) and patients with diabetes whose condition is controlled by sulfonylurea (group II). * $p < 0.05$: significant difference.

Table 2. Anthropometric, and biochemical characteristics of the study population according to the potassium inwardly rectifier 6.2 (Kir6.2) genotype.

	Patients with secondary failure			Patients whose condition is controlled with sulfonylurea		
	Carriers of E allele	Carriers of K allele	<i>p</i>	Carriers of E allele	Carriers of K allele	<i>p</i>
<i>n</i> (men/women)	10/12	14/14	0.26	18/15	7/10	0.77
Age (years)	52.3 ± 2.2	50.9 ± 3.1	0.08	52.6 ± 4.2	51.2 ± 3.7	0.2
BMI (kg/m ²)	30.8 ± 1.7	30.3 ± 1.5	0.32	29.8 ± 1.2	30.3 ± 1.2	0.19
Hemoglobin A1c (%)	9.3 ± 1.3	9.7 ± 1.4	0.28	7.6 ± 0.4	7.7 ± 0.24	0.08
Fasting insulin (μU/ml)	10.6 ± 0.7	10.1 ± 0.6	0.16	8.5 ± 0.4	8.7 ± 0.5	0.93
Fasting glucose (mmol/liter)	11.4 ± 0.7	11.2 ± 0.9	0.36	8.7 ± 0.5	8.7 ± 0.6	0.99
HOMA-IR	4.8 ± 0.6	4.6 ± 0.6	0.22	2.7 ± 0.4	2.7 ± 0.4	0.9
HOMA-β cell	23.8 ± 1.1	23.9 ± 1.7	0.87	28.6 ± 1.5	28.5 ± 1.3	0.91
QUICKI	0.304 ± .005	.306 ± .006	0.21	0.324 ± 0.005	0.324 ± .006	0.94
Total cholesterol (mmol/liter)	5.02 ± 0.32	5.07 ± 0.29	0.57	4.96 ± 0.29	5.1 ± 0.3	0.19
Triglycerides (mmol/liter)	1.89 ± 0.11	170.2 ± 13.6	0.84	1.96 ± 0.12	2.0 ± 0.09	0.13
HDL cholesterol (mmol/liter)	1.1 ± 0.07	43.2 ± 2.3	0.24	1.2 ± 0.05	1.2 ± 0.04	0.89
LDL cholesterol (mmol/liter)	3.54 ± 0.34	120.7 ± 15.1	0.78	3.4 ± 0.3	3.5 ± 0.3	0.29

Data are means ± standard deviation. Differences between means were compared using unpaired Student's *t*-test.

* $p < 0.05$: significantly different.

BMI, body mass index; HDL, high-density lipoprotein; HOMA-IR and HOMA-β cell, homeostasis model assessment of insulin resistance and of β-cell function respectively; LDL, low-density lipoprotein. QUICKI, quantitative insulin check index.

0.002, respectively). Subsequently, there was a decrease in insulin sensitivity, measured by the Quantitative Insulin Sensitivity Check Index (QUICKI), observed in carriers of the Arg⁹⁷² IRS-1 variant compared with those of wild-type allele carriers ($p = 0.03$, 0.003, respectively). The study also demonstrated that the IRS-1 Gly972Arg variant in pancreatic β-cells results in a reduction in insulin secretion estimated by

HOMA-β cell function in both groups ($p = 0.02$, 0.045, respectively) (Table 3).

Discussion

This study investigated the relationship between the E23K variant and the risk for secondary failure to sulfonylurea in Egyptian patients with type 2 diabetes. The study found that the E23K variant was associated with secondary failure to

Table 3. Anthropometric and biochemical characteristics of the study population according to the insulin receptor substrate-1 (IRS-1) genotype.

	Patients with secondary failure			Patients whose condition is controlled with sulfonylurea		
	Carriers of wild allele	Carriers of Arg ⁹⁷² variant	<i>p</i>	Carriers of wild allele	Carriers of Arg ⁹⁷² IRS-1 variant	<i>p</i>
<i>n</i> (men/women)	19/20	5/6	0.74	24/23	2/1	0.77
Age (years)	51.7 ± 3.01	50.6 ± 1.6	0.27	52.1 ± 4.1	52.5 ± 6.9	0.9
BMI (kg/m ²)	30.3 ± 1.5	32.2 ± 1.3	0.009*	29.9 ± 1.3	31.9 ± 0.96	0.03*
Hemoglobin A1c (%)	9.4 ± 1.4	9.9 ± 1.4	0.33	7.6 ± 0.3	7.8 ± 0.3	0.4
Fasting insulin (μU/ml)	9.2 ± 0.6	10.0 ± 0.6	0.009*	7.5 ± 0.5	7.8 ± 0.2	0.01*
Fasting glucose (mmol/liter)	11.2 ± 0.81	11.6 ± 0.68	0.06	8.7 ± 0.5	9.0 ± 0.3	0.1
HOMA-IR	4.6 ± 0.6	5.26 ± 0.46	0.03*	2.9 ± 0.3	3.2 ± 0.09	0.002*
HOMA-β cell	23.8 ± 1.5	26.01 ± 1.2	0.02*	28.7 ± 1.4	27.3 ± 1.3	0.045*
QUICKI	0.306 ± 0.005	0.300 ± 0.003	0.03*	0.326 ± 0.005	0.322 ± 0.001	0.003*
Total cholesterol. (mmol/liter)	5.0 ± 0.01	5.3 ± 0.22	0.02*	4.9 ± 0.3	5.3 ± 0.18	0.04*
Triglycerides (mmol/liter)	1.9 ± 0.12	1.97 ± 0.11	0.09	1.98 ± 0.12	1.99 ± 0.04	0.7
HDL cholesterol. (mmol/liter)	1.1 ± 0.6	1.1 ± 0.07	0.09	1.2 ± 0.05	1.2 ± 0.05	0.8
LDL cholesterol. (mmol/liter)	3.5 ± 0.32	3.8 ± 0.2	0.007*	3.4 ± 0.3	3.8 ± 0.2	0.03*

Data are mean ± standard deviation. Differences between means were compared using unpaired Student's *t*-test.

**p* < 0.05: significantly different.

BMI, body mass index; HDL, high-density lipoprotein; HOMA-IR and HOMA-β cell, homeostasis model assessment of insulin resistance and of β-cell function respectively; LDL, low-density lipoprotein. QUICKI, quantitative insulin check index.

sulfonylurea in patients carrying this variant compared with those who were noncarriers. This finding is supported by the study of Sesti and colleagues [Sesti *et al.* 2006] who reported a higher proportion of K-allele carriers (51.4%) among 208 patients whose condition failed to respond to sulfonylurea-metformin combination therapy and requiring insulin due to uncontrolled hyperglycemia (fasting plasma glucose >300 mg/dl). The data reported here are in contrast with the Prospective Diabetes Study (UKPDS) [Gloyn *et al.* 2001] in which the authors did not find any significant association between the E23K variant and response to sulfonylurea therapy. It is possible that differences in the definition of secondary sulfonylurea failure might account for these divergent results. In the UKPDS report, the authors identified patients whose condition failed to respond to sulfonylurea therapy when their fasting plasma glucose rose to more than 300 mg/dl. Also, the type of sulfonylurea (chlorpropamide in the UKPDS *versus* glibenclamide in the present study), and the clinical characteristics of patients (newly diagnosed patients aged 25–65 years in the UKPDS *versus* patients with known diabetes diagnosed at over 40 years of age in the present study).

The present study demonstrated that the E23K polymorphism is not associated with β-cell dysfunction as estimated by HOMA-β-cell function.

Our observation is consistent with the other studies which provide preliminary evidence that the association of this polymorphism with increased blood glucose levels found in some other studies may be explained by diminished suppression of glucagon secretion in response to hyperglycemia [Tschrirter *et al.* 2002]. This is at variance with results of the studies showing that the E23K polymorphism is associated with impaired glucose-induced insulin release [Hansen *et al.* 2005; Nielsen *et al.* 2003; Hart *et al.* 2002]. Villareal *et al.* (2009) reported that the Kir6.2 E23K variant is associated with multiple insulin secretory defects, including a reduction in overall β-cell responsiveness to glucose and a rightward shift in the dose–response curve between glucose and insulin secretion [Villareal *et al.* 2009].

Furthermore, the study found that the E23K variant was not associated with worsening metabolic control (fasting glucose level, fasting serum insulin, and hemoglobin A_{1c}) across genotyping groups. Our observation is consistent with the studies which found that the E23K polymorphism does not increase impaired glucose tolerance in carriers of the E23K polymorphism [Ezenwaka *et al.* 2005]. Jose and colleagues [Jose *et al.* 2007] observed no significant longitudinal changes in fasting glucose levels across genotypic groups in the metformin arm. This is in apparent contrast with the study of Sesti and

colleagues who found that the E23K variant was associated with worsening metabolic control in the subgroup of patients whose condition was controlled with sulfonylurea [Sesti *et al.* 2006]. Holstein and colleagues suggested that patients with type 2 diabetes carrying the K allele have reduced response to sulfonylurea therapy, which results in increased hemoglobin A_{1c} [Holstein *et al.* 2009].

It should be noted that the number of patients in the present study is relatively small and this might have reduced the statistical power of the tests. However, the results have demonstrated that the presence of the Kir6.2 E23K genotype in Egyptian patients with diabetes did not increase fasting serum glucose levels or glycosylated hemoglobin percentage. This study and others have found that the estimated insulin resistance by HOMA and insulin sensitivity by QUICKI showed a weak association of the K allele with impaired insulin resistance and sensitivity [Jose *et al.* 2007; Tschrutter *et al.* 2002]. This is at variance with the results of a study demonstrating an association of the homozygous K allele with impaired insulin sensitivity [Wan *et al.* 2009]. Analysis of the present data did not show any significant differences in the biochemical risk factors for developing diabetes in patients who were obese and those who were non-obese with the E23K variants, which is consistent with previous reports [Jose *et al.* 2007; Ezenwaka *et al.* 2005]. However, many studies found that the E23K variant is associated with a significant increase in BMI, suggesting that this polymorphism may be diabetogenic through obesity-related mechanisms [Nielsen *et al.* 2003].

The study also investigated the association between a common polymorphism in IRS-1 causing a Gly972Arg change and secondary failure to sulfonylurea and found that the Arg⁹⁷² IRS-1 variant was associated with secondary failure to sulfonylurea. Sesti and colleagues supported these results with the notion that the Arg⁹⁷² IRS-1 variant impairs insulin secretion induced by secretagogues other than glucose [Sesti *et al.* 2004]. Chronic activation of insulin receptor signaling by IRS-1 overexpression in the β -cell inhibited gene expression of endoplasmic reticulum Ca(2+)-ATPase and subsequent inhibition of Ca⁺² uptake leading to an increase in cytosolic Ca⁺² concentrations [Xu *et al.* 2000]. As reported above, and consistent with the results of Stumvoll and colleagues, patients with

diabetes carrying the Arg⁹⁷² IRS-1 polymorphism have a lower β -cell function level, expressed in HOMA- β , than that of patients carrying the wild-type IRS-1 variant in both groups ($p=0.02$, 0.045 respectively) [Stumvoll *et al.* 2001]. The markedly reduced insulin secretion was accompanied by a decreased binding of the p85 subunit of PI3-K to IRS-1. Marchetti and colleagues showed that human pancreatic islets from carriers of the G972R amino acid polymorphism in IRS-1 have reduced insulin content, altered insulin release, and greater number of immature secretory granules [Marchetti *et al.* 2002]. *In vitro* studies have suggested that pancreatic islets isolated from carriers of Arg⁹⁷² IRS-1 exhibit impaired IRS-1-associated PI 3-kinase activity and increased apoptosis. They also appear resistant to the antiapoptotic effect of insulin compared with wild-type controls and so contribute to the risk for secondary failure [Federici *et al.* 2001]. In contrast, Laakso and colleagues did not find any significant association of the Gly-Arg⁹⁷² substitution with maximum insulin secretion capacity in an oral glucose tolerance test in patients with type 2 diabetes [Laakso *et al.* 1994].

This study has demonstrated that the Arg⁹⁷² variant contributes to increase in fasting serum insulin levels among patients with diabetes well controlled with oral therapy and among patients with secondary failure to sulfonylurea ($p=0.009$ and 0.01 , respectively). Subsequently a significant increase in insulin resistance, represented by HOMA-IR ($p=0.03$ and 0.002 , respectively), and a decrease in insulin sensitivity, represented by QUICKI ($p=0.03$ and 0.003 , respectively), were observed.

The present results are in agreement with several other studies [Lin *et al.* 2006; Villuendas *et al.* 2005]. McGettrick and colleagues investigated the detailed molecular mechanism by which this polymorphism may be linked to insulin resistance. They demonstrated that the Arg⁹⁷² variant alters the ability of the recombinant IRS-1 (925–1008) fragment to be phosphorylated at specific tyrosine residues flanking the polymorphism, which normally form high-affinity binding sites for the p85 α regulatory subunit of PI 3 kinase [McGettrick *et al.* 2005]. Other investigations have failed to observe the association between the Arg⁹⁷² IRS-1 polymorphism and insulin resistance [Koch *et al.* 1999; Laakso *et al.* 1994].

Differences in ethnic background and the technique used to assess insulin resistance may explain this discrepancy.

Moreover, the comparably increased prevalence of the Arg⁹⁷² variant in patients with obesity in groups I and II ($p=0.009$ and 0.03 , respectively) is consistent with a role of the Arg⁹⁷² variant in causing insulin resistance in these patients. The same results were reproduced in the study of Baroni and colleagues [Baroni *et al.* 2001]. Other authors have not found an association between obesity and the Arg⁹⁷² variant [Srivastava *et al.* 2008].

This study found that the most remarkable change in the lipid profile of mutation carriers in both groups was the increase in plasma total cholesterol ($p=0.02$, 0.04 , respectively) and low-density lipoprotein cholesterol ($p=0.007$, 0.03 , respectively). Abe and colleagues described significantly higher triglyceride levels in heterozygous carriers of the Arg⁹⁷² variant [Abe *et al.* 1998]. In this respect, IRS-1-mediated activation of PI 3 kinase has been reported to be involved in the antilipolytic effect of insulin. Because it has been demonstrated that the G972R substitution significantly reduces the IRS-1-mediated PI 3-kinase activation, it is conceivable that mutation carriers may have impaired antilipolysis. As a consequence, an increased efflux of free fatty acid from adipose tissue would provide more substrate available for very-low-density lipoprotein triglyceride synthesis by the liver [Baroni *et al.* 1999].

Overall, these results confirm the growing body of evidence reflecting the association between both the E23K variant in the Kir6.2 gene and the Arg⁹⁷² variant in the IRS-1 gene and increased risk for secondary failure to sulfonylurea in patients with type 2 diabetes. The documented and reproducible association of selected polymorphisms in genes that encode drug targets with type 2 diabetes highlights the possible application of human genomics to medicine.

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Conflict of interest statement

The author declares no conflict of interest in preparing this article.

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