The Arg$^{972}$ Variant in Insulin Receptor Substrate-1 Is Associated With an Increased Risk of Secondary Failure to Sulfonylurea in Patients With Type 2 Diabetes

**OBJECTIVE** — The aim of this study was to investigate whether diabetic patients carrying the Arg$^{972}$ insulin receptor substrate-1 (IRS-1) variant are at increased risk for secondary failure to sulfonylurea.

**RESEARCH DESIGN AND METHODS** — A total of 477 unrelated Caucasian type 2 diabetic patients were recruited according to the following criteria: onset of diabetes after age 35 years, absence of ketonuria at diagnosis, and anti-GAD$^2$ antibody. Type 2 diabetes was diagnosed according to the American Diabetes Association criteria. Patients with secondary sulfonylurea failure were defined as those requiring insulin due to uncontrolled hyperglycemia (fasting plasma glucose $>300$ mg/dl) despite sulfonylurea-metformin combined therapy, appropriate diet, and absence of any conditions causing hyperglycemia.

**RESULTS** — Of the total patients, 53 (11.1%) were heterozygous for the Arg$^{972}$ IRS-1 variant, 1 (0.2%) was homozygous, and the remainder (88.7%) were homozygous for the wild-type allele. The genotype frequency of the Arg$^{972}$ IRS-1 variant was 8.7% among diabetic patients well controlled with oral therapy and 16.7% among patients with secondary failure to sulfonylurea (odds ratio 2.1 [95% CI 1.18–3.70], $P = 0.01$). Adjustment for age, sex, BMI, metabolic control, age at diagnosis, duration of diabetes, and Pro12Ala polymorphism of peroxisome proliferator-activated receptor-γ2 gene in a logistic regression analysis with secondary failure to sulfonylurea as a dependent variable did not change this association (2.0 [1.38–3.86], $P = 0.038$).

**CONCLUSIONS** — These data demonstrate that the Arg$^{972}$ IRS-1 variant is associated with increased risk for secondary failure to sulfonylurea, thus representing a potential example of pharmacogenetics in type 2 diabetes.

**Type 2 diabetes** is a progressive disorder, and maintenance of good metabolic control has been demonstrated to reduce the risk of its associated long-term vascular complications and a delay in their onset (1). It has been estimated that each year 5–7% of diabetic patients treated with sulfonylurea convert to insulin treatment progressively as sulfonylurea fails (2,3). This clinical phenomenon has been termed secondary failure to sulfonylurea. Secondary failure to sulfonylurea has been variably attributed to change in body weight, lack of adequate diet regimen, young age at diagnosis, deterioration of insulin sensitivity or presence of anti-islet cell and anti-GAD antibodies (3,4). However, the U.K. Prospective Diabetes Study has demonstrated that the progressive deterioration of glycemic control in type 2 diabetic patients was associated with declining of pancreatic β-cell function despite either conventional treatment with dietary modification or intensive monotherapy treatment (1,3). A progressive deterioration of insulin secretion has been confirmed in smaller studies, and β-cell homeostatic model assessment has been demonstrated to be a better predictor of the insulin-requiring stage in type 2 diabetes than clinical indexes such as duration of diabetes, BMI, insulin sensitivity, or glycemic control (5,6). Factors determining the progressive loss of pancreatic β-cell function that leads to sulfonylurea failure are still unclear. Although hyperglycemia per se may adversely affect β-cell function through a process referred to as glucose toxicity, the possibility that genetic factors may predispose to sulfonylurea failure remains unsettled.

Studies in knockout mice, pancreatic β-cell lines, and human islets of Langerhans have indicated that an important role in the regulation of β-cell function may be played by insulin receptor sub-
insulin receptor acting as a multisite tyrosine phosphorylated substrate for the insulin receptor substrate-1 (IRS-1) (7–12). IRS-1 is a major docking protein for various molecules possessing src homology 2 domains, including the p85 regulatory subunit of phosphatidylinositol (PL) 3-kinase. The activation of these src homology 2 domain proteins initiates signaling cascades, leading to the activation of multiple downstream effectors, which mediate metabolic and anabolic responses.

Almost a decade ago, Almind et al. (13) reported an association between a common polymorphism in IRS-1 causing a Gly972Arg change and type 2 diabetes. These results have been confirmed in some but not all studies (14–16). However, a recent meta-analysis of 27 studies including 3,408 diabetic patients and 5,419 control individuals has revealed that carriers of the Arg972 IRS-1 variant have an increased risk for type 2 diabetes (odds ratio [OR] 1.25 [95% CI 1.05–1.48]) (17). Transfection studies have demonstrated that the Gly972Arg change has functional consequence causing impairment in IRS-1–associated PL 3-kinase activity owing to their defective interaction (18,19). Interestingly, we found that both rat β-cell line transfected with and expressing the Arg972 IRS-1 variant and human pancreatic islets of Langerhans isolated from carriers of this variant exhibited a marked decrease in insulin secretion in response sulfonylurea (19,20). These observations raise the intriguing hypothesis that diabetic patients carrying the Arg972 IRS-1 variant might be at increased risk for secondary failure to sulfonylurea. To test this hypothesis, we analyzed the prevalence of the Arg972 IRS-1 variant in an Italian cohort of 477 type 2 diabetic patients with and without secondary failure to sulfonylurea.

**RESEARCH DESIGN AND METHODS** — The study group consisted of 477 unrelated Caucasian type 2 diabetic patients who were consecutively recruited according to the following criteria: men and women with onset of diabetes after age 35 years, absence of ketonuria at diagnosis, and anti-GAD− antibody. Type 2 diabetes was diagnosed according to the American Diabetes Association criteria (21). 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, clinical problems potentially causing hyperglycemia including infection, thyroid disease, surgery, and treatments able to modify glucose metabolism such as corticosteroids or estrogens. In addition to appropriate diet, all patients were treated with second- or third-generation sulfonylureas at >15 mg/day (i.e., for most patients glibenclamide 5 mg, three tablets/day) until their plasma glucose rose to >300 mg/dl or they developed hyperglycemic symptoms (typically thirst or polyuria). At this point, metformin was added up to 1,700 mg/day in most patients. Patients with secondary sulfonylurea failure were defined as those requiring insulin due to uncontrolled hyperglycemia (fasting plasma glucose >300 mg/dl) despite sulfonylurea-metformin combined therapy, appropriate diet (i.e., iso- or hypocaloric diet depending on presence of overweight), and absence of any conditions causing hyperglycemia. The median duration of therapy with oral agents before failure was 10 years. The ethical committee approved the protocol, and informed written consent was obtained from all participants. All the investigations were performed in accordance with the principles of the Declaration of Helsinki.

**Biochemical assays**

Fasting blood glucose, total and HDL cholesterol, and triglycerides were measured by enzymatic methods (Roche Diagnostics, Mannheim, Germany). Blood HbA1c concentrations were analyzed by high-pressure liquid chromatography (Menarini Diagnostics, Italy) with coefficients of variation <4%.

**DNA analysis**

Genomic DNA was isolated from peripheral blood according to standard procedures. The Gly972Arg of the human IRS-1 sequence was detected by digestion of PCR products with restriction enzyme BstNI as previously described (14,20,22). The Pro12Ala polymorphism in exon B of the peroxisome proliferator–activated receptor (PPAR)−γ gene was detected by digestion of PCR products with restriction enzyme BsrUI as previously described (23).

**Statistical analysis**

Fisher’s exact test was used to test for differences in genotype frequencies. Categorical variables were compared by χ² test. Continuous data are shown as means ± SD. Because only one subject was found to be homozygous for the Arg972 IRS-1 variant, she was combined with the heterozygous group for statistical analysis. Non-normally distributed parameters were logarithmically transformed to approximate a normal distribution. Differences between means were compared using unpaired Student’s t test. All differences were also tested after adjusting for sex, age, and BMI. The Hardy-Weinberg equilibrium between the two genotypes was evaluated by χ² test. The OR for a possible specific influence of the IRS-1 genotype on secondary failure to sulfonylurea was calculated using a logistic regression analysis while adjusting for age, sex, BMI, degree of glycemic control, age at diagnosis, duration of diabetes, and Pro12Ala polymorphism of the PPAR-γ2 gene. All tests were two-sided, and P < 0.05 was considered statistically significant. All analyses were performed using SPSS version 10.0 software program for Windows.

**RESULTS** — The clinical characteristics of the study subjects stratified according to therapy and genotype are reported in Table 1. As expected, patients with secondary failure to sulfonylurea showed significant differences in age (P < 0.001), duration of the disease (P < 0.0001), age of clinical diagnosis (P < 0.0001), fasting plasma glucose levels (P < 0.0001), and HbA1c concentrations (P < 0.03), as compared with patients well controlled with oral therapy. Of the total patients, 53 (11.1%) were homozygous for the Arg972 IRS-1 variant, 1 (0.2%) was homozygous, and the remainder (88.7%) were homozygous for the wild-type allele. The genotype frequency was in the Hardy-Weinberg equilibrium. The genotype frequency of the Arg972 IRS-1 variant was 8.7% among diabetic patients well controlled with oral therapy and 16.7% among patients with secondary failure to sulfonylurea. Thus, the Arg972 IRS-1 variant was associated with secondary failure to sulfonylurea with carriers having a relative risk of 2.1 (95% CI 1.18–3.70, P = 0.01). Adjustment for age, sex, BMI, degree of metabolic control estimated as fasting plasma glucose and HbA1c concentrations, age at diagnosis, and duration of diabetes in a logistic regression analysis with secondary failure to sulfo-
**IRS-1 variant and secondary failure**

**Table 1 — Clinical and biochemical characteristics of the study population according to the IRS-1 genotype**

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Gly/Gly</th>
<th>X/Arg</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>treatment (SU/SU + Met)</strong>*</td>
<td>117/204</td>
<td>109/184</td>
<td>8/20</td>
<td>0.41</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.1 ± 0.6</td>
<td>30.0 ± 0.6</td>
<td>29.3 ± 4.1</td>
<td>0.52</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.94 ± 0.10</td>
<td>0.94 ± 0.10</td>
<td>0.92 ± 0.06</td>
<td>0.28</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>150 ± 59</td>
<td>150 ± 59</td>
<td>152 ± 53</td>
<td>0.84</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.6 ± 2.3</td>
<td>7.6 ± 2.3</td>
<td>7.3 ± 2.4</td>
<td>0.47</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>204 ± 45</td>
<td>204 ± 46</td>
<td>203 ± 29</td>
<td>0.82</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>46 ± 14</td>
<td>45 ± 14</td>
<td>51 ± 14</td>
<td>0.12</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>162 ± 101</td>
<td>163 ± 102</td>
<td>153 ± 93</td>
<td>0.63</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Gly/Gly</th>
<th>X/Arg</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patients well controlled with oral agents</strong></td>
<td>80/76</td>
<td>66/64</td>
<td>14/12</td>
<td>0.77</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.4 ± 5.1</td>
<td>29.4 ± 5.0</td>
<td>29.4 ± 5.1</td>
<td>0.95</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.93 ± 0.08</td>
<td>0.93 ± 0.08</td>
<td>0.94 ± 0.08</td>
<td>0.93</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>194 ± 83†</td>
<td>195 ± 85</td>
<td>191 ± 76</td>
<td>0.84</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>7.7 ± 1.7</td>
<td>7.2 ± 2.8</td>
<td>8.1 ± 2.3</td>
<td>0.26</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>47 ± 12</td>
<td>47 ± 12</td>
<td>46 ± 14</td>
<td>0.81</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>145 ± 64</td>
<td>145 ± 64</td>
<td>135 ± 103</td>
<td>0.39</td>
</tr>
</tbody>
</table>

Data are means ± SD. Differences between means were compared using unpaired Student’s t test. *The last treatment with oral agents before insulin treatment is reported for the group of patients with secondary failure (SU, sulfonylurea; Met, metformin). †P < 0.001; ‡P < 0.0001; §P < 0.05 vs. patients well controlled with oral agents.

Sulfonylurea as a dependent variable did not change this association (OR 2.2 [95% CI 1.16–4.46], P = 0.016). To further exclude the influence of duration of diabetes in our study population, we analyzed subgroups matched for sex, age, BMI, and duration of diabetes (50 men/33 women vs. 49 men/48 women, P = 0.19; 64.9 ± 10 vs. 67 ± 8 years of age, P = 0.09; 29.0 ± 5.5 vs. 29.5 ± 2.7 kg/m², P = 0.51; 20.3 ± 4.7 vs. 21.1 ± 5.1 years of diabetes duration, P = 0.51; respectively, for the diabetic patients well controlled with oral therapy and patients with secondary sulfonylurea failure). The genotype frequency of the Arg972 IRS-1 variant was 7.2% among diabetic patients well controlled with oral therapy and 17.5% among patients with secondary failure to sulfonylurea. Thus, also in this subgroup analysis, which takes into account major confounding factors, the Arg972 IRS-1 variant was associated with secondary failure to sulfonylurea with carriers having a relative risk of 2.7 (1.02–7.28, P = 0.045). There were no differences in clinical and metabolic characteristics between patients carrying the Arg972 variant and homozygous wild-type patients within each of the two groups, i.e., patients well controlled with oral therapy and patients with secondary failure to sulfonylurea (Table 1). It has been reported that the Ala allele of the Pro12Ala polymorphism in the PPAR-γ2 gene is associated with a decreased risk of type 2 diabetes (24,25). These results have been confirmed in some, but not all, studies (26–30). PPAR-γ is the target of thiazolidinediones, a new class of insulin-sensitizing agents used clinically to treat type 2 diabetes. In vitro, the Pro12Ala change is associated with reduced transcriptional activity of PPAR-γ2 (24), thus raising the possibility that the Pro12Ala variant could influence the response to oral hypoglycemic agents. Although no patient in our study was treated with thiazolidinediones because these drugs were not available in Italy at the time of recruitment, we analyzed the prevalence of Pro12Ala variant in our cohort of patients. The genotype frequency of the Ala variant was 12.3% among diabetic patients well controlled with oral therapy and 17.6% among patients with secondary failure to sulfonylurea. Addition of the Pro12Ala polymorphism to the logistic regression analysis model, including as confounding factors age, sex, BMI, degree of metabolic control, age at diagnosis, and duration of diabetes, did not change the association of the Arg972 IRS-1 variant and the risk for secondary failure to sulfonylurea in an Italian cohort of type 2 diabetic patients. We found that the Arg972 IRS-1 variant was associated with secondary failure to sulfonylurea with patients carrying this variant having a relative risk of 2.0 as compared with noncarriers also after adjustment for age, insulin receptors, BMI, sex, age at diagnosis, and duration of diabetes. The Arg972 IRS-1 variant was not associated with secondary failure to sulfonylurea with patients carrying this variant having a relative risk of 2.0 as compared with noncarriers also after adjustment for age, BMI, sex, and age at diagnosis. In conclusion, our study supports the hypothesis that the Arg972 variant in IRS-1 is associated with secondary failure to sulfonylurea in an Italian cohort of type 2 diabetic patients. Furthermore, these results suggest that the Arg972 IRS-1 variant may influence the response to oral hypoglycemic agents by affecting the expression of PPAR-γ2, which in turn regulates the transcriptional activity of thiazolidinediones. These findings have implications for the treatment of type 2 diabetes, where thiazolidinediones are commonly used as add-on therapy to sulfonylurea. Further research is needed to validate these results and to elucidate the mechanisms underlying the association between Arg972 IRS-1 variant and secondary failure to sulfonylurea.
sex, BMI, metabolic control, age at diagnosis, duration of diabetes, and Pro12Ala polymorphism of the PPAR-γ2 gene. It has been reported that patients with apparent secondary failure to sulfonylurea are positive for anti-GAD antibody, and they tend to develop insulin deficiency requiring insulin treatment in a short period (4,32). Interestingly, we have recently reported that the frequency of the Arg972 IRS-1 variant is significantly increased in patients with type 1 diabetes compared with nondiabetic individuals, conferring an OR of 2.5 (22). However, the possibility that patients with type 1 diabetes or latent autoimmune diabetes in adults might account for the increased prevalence of the Arg972 IRS-1 variant among patients with secondary failure to sulfonylurea was excluded a priori by recruiting exclusively anti-GAD patients.

There is evidence that the type of sulfonylurea may influence the development of secondary failure (3). Unfortunately, our data do not allow comparisons between the different types of sulfonylurea due to the small subgroups taking different sulfonylureas. Further longitudinal studies comparing the different types of sulfonylureas are needed to address this issue.

Sulfonylureas stimulate insulin release from pancreatic β-cells through the interaction with a specific plasma membrane receptor (SUR1) coupled to the ATP-sensitive K+ channel, leading to its closure. This causes depolarization of the β-cell membrane, leading to opening of voltage-sensitive Ca2+ channels, Ca2+ influx, and a rise in intracellular Ca2+, which stimulates exocytosis of insulin-containing granules. Several experimental studies support the notion that IRS-1 plays an important role in insulin secretion by regulating intracellular calcium homeostasis (8–11). Overexpression of IRS-1 in βTC6-F7 or RIN 1046-38 insulinoma cell lines resulted in an increased fractional insulin secretion in response to glucose or sulfonylurea (8,19). This event was associated with an increase in cytosolic Ca2+ concentrations due to inhibition of Ca2+ uptake by the endoplasmic reticulum through the activation of PI 3-kinase associated with IRS-1 (8,10). Further investigation revealed that the increase in cytosolic Ca2+ was the result of the inhibition of the sarcoendoplasmic reticulum Ca2+-ATPase, a calcium pump that plays a major role in the sequestration of Ca2+ into the endoplasmic reticulum lumen (11). In isolated IRS-1-/-/ knockout β-cells, insulin exocytosis and cytosolic Ca2+ concentrations were decreased, further supporting the role of IRS-1 in regulating insulin secretion and intracellular Ca2+ homeostasis (9). Taken together, these results are consistent with the possibility that the Arg972 IRS-1 variant might impair insulin secretion stimulated by sulfonylureas by affecting intracellular calcium homeostasis, thus contributing to increase the risk to develop secondary failure to these oral agents. In vitro studies have suggested that sulfonylureas may induce apoptosis of pancreatic β-cell (33). Interestingly, we have recently demonstrated that human pancreatic β-cells isolated from donors carrying the Arg972 IRS-1 variant exhibit an increased apoptosis compared with their wild-type counterparts, and they are also resistant to the antiapoptotic effects of insulin (34). These data raise the possibility that another potential mechanism, whereby the Arg972 IRS-1 variant could contribute to the risk for secondary failure, might be related to increased susceptibility to apoptosis of pancreatic β-cells chronically exposed to sulfonylureas.

In conclusion, the present data demonstrate that the common Arg972 IRS-1 variant is associated with increased risk for secondary failure to sulfonylurea in patients with type 2 diabetes. Experimental studies support the notion that this IRS-1 variant may influence the variability in patients’ response to sulfonylureas, thus representing a potential example of pharmacogenetics in type 2 diabetes.

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References


IRS-1 variant and secondary failure


