Polymorphisms of the Insulin Receptor Substrate-2 in Patients with Type 2 Diabetes

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We investigated the significance of Gly1057Asp and Leu647Val insulin receptor substrate (IRS)-2 polymorphisms in two Italian cohorts comprising 186 glucose-tolerant subjects and 240 subjects with type 2 diabetes from the Lazio region (i.e. representative of central Italy), and 123 glucose-tolerant subjects from the Sicily region (i.e. representative of south Italy). The allelic frequency of Gly1057Asp variant did not differ between diabetics (32.9%) and nondiabetic subjects, whatever their ethnicity was (35.8% and 33.7% from Lazio and Sicily, respectively). As compared with Gly/Gly subjects within each group, Asp/Asp individuals showed no differences in quantitative traits, including fasting insulin and C-peptide, and several indices of insulin sensitivity and secretion. Only one of the diabetic patients was heterozygous for the Leu647Val variant, and none of the control subjects carried this variant. This patient had three children who were also heterozygous for this variant. They were glucose tolerant, and their insulin sensitivity and insulin secretion indices were within the range of age-matched controls. We also analyzed IRS-2 function in fibroblasts from carriers of Gly1057Asp or Leu647Val variant. No defects in IRS-2 expression, insulin-stimulated phosphorylation, or binding to the p85 subunit of phosphatidylinositol 3-kinase were observed. These results strongly argue against a major role of IRS-2 polymorphisms in the pathogenesis of type 2 diabetes. (J Clin Endocrinol Metab 88: 317–322, 2003)

Insulin Receptor Substrate (IRS)-1 and IRS-2 proteins are key mediators in insulin signaling, and they play a central role for maintaining cellular functions such as growth, survival, and metabolism (1, 2). Tyrosine phosphorylated IRSs act as docking proteins between the insulin receptor and a complex network of intracellular signaling molecules containing Src homology 2 domains, including the p85 subunit of phosphatidylinositol (PI) 3-kinase (3, 4). IRS-2 knockout mice exhibit a phenotype similar to human type 2 diabetes, characterized by insulin resistance with abnormal glucose tolerance at birth culminating in the development of fasting hyperglycemia in later age (5). Previous studies have identified several polymorphisms in the IRS-2 gene (6–12). One of these is a frequent variant causing a Gly1057Asp substitution, whereas four others are rare variants causing an Ala157Thr substitution, a Leu647Val substitution, a Gly879Ser substitution, and an insertion AAC (Asn) in the Asn repeat sequence centered around codons 29–36 (10). In different populations, although the Leu647Val variant was found exclusively in type 2 diabetic patients (7), allelic frequencies of both Gly1057Asp and Gly879Ser variants were similar in diabetic and control individuals (6–12). By contrast, in an Italian population representative of East Coast Italy, a gene-environment interaction has been reported with homozygous for the Gly1057Asp variant being exposed to either a lower or a higher risk of type 2 diabetes according to body mass index (BMI) less than or greater than or equal to 27 kg/m² (9). The reason for this discrepancy is not known. It may certainly reside in the different ethnicity of the populations studied but it may also be explained by a false positive result due to population stratification in Italy. In the present study, to minimize the risk of population stratification, we tested for association between IRS-2 polymorphisms and insulin resistance in two cohorts of independent Italian populations of different ethnicity (i.e. from Lazio, representative of central Italy; and from Sicily, representative of south Italy; Ref. 13). The cohort from Lazio was also tested for association between IRS-2 variants and type 2 diabetes. In addition, to more deeply investigate the biological significance, if any, of the IRS-2 polymorphisms detected in our cohorts, cultured fibroblasts from carriers of Gly1057Asp or Leu647Val variants were obtained, and functional studies on IRS-2 expression and function were performed.

Subjects and Methods

Subjects
A total of 240 patients with type 2 diabetes and 186 glucose-tolerant subjects were recruited in the Lazio region of Italy. The subjects were consecutively recruited from the Department of Internal Medicine of the
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### TABLE 2. Biochemical and clinical characteristics of the study populations according to the Gly1057Asp IRS-2 genotype

<table>
<thead>
<tr>
<th></th>
<th>Glucose-tolerant subjects–Lazio</th>
<th>Glucose-tolerant subjects–Sicily</th>
<th>Type 2 diabetes–Lazio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gly/Gly</td>
<td>Gly/Asp</td>
<td>Asp/Asp</td>
</tr>
<tr>
<td>Total no. subjects</td>
<td>n = 77</td>
<td>n = 85</td>
<td>n = 24</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>48.4 ± 12.9</td>
<td>49.1 ± 14.9</td>
<td>48.1 ± 14.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.44 ± 8.31</td>
<td>29.23 ± 7.19</td>
<td>30.62 ± 6.18</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.85 ± 0.07</td>
<td>0.86 ± 0.08</td>
<td>0.88 ± 0.07</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/liter)</td>
<td>4.76 ± 0.58</td>
<td>4.92 ± 0.81</td>
<td>4.64 ± 0.55</td>
</tr>
<tr>
<td>Fasting serum insulin (pmol/liter)</td>
<td>66.64 ± 3.84</td>
<td>66.98 ± 38.66</td>
<td>78.38 ± 41.57</td>
</tr>
<tr>
<td>Fasting serum C-peptide (ng/ml)</td>
<td>2.21 ± 1.38</td>
<td>2.16 ± 0.98</td>
<td>2.05 ± 1.00</td>
</tr>
<tr>
<td>HbA1c %</td>
<td>5.26 ± 0.67</td>
<td>5.25 ± 1.03</td>
<td>5.26 ± 0.54</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>190.64 ± 45.12</td>
<td>211.57 ± 50.99</td>
<td>202.58 ± 34.11</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>53.34 ± 14.02</td>
<td>51.69 ± 15.41</td>
<td>50.90 ± 11.93</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>121.17 ± 65.66</td>
<td>122.57 ± 63.91</td>
<td>114.38 ± 42.38</td>
</tr>
<tr>
<td>HO-MIA</td>
<td>2.41 ± 1.39</td>
<td>2.48 ± 1.53</td>
<td>2.68 ± 1.32</td>
</tr>
<tr>
<td>Ins0/Glu0</td>
<td>13.87 ± 6.79</td>
<td>13.62 ± 7.81</td>
<td>17.21 ± 10.49</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.34 ± 0.03</td>
<td>0.34 ± 0.03</td>
<td>0.34 ± 0.02</td>
</tr>
<tr>
<td>β-cell HOMA</td>
<td>217.70 ± 197.99</td>
<td>182.47 ± 138.52</td>
<td>315.08 ± 373.02</td>
</tr>
<tr>
<td>Stumvoll index</td>
<td>1231.92 ± 616.17</td>
<td>1123.11 ± 536.77</td>
<td>1319.81 ± 424.03</td>
</tr>
</tbody>
</table>

Values shown are the means ± SD. ANOVA multiple comparisons—Bonferroni test. HbA1c, Glycosylated hemoglobin; ND, not determined; Ins0/Glu0, fasting insulin/glucose.
sensitivity or insulin secretion were detected between the two heterozygous siblings and age-matched wild-type subjects.

To determine whether Gly1057Asp and Leu647Val variants have any repercussion on IRS-2 function, fibroblasts from carriers of Gly/Gly, Gly/Asp, or Asp/Asp genotype...
and the heterozygous Leu647Val carrier were analyzed. IRS-2 protein levels were similar in cells from carriers of the three genotypes (Fig. 1). The amount of IRS-2 did not differ in fibroblasts expressing the Leu647Val IRS-2 variant as compared with wild type (Fig. 1). Insulin-stimulated tyrosine phosphorylation of IRS-2 was similar in fibroblasts expressing Gly/Gly, Gly/Asp, or Asp/Asp genotype (Fig. 1). There were no significant differences in the extent of phosphorylation between cells expressing IRS-2 wild type or the Leu647Val IRS-2 variant. Next, we investigated whether the IRS-2 variants had any influence on insulin-stimulated association of p85 subunit of PI 3-kinase to IRS-2. Both basal and insulin-stimulated binding of p85 subunit to IRS-2 was similar in fibroblasts expressing Gly/Gly, Gly/Asp, or Asp/Asp genotype (Fig. 1). Accordingly, the interaction between IRS-2 and p85 subunit of PI 3-kinase was similar in cells expressing Leu647Val IRS-2 variant as compared with wild type (Fig. 1).

Discussion

We found that the frequent Gly1057Asp IRS-2 variant showed no association with the common form of type 2 diabetes. This variant did not appear to affect any clinical and biochemical features in both glucose-tolerant and type 2 diabetic subjects, nor did it seem to have any impact on insulin secretion and insulin sensitivity. Our results confirm those reported in different populations, including Danish, Swedish, Finnish, Chinese, German, and Dutch (6, 7, 10–12), but are at variance with those reported in a study of a different sample of the Italian population (9). In this study, it was found that the Gly1057Asp IRS-2 variant is associated with a lower prevalence of type 2 diabetes in lean subjects, and with a tendency toward an increased prevalence of type 2 diabetes in overweight subjects. Moreover, Asp/Asp type 2 diabetic patients exhibited a trend toward lower insulin sensitivity as deduced by increase in fasting C-peptide concentrations (9). It is possible that regional differences in the distribution of this genotype may explain this discrepancy, as has been shown for the Gly972Arg IRS-1 variant (20) and the glucagon receptor Gly40Ser variant (21). In addition, differences in the age of the study populations might account for the discrepancy between the present and the previous results. Lower fasting insulin and C-peptide levels have been observed in Asp/Asp middle-aged Danish subjects as compared with a young Danish population (7), thus raising the possibility that the potential effect of this IRS-2 variant is not detectable in early adult life but develops as a result of age-related modifications in insulin sensitivity. However, this possibility seems unlikely because no differences in insulin and C-peptide levels between Gly/Gly and Asp/Asp carriers were detected either in our three groups of subjects, which differ for mean age, or in a Swedish population aged 70 yr (7). Moreover, differences in the methods used to assess insulin sensitivity (HOMA and FGIR vs. fasting C-peptide levels) may account for the disparity. Fasting C-peptide used as a surrogate for insulin sensitivity cannot explain more than 30–40% of the variance in glucose-derived insulin sensitivity, whereas HOMA and FGIR correlation with clamp is about 0.80 (22, 23). We also identified a rare Leu647Val variant of IRS-2 in only one of the diabetic patients examined and in his offspring. The offspring carrying this polymorphism showed no diabetes or impaired glucose tolerance, although we cannot exclude the possibility that they will develop type 2 diabetes later in life. Because only one patient carried this variant, the present data do not allow a statement on whether this polymorphism contributes to the development of rare cases of type 2 diabetes.

We evaluated the functional impact of the IRS-2 variants in fibroblasts from carriers of the different genotypes. The Gly1057Asp polymorphism did not alter the level of expression or the extent on insulin-stimulated tyrosine phosphorylation of IRS-2, although its location between two putative tyrosine phosphorylation sites (Tyr1032 and Tyr1061) might be predicted to alter IRS-2 phosphorylation. Moreover, this variant did not cause any impairment in the ability of IRS-2 to bind the p85 regulatory subunit of PI 3-kinase. Although we cannot rule out the possibility that there might be defects in IRS-2 function that were too subtle to be detected by our experimental approach, results support the notion that the Gly1057Asp variant does not affect IRS-2 function. The Leu647Val substitution is located close to tyrosine residue 653, which lies in a YMXM motif involved in the binding of p85 subunit of PI 3-kinase. However, we did not detect any abnormality in insulin-stimulated phosphorylation of Leu647Val IRS-2 or in its ability to bind the p85 subunit of PI 3-kinase. Although the antiphosphotyrosine blotting technique is not a sensitive method for detecting a selective defect in the phosphorylation of a single tyrosine residue, our results are consistent with a previous study in which the binding of the IRS-2 variant to p85 subunit of PI 3-kinase was measured using the yeast two-hybrid system (7). Although these data suggest that Leu647Val variant does not affect insulin signaling, the pathogenic role of this polymorphism remains unsettled.

In conclusion, the common Gly1057Asp IRS-2 and the rare Leu647Val IRS-2 variants do not appear to affect insulin secretion and insulin sensitivity or to cause major defects in the function of IRS-2. Although we cannot totally rule out the possibility that IRS-2 variants may induce minor defects that were not detected by our sample size and laboratory assays, the present results strongly argue against a major role of IRS-2 polymorphisms in the pathogenesis of type 2 diabetes.

Acknowledgments

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