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

ROSSELLA D'ALFONSO, MARIA ADELAIDE MARINI, LUCIA FRITTITTA, ROBERTO SORGE, SIMONA FRONTONI, OTTAVIA PORZIO, LUISA MARINA MARIANI, DAVIDE LAURO, SERGIO GAMBARDELLA, VINCENZO TRISCHITTA, MASSIMO FEDERICI, RENATO LAURO, AND GIORGIO SESTI

Laboratory of Molecular Medicine, Department of Internal Medicine (R.D., M.A.M., S.F., O.P., L.M.M., D.L., S.G., M.F., R.L.), and Department of Neuroscience (R.S.), University of Rome-Tor Vergata, 00133 Rome, Italy; Institute of Internal Medicine, Endocrine and Metabolic Disease (L.F.), University of Catania, Garibaldi Hospital, 95123 Catania, Italy; Unit of Endocrinology (V.T.), Scientific Institute Casa Sollievo della Sofferenza, 71013 San Giovanni Rotondo, Italy; Department of Clinical Science (V.T.), University of Rome–"La Sapienza", 00185 Rome, Italy; and Dipartimento di Medicina Sperimentale e Clinica (G.S.), Università di Catanzaro-Magna Græcia, 88100 Catanzaro, Italy

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/m^2 (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

Abbreviations: BMI, Body mass index; FGIR, fasting glucose to insulin ratio; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; IRS, insulin receptor substrate; PI, phosphatidylinositol; QUICKI, quantitative insulin sensitivity check index.

University of Rome-Tor Vergata to participate in this study. Type 2 diabetes was diagnosed according to the American Diabetes Association criteria (14). Another Italian group consisting of 123 subjects who were residents of Sicily was recruited from the Institute of Internal Medicine, Endocrine and Metabolic Diseases, University of Catania. To estimate insulin sensitivity and β-cell function, we used several described indices derived from either fasting or oral glucose tolerance test (OGTT) measurements of glucose and insulin. Insulin sensitivity was estimated by using the homeostasis model assessment (HOMA) index (15), fasting glucose to insulin ratio (FGIR; Ref. 16), and the quantitative insulin sensitivity check index (QUICKI; Ref. 17). β-cell function was estimated by both the HOMA index and the Stumvoll index (15, 18). The study was approved by the institutional ethics committee, and informed consent was obtained from each subject in accordance with principles of the Declaration of Helsinki.

DNA analysis

Genomic DNA was isolated from peripheral blood according to standard procedures. The Gly1057Asp polymorphism was detected as previously described (7). The nucleotide substitution at codon 647 of the human IRS-2 was determined by digesting PCR products with restriction enzyme *Bst*NI (New England Biolabs, Inc., Beverly, MA). The primers used were 5′-GAG GGC TGC GCA AGA GGA CCT-3′, complementary to nucleotides 1706–1726 of IRS-2 as upstream primer, and 5′-CCC TGG GCT GCA AAA TCT GCT T-3′, complementary to nucleotides 2059–2080 as downstream primer.

Expression of IRS-2, tyrosine phosphorylation of IRS-2, and its association with the p85 subunit of PI 3-kinase in cultured fibroblasts

Skin fibroblasts were grown in monolayers at 37 C in RPMI 1640, supplemented with 10% fetal calf serum, and used for experiments between the 8th and 13th passages. After overnight incubation in serum-free medium, fibroblasts were incubated in the presence or absence of 100 nM insulin for 5 min at 37 C. The cells were lysed for 1 h at 4 C in buffer containing 150 mM NaCl, 50 mM Tris-HCl (pH 7.4), 10 mM EDTA, 10 mM $Na_4P_2O_4$, 2 mM sodium orthovanadate, 10 mM NaF, 2 mM phenylmethylsulfonyl fluoride, 1% Triton X-100, and protease inhibitors. Aliquots of the supernatant were incubated for 16 h at 4 C with anti-IRS-2 antibody, and immune complexes were collected by incubation with protein A-Sepharose. Equal amounts of cell lysates or immunoprecipitated proteins were subjected to 7% SDS-PAGE, transferred to polyvinylidene difluoride membrane, and blotted with primary antibodies. Proteins were detected by enhanced chemiluminescence, and band densities were quantified by densitometry. Anti-IRS-2 and p85 subunit of PI 3-kinase antibody were purchased from Upstate Biotechnology, Inc. (Lake Placid, NY). The antiphosphotyrosine antibody was obtained from Transduction Laboratories, Inc. (Lexington, KY).

Statistical analysis

Data for continuous variables were expressed as mean ± SD. Comparisons of continuous variables among groups were performed by ANOVA. For comparing discrete variables, χ^2 analysis was used. The distribution of alleles and genotypes among study groups was compared by χ^2 tests. A multivariate analysis was used to find out whether there was an independent association between the Asp allele and hormonal and biochemical variables, including HOMA indices, after adjustment for other modulators of insulin sensitivity and insulin secretion such as gender, age, BMI, cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides. The association of concomitant variables with genotypes was tested by one-way ANOVA. All comparisons with *P* value less than 0.01 were considered statistically significant, according to recently published guidelines (19). All analyses were performed using SPSS, Inc. (Chicago, IL) software program version 10.0 for Windows.

Results

Type 2 diabetic patients were older, showed higher waist to hip ratio, and had significantly higher levels of glycosylated hemoglobin and fasting glucose and triglycerides, and lower levels of HDL cholesterol compared with the two glucose-tolerant groups ($P < 0.01$). Furthermore, diabetic patients appeared to be insulin resistant, as estimated by HOMA and QUICKI, and had an impaired insulin secretion as estimated by β-cell HOMA and Stumvoll index ($P < 0.01$). The frequency of Asp allele was slightly, but not significantly, lower in type 2 diabetic patients than in the two glucose-tolerant groups (Table 1). There was no difference in genotype frequency, which was in the Hardy-Weinberg equilibrium. In both the type 2 diabetic patients and the glucose-tolerant groups, no significant differences in BMI, waist to hip ratio, fasting glucose levels, glycosylated hemoglobin concentrations, total cholesterol, HDL cholesterol, triglycerides, fasting insulin and C-peptide levels, HOMA, QUICKI, or FGIR, and β-cell HOMA and Stumvoll index were observed among the three genotypes (Table 2). After adjusting, in a multivariate analysis, for the confounding effect of well known modulators of insulin sensitivity (*i.e.* gender, age, BMI, and plasma lipids), no significant differences in insulin sensitivity, insulin secretion, hormonal and biochemical findings were detected between Gly/Gly, Gly/Asp, and Asp/Asp genotypes in the three groups of subjects. After stratifying for different categories of BMI, the frequency of Asp allele was slightly (0.26), but not significantly, lower in diabetic patients with BMI below 27 kg/m^2 than in the two glucose-tolerant groups (0.36 and 0.34 for subjects from Lazio and Sicily, respectively; $P = 0.17$), whereas in subjects with BMI equal to or higher than 27 kg/m^2 Asp allele frequency did not differ between the three groups of subjects (0.35, 0.33, and 0.37 for glucose-tolerant subjects from Lazio, from Sicily, and type 2 diabetics, respectively). After stratifying for different categories of BMI, no significant differences in hormonal and biochemical findings, or indices of insulin sensitivity or insulin secretion were detected between Gly/Gly, Gly/Asp, and Asp/Asp genotypes in the three groups of subjects. The Leu647Val IRS-2 variant was detected in one of 240 diabetic patients and in none of the glucose-tolerant subjects. The proband was a man, now aged 45 yr, with onset of diabetes at age 43 yr. No DNA was available from parents because his father had died at age 52 yr for duodenal carcinoma, whereas his mother had died at age 72 yr for myocardial infarction. His three children appeared healthy; they were heterozygous for the Leu647Val IRS-2 variant, whereas his spouse was wild type. The two older siblings had normal fasting glucose levels and normal 2-h post-load glucose concentrations. No differences in insulin

TABLE 1. Frequency of the Gly1057Asp IRS-2 variant in the study populations

	NGT–Lazio	NGT–Sicily	Type 2 diabetes–Lazio
Allele frequency			
Gly	239 (64.2)	163 (66.3)	322 (67.1)
Asp	133 (35.8)	83 (33.7)	158 (32.9)
Genotype frequency			
Gly/Gly	77 (41.4)	54 (43.9)	108 (45.0)
Gly/Asp	85 (45.7)	55 (44.7)	106 (44.2)
Asp/Asp	24 (12.9)	14 (11.4)	26 (10.8)

Data represent number of subjects (%). NGT, Normal glucose tolerant.

TABLE 2. Biochemical and clinical characteristics of the study populations according to the Gly1057Asp IRS-2 genotype

	Glucose-tolerant subjects–Lazio			Glucose-tolerant subjects–Sicily			Type 2 diabetes–Lazio		
	Gly/Gly	Gly/Asp	Asp/Asp	Gly/Gly	Gly/Asp	Asp/Asp	Gly/Gly	Gly/Asp	Asp/Asp
Total no. subjects	n = 77	n = 85	n = 24	n = 54	n = 55	n = 14	n = 108	n = 106	n = 26
Age (yr)	48.4 ± 12.9	49.1 ± 14.9	48.1 ± 14.6	36.6 ± 11.1	34.5 ± 13.1	34.1 ± 11.4	63.8 ± 9.3	62.1 ± 11.2	63.9 ± 9.9
BMI (kg/m^2)	30.44 ± 8.31	29.23 ± 7.19	30.62 ± 6.18	33.56 ± 3.24	29.57 ± 10.21	31.72 ± 10.95	28.86 ± 6.07	29.43 ± 5.83	30.48 ± 4.16
Waist/hip ratio	0.85 ± 0.07	0.86 ± 0.08	0.88 ± 0.07	0.84 ± 0.08	0.85 ± 0.08	0.92 ± 0.12	0.91 ± 0.07	0.92 ± 0.12	0.94 ± 0.07
Fasting plasma glucose (mmol/liter)	4.76 ± 0.58	4.92 ± 0.61	4.64 ± 0.55	4.97 ± 0.97	5.00 ± 0.93	5.17 ± 0.81	9.25 ± 3.33	8.82 ± 3.46	10.67 ± 4.63
Fasting serum insulin (pmol/liter)	66.64 ± 3.84	66.98 ± 38.66	78.38 ± 41.57	65.59 ± 51.36	59.59 ± 46.24	66.28 ± 50.10	82.60 ± 46.73	77.46 ± 41.78	109.50 ± 26.10
Fasting serum C-peptide (ng/ml)	2.21 ± 1.38	2.16 ± 0.98	2.05 ± 1.00	ND	ND	ND	2.51 ± 1.18	2.61 ± 1.73	3.11 ± 0.75
HbA1c %	5.26 ± 0.67	5.25 ± 1.03	5.26 ± 0.54	ND	ND	ND	7.60 ± 2.81	7.59 ± 2.26	8.01 ± 2.50
Total cholesterol (mg/dl)	190.64 ± 45.12	211.57 ± 50.99	202.58 ± 34.11	201.16 ± 41.69	205.14 ± 43.85	196.38 ± 33.59	216.24 ± 46.96	198.19 ± 44.42	217.68 ± 45.32
HDL cholesterol (mg/dl)	53.34 ± 14.02	51.69 ± 15.41	50.90 ± 11.93	48.31 ± 11.23	48.05 ± 11.32	49.18 ± 17.30	42.41 ± 0.41	41.26 ± 13.29	45.89 ± 10.89
Triglycerides (mg/dl)	121.17 ± 65.66	122.57 ± 63.91	114.38 ± 42.38	120.80 ± 91.58	99.97 ± 56.04	90.08 ± 28.36	170.30 ± 98.85	166.62 ± 10.19	193.96 ± 110.41
HOMA	2.41 ± 1.39	2.48 ± 1.53	2.68 ± 1.32	2.72 ± 2.41	2.39 ± 2.30	2.27 ± 1.86	4.64 ± 2.58	4.44 ± 3.22	6.66 ± 5.74
Ins0'/Glu0'	13.87 ± 6.79	13.62 ± 7.81	17.21 ± 10.49	12.92 ± 8.52	11.47 ± 7.84	12.42 ± 10.42	11.54 ± 7.58	10.90 ± 5.53	16.65 ± 5.15
QUICKI	0.34 ± 0.03	0.34 ± 0.03	0.34 ± 0.02	0.35 ± 0.04	0.36 ± 0.04	0.35 ± 0.04	0.31 ± 0.03	0.32 ± 0.03	0.30 ± 0.03
β-cell HOMA	217.70 ± 197.99	182.47 ± 138.52	315.08 ± 373.02	137.59 ± 78.94	138.83 ± 96.12	158.06 ± 131.26	82.59 ± 66.95	85.14 ± 64.59	160.04 ± 93.35
Stumvoll index	1231.92 ± 616.17	1123.11 ± 536.77	1319.81 ± 424.03	ND	ND	ND	ND	ND	ND

Values shown are the means ± SD. ANOVA multiple comparisons—Bonferroni test. HbA1c, Glycosylated hemoglobin; ND, not determined; Ins0'/Glu0', fasting insulin/glucose.

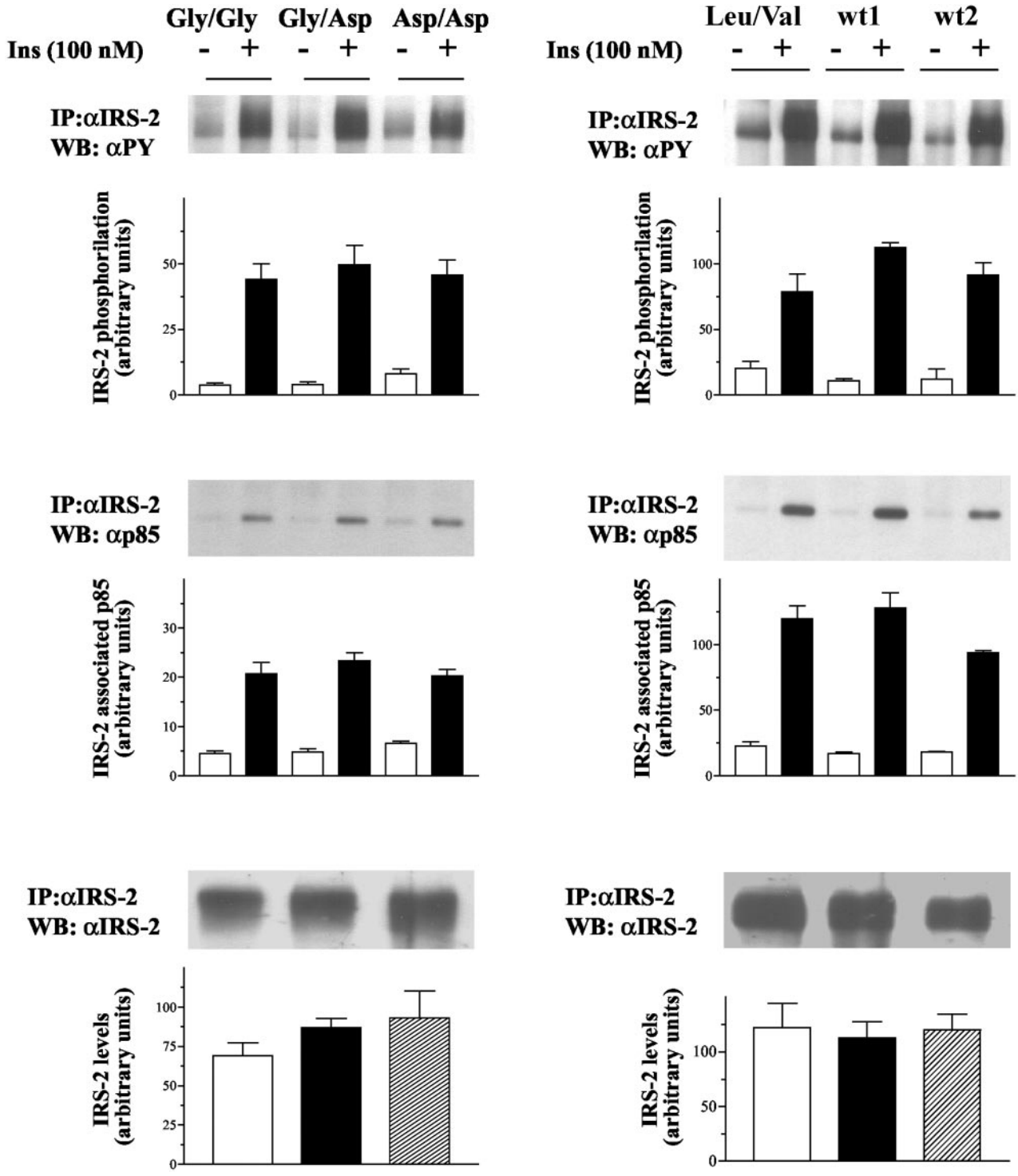

FIG. 1. Expression of IRS-2, tyrosine phosphorylation, and its association with the p85 subunit of PI 3-kinase in cultured fibroblasts. Fibroblasts from Gly/Gly, Gly/Asp, and Asp/Asp carriers (*left panels*) or from wild-type (wt) and heterozygous Leu647Val carriers (*right panels*) were incubated in the presence (*black bars*) or absence (*white bars*) of 100 nM insulin for 5 min. Equal amounts of proteins were immunoprecipitated with anti-IRS-2 antibody, subjected to SDS-PAGE, and immunoblotted with antiphosphotyrosine (*top panels*), anti-p85 (*middle panels*), or anti-IRS-2 antibody (*bottom panels*). Proteins were detected by enhanced chemiluminescence. The autoradiographs shown are representative of three independent experiments. IP, Immunoprecipitated; WB, Western blotting.

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.

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Address all correspondence and requests for reprints to: Giorgio Sesti, M.D., Dipartimento di Medicina Sperimentale e Clinica, Università Magna Græcia di Catanzaro, Via Tommaso Campanella, 115 88100 Catanzaro, Italy. E-mail:sesti@unicz.it.

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References

1. **Sun XJ, Rothenberg P, Kahn CR, Baker JM, Araki E, Wilden PA, Cahill DA** 1991 Structure of the insulin receptor substrate IRS-1 defines a unique signal transduction protein. Nature 352:73–77
2. **Sun XJ, Wang LM, Zhang Y, Yenush L, Myers Jr MG, Glasheen E, Lane WS, Pierce JH, White MF** 1995 Role of IRS-2 in insulin and cytokine signalling. Nature 377:173–177

3. **Saltiel AR, Kahn CR** 2001 Insulin signalling and the regulation of glucose and lipid metabolism. Nature 414:799–806
4. **Giovannone B, Scaldaferri ML, Federici M, Porzio O, Lauro D, Fusco A, Sbraccia P, Borboni P, Lauro R, Sesti G** 2000 Insulin receptor substrate (IRS) transduction system: distinct and overlapping signaling potential. Diabetes Metab Res Rev 16:434–441
5. **Withers DJ, Gutierrez JS, Towery H, Burks DJ, Ren JM, Previs S, Zhang Y, Bernal D, Pons S, Shulman GI, Bonner-Weir S, White MF** 1998 Disruption of IRS-2 causes type 2 diabetes in mice. Nature 391:900–904
6. **Bernal D, Almind K, Yenush L, Ayoub M, Zhang Y, Rosshani L, Larsson C, Pedersen O, White MF** 1998 Insulin receptor substrate-2 amino acid polymorphisms are not associated with random type 2 diabetes among Caucasians. Diabetes 47:976–979
7. **Almind K, Frederiksen SK, Bernal D, Hansen T, Ambye L, Urhammer S, Ekstrom CT, Berglund L, Reneland R, Lithell H, White MF, Van Obberghen E, Pedersen O** 1999 Search for variants of the gene-promoter and the potential phosphotyrosine encoding sequence of the insulin receptor substrate-2 gene: evaluation of their relation with alterations in insulin secretion and insulin sensitivity. Diabetologia 42:1244–1249
8. **Bektas A, Warram JH, White MF, Krolewski AS, Doria A** 1999 Exclusion of the insulin receptor substrate-2 (IRS-2) as a major locus for early-onset autosomal dominant type 2 diabetes. Diabetes 48:640–642
9. **Mammarella S, Romano F, Di Valerio A, Creati B, Esposito DL, Palmirotta R, Capani F, Vitullo P, Volpe G, Battista P, Della Loggia F, Mariani-Costantini R, Cama A** 2000 Interaction between the G1057D variant of IRS-2 and overweight in the pathogenesis of type 2 diabetes. Hum Mol Genet 9:2517–2521
10. **Wang H, Rissanen J, Miettinen R, Karkkainen P, Kekalainen P, Kuusisto J, Mykkanen L, Karhapaa P, Laakso M** 2001 New amino acid substitutions in the IRS-2 gene in Finnish and Chinese subjects with late-onset type 2 diabetes. Diabetes 50:1949–1951
11. **Fritsche A, Madaus A, Renn W, Tschritter O, Teigeler A, Weisser M, Maerker E, Machicao F, Haring H, Stumvoll M** 2001 The prevalent Gly1057Asp polymorphism in the insulin receptor substrate-2 gene is not associated with impaired insulin secretion. J Clin Endocrinol Metab 86:4822–4825
12. **'t Hart LM, Nijpels G, Dekker JM, Maassen JA, Heine RJ, van Haeften TW** 2002 Variations in insulin secretion in carriers of gene variants in IRS-1 and -2. Diabetes 51:884–887
13. **Piazza A, Cappello N, Olivetti E, Rendine SA** 1988 Genetic history of Italy. Ann Hum Genet 52:203–213
14. 1998 Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 21:S5–S19
15. **Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC** 1985 Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412–419
16. **Legro RS, Finegood D, Dunaif A** 1998 A fasting glucose to insulin ratio is a useful measure of insulin sensitivity in women with polycystic ovary syndrome. J Clin Endocrinol Metab 83:2694–2698
17. **Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, Quon MJ** 2002 Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab 85:2402–2410
18. **Stumvoll M, Mitrakou A, Pimenta W, Jenssen T, Yki-Järvinen H, Van Haeften I, Renn W, Gerich J** 2000 Use of the oral glucose tolerance test to assess insulin release and insulin sensitivity. Diabetes Care 23:295–301
19. 1999 Freely associating. Nat Genet 22: 1–2
20. **Sesti G, Federici M, Hribal ML, Lauro D, Sbraccia P, Lauro R** 2001 Defects of the insulin receptor substrates (IRSs) system in human metabolic disorders. FASEB J 15:2099–2111
21. **Ristow M, Busch K, Schatz H, Pfeiffer A** 1996 Restricted geographical extension of the association of a glucagon receptor gene mutation (Gly40Ser) with non-insulin-dependent diabetes mellitus. Diabetes Res Clin Practice 32: 183–185
22. **Laakso M** 1993 How good a marker is insulin level for insulin resistance? Am J Epidemiol 137:959–965
23. **Bonora E, Targher G, Alberiche M, Bonadonna RC, Saggiani F, Zenere MB, Monauni T, Muggeo M** 2000 Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. Diabetes Care 23:57–63