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The *G972R* variant of the Insulin Receptor Substrate-1 *(IRS-1)* gene, body fat distribution and insulin-resistance

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Abstract

Aims/hypothesis. Insulin resistance is recognised as the core factor in the pathogenesis of Type II (non-insulin-dependent) diabetes mellitus, hypertension and atherosclerosis. Several studies indicate the possible role of mutations of the insulin receptor substrate-1 (*IRS-1*) gene in the pathogenesis of insulin-resistance and suggest a possible interaction between the *IRS-1* gene and obesity, either by an effect on the development of obesity or by causing or aggravating the obesity-associated insulin resistance. Therefore, the prevalence of the *G972R* mutation of the *IRS-1* gene was compared in 157 non-diabetic obese subjects (BMI > 30 m/kg^2) and in 157 lean subjects (BMI < 28 m/kg^2). By investigating the relation between this *IRS-1* mutation, measures of obesity and metabolic parameters, we explored the possible influence of this mutation on body fat distribution and insulin resistance.
Methods. The *G972R* mutation was detected by PCR amplification and BstN-1 restriction enzyme digestion. Data were analysed by univariate and multivariate analysis.
Results. The *G972R* allele was significantly more frequent in obese subjects than in lean subjects ($p < 0.002$); however, no difference was found between centrally and peripherally obese subjects. Obese *G972R* carriers had significantly higher BMI ($p < 0.001$), fasting insulin ($p < 0.01$), triglycerides ($p < 0.03$) and $HOMA_{IR}$ ($p < 0.001$) than obese non-carriers. No differences were observed between *G972R* carriers and non-carriers among control subjects. Multivariate analysis confirmed that the *IRS-1 G972R* mutation was significantly and independently associated with reduced insulin sensitivity ($p < 0.009$) in the obese group.
Conclusion/interpretation. The *G972R* mutation of the *IRS-1* gene associates with obesity, but not with fat distribution, in this Italian cohort, and within the obese subjects this *IRS-1* variant strongly associates with metabolic parameters suggesting greater insulin-resistance. These findings indicate a possible interaction between the *IRS-1* variant and obesity in worsening insulin sensitivity. [Diabetologia (2001) 44: 367–372]

Keywords $HOMA_{IR}$, *IRS-1*, BMI, Type II diabetes, central obesity, peripheral obesity, bioeletric impedance, *G972R*.

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Abbreviations: $HOMA_{IR}$, Homeostasis model assessment for insulin resistance; *IRS-1*, insulin-receptor substrate-1; *G972R*, Glycine 972 Arginine; FFM, fat free mass; FAT, fat mass; WC, waist circumference; TBW, total body water; CAD, coronary artery disease.

Insulin resistance is generally recognised as a core factor in the pathogenesis of common disorders such as Type II (non-insulin-dependent) diabetes mellitus, hypertension and premature atherosclerosis. Familial transmission and variations in ethnic distribution suggest that insulin resistance is genetically determined. It is likely that impaired insulin action results from several inherited mutations in a variety of genes, each with subtle or minor effects.

In the search for susceptibility loci predisposing to insulin resistance, previous studies have identified a glycine to arginine substitution at codon 972 (*G972R*) in the insulin receptor substrate-1 (*IRS-1*) gene as possibly being associated with impaired insulin action. The IRS-1 functions as a key proximal signalling molecule for both insulin receptor and the insulin-like growth factor-1 receptor signalling pathways. When phosphorilated, IRS-1 binds with high affinity proteins with src homology-2 domains, including phosphatidylinositol-3 kinase (PI-3 kinase) [1]. In vitro studies have shown that this mutation is associated with a substantial reduction in insulin-stimulated PI-3 kinase activity and in binding of the P85 regulatory subunit of the PI-3 kinase to IRS-1 [2]. In transgenic animals, *IRS-1* deficiency was associated with insulin resistance [3–4] and mice with null mutations in both insulin receptor and *IRS-1* genes were shown to be insulin resistant with compensatory beta-cell hyperplasia [5]. It was originally reported that the *IRS-1* codon 972 variant did not confer insulin resistance in subjects with Type II diabetes [6]. A more recent work [7] found, however, a 50 % decrease in insulin sensitivity in a small cohort of obese carriers of the *G972R* mutation. In addition, we recently found that the *G972R* mutation of the *IRS-1* gene was associated with a significantly increased risk of coronary artery disease (CAD) [8], particularly in obese subjects. Although these findings strongly suggest an interaction between this *IRS-1 G972R* mutation and obesity, no study has evaluated the possible effect of this gene variant on the development of obesity, particularly visceral obesity with which insulin resistance is predominantly associated. Moreover, the effect of the *IRS-1* variant in aggravating the obesity-associated insulin resistance has not been fully explained.

To address these questions, we compared the prevalence of the *G972R* mutation in non-diabetic obese subjects and a group of lean subjects. Moreover, by investigating the relation between this *IRS-1* mutation, measures of obesity (including body fat distribution, fat mass, fat free mass, FAT/FFM and basal metabolic rate) and metabolic parameters, we explored the possible influence of the *G972R* mutation on the relation between body fat distribution and insulin resistance.

Subjects and methods

Subjects. A total of 314 Caucasian subjects were studied. All subjects were recruited in the Lazio region of Italy, mostly from Rome and its surrounding towns. The 157 obese subjects were consecutively recruited from the obesity clinic of the Department of Clinical Science, University of Rome "La Sapienza". All obese patients were selected on the basis of a BMI higher than 30 kg/m^2, in order to recruit strictly obese subjects, according to previously suggested criteria [9]. Body fat distribution was assessed by waist circumference (WC). The cut-off points chosen to differentiate central from peripheral obesity were the following: WC higher than 94 for men and WC higher than 88 for women. These limits involve a trade-off between sensitivity and specificity and were recently described [10]. Furthermore, these limits take into account the metabolic complications of the android biotype. Of these obese subjects, 105 underwent bioeletric impedance to determine fat-free mass, fat mass, basal metabolic rate and per cent total body water (Datasystem vers. 1, Medigroup, Milan, Italy). Total fat mass was calculated by subtracting fat-free mass (FFM) from total body weight. The accuracy of the FFM measurement was increased by using a multifrequency bioimpedence (1–5–10–50–100 KHz) and applying the equation described previously [11]. Exclusion criteria were: (1) the presence of Type II diabetes or first-grade relatives with Type II diabetes; (2) the presence of thyroid, liver or renal disease; (3) the presence of coronary artery disease (CAD).

Control subjects were 157 unrelated individuals selected at random from a population of individuals screened for CAD risk factors. Exclusion criteria were: (1) the presence of a BMI higher than 28; this cut-off point was set to avoid the risk of an overlap between the two groups of patients and control subjects, when recruiting moderately overweight subjects; (2) the presence of Type II diabetes or first-grade relatives with Type II diabetes; (3) the presence of CAD. The latter was excluded by use of the Rose questionnaire and electrocardiograms (Minnesota coding) [12]. In both obese and control subjects a complete medical history was obtained by questionnaire, which included questions about smoking habits, history of hypertension and Type II diabetes and current medication used. Diagnosis of Type II diabetes was based on history of hypoglycaemic treatment and a repeated fasting blood glucose higher than 126 mg/dl or both [13]; that of hypertension was based on the presence of high systolic (> 160 mmHg) and diastolic (> 95 mmHg) blood pressure or both and the current use of antihypertensive medications or both.

Methods. The presence of glycine to arginine substitution at codon 972 of *IRS-1* gene was determined as described previously [8]. Genotypes were scored by two independent investigators who did not know whether the samples were from a case patient or from a control subject. Ambiguous samples were analysed a second time.

Plasma insulin concentrations were measured on frozen samples using a radioimmunoassay (Biodata Insulin Kit, Milan, Italy) with an inter-assay coefficient of variation of 7.5 %.

Homeostasis model assessment for insulin resistance ($HOMA_{IR}$) and for the percentage of beta-cell function were calculated as described previously [14].

Categorical variables were compared by χ^2 test or Fisher's exact test. Differences between continuous variables were evaluated by two-tailed Student's *t*-test. Genotype distributions between the study groups were compared by $2 \cdot 2$ and $2 \cdot 3$ contingency tables and χ^2 analysis. Multiple regression analysis was used to find out whether there was an independent association between the *G972R* mutation and insulin resistance ($HOMA_{IR}$), after adjustment for other modulators known to affect both conditions (sex, age, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, BMI). A first-order interaction between obesity (BMI) and the *IRS-1* variant was also included in the regression model. P-values < 0.05 were taken as statistically significant. All analyses were carried out using SPSS software programme Version 7.0.

Table 1. Clinical characteristics of study subjects

	Obese subjects (n = 157)	Lean subjects (n = 157)	p value
Age (years)	51.6 ± 1.2	57.9 ± 0.9	< 0.001
Sex (M/F)	53/104	72/85	< 0.03
Body mass index (kg/m^2)	33.2 ± 0.5	24.6 ± 0.2	< 0.0001
Smokers (%)	21.1	15.9	NS
Hypertensive (%)	38.2	29.0	NS
Blood glucose (mmol/l)	4.80 ± 0.1	4.30 ± 0.1	NS
Fasting plasma insulin (pmol/l)	135.5 ± 10.0	50.6 ± 2.8	< 0.0001
HOMA $_{IR}$	3.88 ± 0.2	1.55 ± 0.1	< 0.0001
% β-cell function (log)	2.39 ± 0.04	2.33 ± 0.04	NS
Plasma lipids (mmol/l)			
Total cholesterol	5.66 ± .09	5.86 ± 0.15	NS
Total triglycerides	1.80 ± 0.10	1.73 ± 0.09	NS
HDL cholesterol	1.33 ± 0.03	1.43 ± 0.03	NS
LDL cholesterol	3.52 ± 0.08	3.55 ± 0.07	NS

Data are given as means ± SEM. All p values were corrected for differences in age, sex and BMI
The statistical analysis of HOMA $_{IR}$, total triglycerides and plasma insulin were done on log-transformed values but the untransformed values are given in the table. Continuous variables were compared by t test and categorical variables by χ^2 test

Table 2. Genotype distributions and allele frequencies for the *G972R* mutation in the *IRS-1* gene in obese subjects and control subjects

	Genotypes				Allele frequencies	
	n	*GG*	*GR*	*RR*	*G*	*R*
Total obese subjects[a]	157	125 (79.6%)	31 (19.7%)	1 (0.01%)	0.895	0.105
– Central obese subjects[b]	65	50 (76.9%)	14 (21.5%)	1 (1.6%)	0.876	0.124
– Peripheral obese subjects[c]	40	31 (77.5%)	9 (22.5%)	0	0.887	0.113
Lean subjects	157	144 (91.7%)	13 (8.3%)	0	0.958	0.042

"Total obese subjects" includes 50 patients who did not undergo biolectric impedance analysis
[a] "Total obese vs control subjects": genotypes $\chi^2 = 9.36$, df = 2, $p < 0.002$; allele frequencies $\chi^2 = 8.43$, df = 1, $p < 0.004$
[b] "Central obese vs control subjects": genotypes $\chi^2 = 10.21$, df = 2, $p < 0.006$; allele frequencies $\chi^2 = 10.04$, df = 1, $p < 0.001$
[c] "Peripheral obese vs control subjects": genotypes $\chi^2 = 5.41$, df = 1, $p < 0.02$; allele frequencies $\chi^2 = 6.11$, df = 1, $p < 0.01$
"Central obese subjects vs peripheral obese subjects": genotypes $\chi^2 = 0.71$, df = 2, p = NS; allele frequencies $\chi^2 = 0.05$, df = 1, p = NS.
Cut-off points for centrally obese subjects were: WC ≥ 94 for men and WC ≥ 88 for women

Results

The clinical characteristics of the study subjects are shown in Table 1. The obese subjects showed higher fasting insulin ($p < 0.0001$) and HOMA$_{IR}$ ($p < 0.0001$), indicating the presence of insulin resistance in this group. These differences remained significant after adjustment for age, sex and BMI. The percentage beta-cell function, fasting glucose and plasma lipids were not different between the two groups. Of the obese subjects, 105 were studied by bioeletric impedance, and were divided into subjects with central and peripheral obesity, according to their body fat distribution. This was assessed by waist circumference (WC), which provides a measure of upper body fat deposition and correlates with an increased risk of metabolic and cardiovascular complications [10]. When compared with subjects with peripheral obesity, those with central obesity had a higher BMI (35.5 ± 1.7 vs 32.7 ± 0.9, $p < 0.002$), a larger WC (108.9 ± 5.0 vs 90.6 ± 2.4, $p < 0.0007$), a higher fat mass and a lower FFM ($p < 0.007$, data not shown). Furthermore, HOMA $_{IR}$ was significantly higher in the subjects with central obesity (5.73 ± 0.6 vs 3.99 ± 0.3; $p < 0.04$), together with total cholesterol, total triglycerides and LDL cholesterol ($p < 0.01$, data not shown). Overall, these data confirm the expected finding of a worse metabolic profile in subjects with central obesity than in those with peripheral obesity.

Genotype distributions and allele frequencies in obese and control subjects are shown in Table 2. Both groups were in Hardy-Weinberg equilibrium. The mutant *G972R* allele was significantly more frequent in obese subjects than in lean control subjects ($p < 0.002$) and no differences related to sex and age

Table 3. Comparison of measures of body fatness and of metabolic parameters according to the *G972R* genotypes

	Obese Subjects		Lean Subjects	
	GR + RR (n = 32)	GG (n = 125)	GR + RR (n = 13)	GG (n = 144)
Age (years)	46.0 ± 2.5[d]	52.9 ± 1.4	63.6 ± 1.0	57.4 ± 3.2
Body mass index (kg/m^2)	36.5 ± 1.1[a]	32.3 ± 0.4	24.2 ± 0.7	24.6 ± 1.7
Blood glucose (mmol/L)	5.16 ± 0.2	4.69 ± 0.1	4.23 ± 0.9	4.26 ± 1.6
Fasting plasma insulin (pmol/l)	200.7 ± 25.8[b]	119.0 ± 10.7	45.1 ± 3.5	55.9 ± 2.8
$HOMA_{IR}$	6.32 ± 0.8[c]	3.28 ± 0.2	1.19 ± 0. 4	1.59 ± 0.4
%β-cell function (log)	2.51 ± 0.07	2.36 ± 0.04	2.22 ± 0.1	2.34 ± 0.2
Plasma lipids (mmol/l)				
Total cholesterol	5.69 ± 0.26	5.65 ± 0.10	5.65 ± 0.22	5.88 ± 0.18
Total triglycerides	2.11 ± 0.36	1.73 ± 0.10	1.72 ± 0.09	1.78 ± 0.26
HDL cholesterol	1.23 ± 0.07	1.35 ± 0.03	1.51 ± 0.04	1.44 ± 0.04
LDL cholesterol	3.32 ± 0.2	3.55 ± 0.11	3.33 ± 0.22	3.57 ± 0.07

Data are given as means ± SEM. *GG + GR* = carriers; *GG* = non-carriers of *IRS-1* mutation.

[a] $p < 0.001$ obese carriers of *G972R* mutation vs obese non-carriers

[b] $p < 0.01$ obese carriers of *G972R* mutation vs obese non-carriers, adjusted for age and BMI.

[c] $p < 0.001$ obese carriers of *G972R* mutation vs obese non-carriers, adjusted for age and BMI.

[d] $p < 0.03$ obese carriers of *G972R* mutation vs obese non-carriers

Differences between control carriers vs control non-carriers were all non-significant

The statistical analysis of $HOMA_{IR}$, total triglycerides and plasma insulin were done on log-transformed values but the untransformed values are given in table. Continuous variables were compared by t test and categorical variables by χ^2 test

Table 4. Multiple regression analysis of modulators of insulin sensitivity ($HOMA_{IR}$) in study groups

	Obese subjects (n = 157)		Lean subjects (n = 157)	
	Standardised regression coefficient	p value	Standardised regression coefficient	p value
Age (years)	–0.07	0.50	0.14	0.12
Sex	0.13	0.15	0.03	0.73
Body mass index (kg/m^2)	0.45	< 0.0001	0.11	0.25
Total triglycerides	0.23	< 0.013	0.05	0.60
HDL cholesterol	0.023	0.82	–0.07	0.47
LDL cholesterol	–0.001	0.99	–0.06	0.55
IRS-1 status[a]	0.26	< 0.009	0.10	0.28
BMI · *IRS-1* status	0.536	< 0.0001	–0.64	0.48

[a] wild type vs heterozygous for the *G972R* mutation

were found between the two groups. To test whether the *G972R* mutation could be associated with body fat distribution, we compared frequencies between the two sub-groups of obese subjects; however no significant difference was found between centrally obese and peripherally obese subjects. Similarly, the 105 obese carriers and non-carriers studied by bioelectric impedance did not differ in all the other measures of adiposity (FFM, FAT, % TBW, basal metabolic rate). Both sub-groups were significant and independently different from the control subjects (Table 2).

Assuming a dominant model of inheritance of the *IRS-1 G972R* mutation (only one homozygous detected for this mutation), we compared metabolic parameter between carriers (GR + RR) and non-carriers (GG) (Table 3). Obese carriers were younger and had a significantly higher BMI ($p < 0.001$). After adjustment for these differences, obese subjects carrying the *IRS-1* variant showed higher plasma fasting insulin ($p < 0.01$), plasma tryglicerides ($p < 0.03$) and $HOMA_{IR}$ ($p < 0.01$) than obese non-carriers. Conversely, no differences were observed between *G972R* carriers and non-carriers among lean control subjects. These findings suggest that the *G972R* mutation affects insulin sensitivity in obese subjects. It should also be noted that higher triglycerides concentrations (although not significant) were observed in obese carriers, and this agrees with our previous observation [8].

To estimate the independent contribution of the *IRS-1* variant to insulin sensitivity (measured by $HOMA_{IR}$) we carried out multivariate analysis in obese and lean subjects to control for the confounding effect of well-known modulators of insulin resistance (sex, age, BMI, plasma lipids). In the regression model, we also included the interaction between BMI and the *G972R* mutation (Table 4). The results of multivariate analysis confirmed that the *IRS-1 G972R* mutation was an independent predictor of insulin sensitivity ($p < 0.009$) measured by $HOMA_{IR}$ in obese

subjects. Moreover BMI ($p < 0.0001$) and triglycerides ($p < 0.01$) were associated with insulin sensitivity in these subjects. The interaction between obesity and *IRS-1* carrier status was even a stronger predictor of insulin resistance ($p < 0.0001$). Conversely none of these factors were associated with insulin sensitivity in lean control subjects.

Discussion

These data show that in our Italian cohort the *G972R* mutation of the *IRS-1* gene is strongly associated with obesity, with almost 20% of the obese subjects carrying this mutation. This frequency is similar to that found in previous studies [15, 16] in obese or insulin-resistant subjects, where 18–25% had the mutation. Other authors have not found an association between obesity and the G972 variant [17]. This study was, however, done on a different cohort (all female, much younger), and the control subjects were older women (mean age 59 years) showing a frequency of the *G972R* variant higher than that reported in the other association studies.

The frequency of the *IRS-1* mutation was equally distributed between subjects with central and peripheral obesity, indicating that this mutation is probably not involved in body fat distribution. In univariate analysis this mutation associates with reduced insulin sensitivity in obese subjects, with carriers being two times more insulin resistant than non-carriers. This finding was confirmed by multivariate analysis showing that among obese subjects the *IRS-1* variant was a significant, independent predictor of insulin resistance. These conclusions, extending previous observations [7], suggest an apparent synergistic interaction between the *G972R* mutation and obesity to worsen insulin resistance. Our results are also in agreement with others recently presented [18] that found this variant to be associated with an increase in BMI in a large African-American cohort. This study did not find any association between the *G972R* mutation and severe obesity (BMI > 40) but the discrepancy could be explained by the fact that severe obesity could recognise other hormonal and environmental influences. It also did not find any effect of *IRS-1* variant on plasma insulin concentrations [18]; fasting insulin used as a surrogate index for insulin sensitivity cannot, however, explain more than 30–40% of the variance in glucose-derived insulin sensitivity [19], whereas $HOMA_{IR}$ correlation with clamp is about 0.80 [20]. Therefore, a better measure of insulin sensitivity might have increased the possibility of finding an association.

Two other studies have attempted to correlate the *G972R* variant to insulin sensitivity by using hyperinsulinaemic euglycaemic clamp in carriers of the mutation [21, 22], without postive results. Both studies, however, had low BMI cut-off values. In particular, one report [21] studied Type II diabetic Japanese patients with a mean BMI of 22.1, and since overt diabetes is probably the result of several defects in insulin action, it is possible that the effect of the *G972R* mutation might have been diluted. The other study [22] was also carried out on subjects with a much lower BMI (mean BMI = 25) than our subjects, thus making it difficult to detect the possible interaction between obesity and the *IRS-1* variant.

A possible effect of the *G972R* mutation of the *IRS-1* gene on impairing insulin secretion has been proposed [23]. Compensatory beta-cell hyperplasia in mice with null mutation in the *IRS-1* gene has, however, been shown by others [5]. Several human studies [7, 18, 21], have also failed to find differences in fasting plasma insulin and C peptide or both in carriers of the *G972R* mutation. In our cohort, we did not find any difference in insulin levels or in the percent of beta-cell function between our groups. The question of the effect of the *G972R* mutation on insulin secretion remains open and further studies are necessary.

The mechanisms behind the interaction between the *IRS-1* gene variant, obesity and insulin resistance are still not known. A defective response to known modulators of insulin signalling, such as TNF-α, an increased serine phosphorylation which would predict impaired insulin signalling or a defective interaction with IRS-1 direct substrates, such as PI3-K, have all been suggested. Molecular studies of the effect of the *G972R* mutation are warranted to answer these questions.

In conclusion, the *G972R* mutation of the *IRS-1* gene strongly associates with obesity in this Italian cohort, and, within the obese group, it was associated with metabolic parameters suggesting greater insulin resistance. This association indicates a possible interaction between the *IRS-1* variant and obesity to worsen insulin sensitivity. These observations support our previous observation that this *IRS-1* gene variant could increase coronary risk in obese subjects by favouring pro-atherogenic metabolic abnormalities.

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