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The *Gly*482*Ser* missense mutation of the *Peroxisome Proliferator – activated receptor* γ *coactivator* -1α $(PGC-1\alpha)$ gene associates with reduced insulin sensitivity in normal and glucose-intolerant obese subjects

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Abstract. Among the putative candidate genes for insulin resistance, the peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) is a transcriptional coactivator of PPARγ and α, regulating a wide range of processes involved in energy production and utilization, such as thermogenesis, liver gluconeogenesis, glucose uptake in muscle. In population studies a *Gly482Ser* substitution in *PGC-1*α has been reported to be associated with increased risk of type diabetes 2 and insulin resistance. In the present study we have analysed the association between the *Gly482Ser* missense mutation of the *PGC-1*α gene and insulin sensitivity and glucose tolerance in a population of obese non-diabetic subjects.
The *Gly482Ser* SNPs was detected by PCR-RFLP in a cohort of 358 Caucasian obese subjects (223 with normal glucose tolerance (NGT) and 125 with impaired glucose tolerance (IGT).
We observed a significant association ($p < 0.007$) between carriers of the *Gly482Ser* variant of the *PGC-1*α gene and insulin resistance measured by $\mathrm{HOMA_{IR}}$. Multivariate analysis confirmed that the *Gly482Ser* SNP was a significant ($p < 0.02$) determinant of decreased insulin sensitivity, independently from other well-known modulators of insulin action.
In conclusion, we have found significant association between the *Gly482Ser* variant of the *PGC-1*α gene and reduced insulin sensitivity in obese subjects. This association resulted independent from all other known modulators of insulin resistance, and suggests a primary role for the *PGC-1*α gene on the genetic susceptibility to insulin resistance in obesity.

Keywords: $PGC-1\alpha$ gene, obesity, HOMAIR, BMI (body mass index), single nucleotide polymorphisms (SNPs)

1. Introduction

Insulin resistance precedes and predicts the development of common disorders such as type 2 diabetes, hypertension and atherosclerosis. Familial transmission and variations in ethnic distribution suggest that insulin resistance is genetically determined [17], and it is likely that impaired insulin action results from several inherited mutations in a variety of genes, each with different effects.

Among the putative candidate genes for insulin resistance, the peroxisome proliferator-activated receptor γ coactivator-1α (*PGC-1*α) is a transcriptional coactivator of peroxisome proliferator-activated receptor (PPARs) γ and α, regulating a wide range of processes involved in energy production and utiliza-

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tion, such as thermogenesis, liver gluconeogenesis, glucose uptake in muscle, and skeletal muscle fiber-type switching [7,9]. The *PGC-1*α gene is induced in the liver by fasting and stimulates hepatic gluconeogenesis by increasing gene transcription of phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) [6–19]. Furthermore, in the liver, β-oxidation of fatty acids and ketogenesis are other adaptative mechanisms in the fasted state, and *PGC-1*α has been shown to induce several key genes involved in both these processes, thus playing a more general and important role in the fasted state [13]. *PGC-1*α has also been shown to increase glucose transport in muscle by up regulating the glucose transporter GLUT4 [9].

Molecular studies have shown that *PGC-1*α interacts with several nuclear receptors involved in glucose metabolism. For example it has been shown that the hepatocyte nuclear factor-4α (HNF4α) is absolutely required for *PGC-1*α to induce the expression of the key gluconeogenic genes PEPCK and G6Pase. Also the interaction with the forkhead transcription factor FOXO1 appears to be important for insulin-regulated hepatic gluconeogenesis [12].

Furthermore, in support of the key role played by *PGC-1*α it has been shown that its expression is greatly increased in mouse models of diabetes, such as the ob/ob mouse, contributing to the insulin resistance of these animals [5]. Finally, adenovirus-mediated delivery of recombinant *PGC-1*α in primary hepatocytes determines a dramatic rise in the expression of key gluconeogenic enzymes in the liver and the subsequent production of glucose [5].

Previous studies have reported linkage between the *PGC-1*α genomic region on chromosome *4p15.1* and fasting serum insulin concentration in Pima Indians [11], and this region has also been linked to increased BMI in Mexican Americans [1].

In population studies a *Gly482Ser* missense mutation in *PGC-1*α has been reported to be associated with increased risk of type diabetes 2 in a large Danish population [2]. Association has also been reported between the *Gly482Ser* substitution and insulin resistance in Japanese subjects [4]. Finally, association of this SNP with altered lipid oxidation and reduced early insulin release has been reported in Pima Indians [10].

Recently, however, the *Gly482Ser* variant was found not associated with diabetes-related traits in non-diabetic German and Dutch populations [16]. Different ethnicities, bias in the selection of the study groups or in the clinical parameters under study are all possible explanations for these contrasting results.

To help to clarify if the *PGC-1*α gene plays a primary role in insulin resistance, we have investigated in a population of obese, non-diabetic subjects the possible association of the *Gly482Ser* polymorphism with insulin sensitivity and glucose tolerance.

2. Research design and methods

2.1. Subjects

A total number of 358 Caucasian obese subjects (223 with normal glucose tolerance (NGT) and 125 with impaired glucose tolerance (IGT), were recruited from the Day-Hospital of the Division Endocrinology of the Department of Clinical Sciences, University of Rome "La Sapienza". All were unrelated individuals with a BMI $>$ 28 Kg/m^2, and all subjects underwent a 75 g oral glucose tolerance test (OGTT).

In order to avoid the confounding effect of multiple environmental, pharmacological and genetic factors on insulin sensitivity, one of the exclusion criteria was the presence of type 2 diabetes. Diagnosis of type 2 diabetes was based on history of hypoglycaemic treatment and/or confirmed fasting blood glucose $>$ 126 mg/dl (7.0 mmol/l) [15].

2.2. Biological measurements

Plasma glucose was determined by the glucose oxidase method (Autoanalyzer, Beckman Coulter, USA; coefficient of variation (CV), 1.9 $\pm$ 0.2%). Plasma insulin concentration was measured on frozen samples using a radio immunoassay (Biodata Insulin Kit, Milan, Italy) with an interassay CV of 7.5%. Cholesterol and triglyceride concentrations in total plasma were measured with a Technicon RA-1000 Autoanalyzer. High-density lipoprotein (HDL) cholesterol was determined in the whole plasma after precipitation of apoB-containing lipoproteins with phosphotungstic acid/$MgCl_2$.

The OGTT was performed after a 10-h overnight fast: subjects ingested a solution containing 75 g dextrose, and venous blood samples were obtained after 0 and 120 min to measure plasma glucose and plasma insulin. Insulin-resistance was estimated with the homeostasis model assessment ($HOMA_{IR}$ = fasting glucose (mmol/l) $\times$ fasting insulin (μU/ml)/22.5 [8]. The HOMA %B index was used to estimate beta-cell function as described [18]. This HOMA %B index has been reported to have a good correlation with insulin release estimated from the hyperglycaemic clamp and from the acute insulin response from the intravenous glucose tolerance test (IVGTT) [18].

2.3. Genotyping

Genomic DNA was extracted from peripheral blood lymphocytes and the 500 bp fragment of the *PGC-1α* gene containing the *Gly482Ser* SNP was amplified by modification of the PCR method described by Hara et al. [4]. PCR conditions were as follows: 34 cycles of denaturation at 94°C for 30 s, annealing at 54°C for 30 s, and extension at 72°C for 45 s. The sequences of the new primers to detect the *Gly482Ser* polymorphism were 5'-tttggaggcaagcaagcag-3' and 5'-tattagggttttgccaagg-3'. By use of Webcutter 2.0 (www.firstmarket.com/cutter/cut2.html), it was found that the *Gly482Ser* variant of the *PGC1 gene* creates an Msp1 restriction site, resulting in the presence of two bands of 380 bp and 120 bp. Thus, restriction enzyme digestion of the 500 bp PCR fragment of the intron 4 was carried out at 37°C for 2 h in 22 μl reactions containing 12 μl PCR product, 2.2 μl buffer 10x and 6 units of Msp1 restriction enzyme. Fragments were analysed on 3.5% high-resolution agarose gel stained with ethidium bromide.

2.4. Statistical analysis

Categorical variables were compared by chi-square or Fisher's exact test. Differences between continuous variables were evaluated by two-tailed Student's t-test. Logarithmic transformation was used to normalize distributions of $\mathrm{HOMA_{IR}}$, plasma insulin, HOMA %B index, HDL cholesterol, and triglycerides. Genotype distributions and allele frequencies between the study groups were compared by 2 X 2 and 2 X 3 contingency tables and chi-square analysis.

To evaluate the possible influence of the *Gly482Ser* SNP on insulin resistance (measured by $\mathrm{HOMA_{IR}}$) and on β-cell function (HOMA %B), multiple linear regression analysis was employed to adjust for possible confounding factors known to have an effect on insulin resistance and β-cell function such as age, gender, BMI, and lipids. p values < 0.05 or less were taken as statistically significant. All statistical analyses were performed with SPSS statistical package.

3. Results

The study group comprised 223 NGT and 125 IGT obese subjects. The clinical characteristics of the two groups are described in Table 1. IGT obese subjects, as expected, had significantly higher BMI, systolic and diastolic blood pressure, fasting and 120' blood glucose, fasting and 120' plasma insulin, $\mathrm{HOMA_{IR}}$, and HDL cholesterol, but did not differ in gender, total cholesterol, triglycerides and HOMA %B.

Genotype distributions and allele frequencies of the *Gly482Ser* SNP of the *PGC-1α* gene were not different between NGT and IGT subjects (Table 2), similarly to what reported by Stumvoll and co-workers [16].

Clinical and metabolic parameters were then compared between genotypes of the *Gly482Ser* SNP of the *PGC-1α* gene. A significant difference in parameters of insulin-resistance was observed when carriers of the variant (both *Gly/Ser* heterozygous and *Ser/Ser* homozygous) where compared to carriers of the *Gly/Gly* genotype (Table 3). In particular, heterozygous and homozygous carriers of the *Gly482Ser* SNP had higher $\mathrm{HOMA_{IR}}$ ($p < 0.01$), and higher fasting plasma insulin ($p < 0.01$). Also a significant difference in HOMA %B ($p < 0.03$) was observed between carriers and non-carriers of the *Gly482Ser* variant, with non-carriers showing a significant lower β-cell function. No difference was observed between heterozygous *Gly/Ser* and homozygous *Ser/Ser* genotypes when compared for any of the clinical and metabolic parameters analyzed.

The same analysis was performed in the NGT and IGT groups independently. In NGT subjects a significant association between carriers of the *Ser* allele and $\mathrm{HOMA_{IR}}$ ($p < 0.01$), fasting insulin ($p < 0.005$) and HOMA %B ($p < 0.01$) was observed (data not shown). In IGT subjects, however, this association did not reach significance, although a similar trend was found, with IGT carriers of the *Ser* allele being more insulin resistant.

To confirm the independent contribution of the *PGC-1α Gly482Ser* variant to insulin sensitivity we performed multiple linear regression analysis controlling for the confounding effect on $\mathrm{HOMA_{IR}}$ of well-known modulators of insulin resistance (age, gender, BMI, HDL-cholesterol. and triglycerides). A second regression model was used to evaluate if the glucose tolerance status could influence the analysis. This in order to verify if IGT status, which is probably more strongly related to insulin-resistance, could play a role as confounding factor.

Both regression models confirmed that the *Gly482Ser* variant was independently associated with higher $\mathrm{HOMA_{IR}}$ (p=0.024), together with BMI ($p < 0.0001$) and gender ($p < 0.002$) (data not shown). When IGT status was added to the analysis, all associations remained significant, including the glucose tolerance status ($p < 0.0001$). This second model better

Table 1
Clinical characteristics of study subjects NGT and IGT

	NGT subjects ($n = 223$)	IGT subjects ($n = 125$)	p-value
Age (yrs)	36 (29–50)	48 (38–55)	< 0.0001
Gender (M/F)	61/162	42/83	NS
Body Mass Index (kg/m^2)	37.7 (31.9–44)	39.9 (34.6–47)	0.006
Systolic blood pressure (mmHg)	125 (115–140)	130 (130–140)	0.03
Diastolic blood pressure (mmHg)	80 (75–90)	90 (85–95)	0.03
Blood glucose 0'(mg/dl)	84 (77–91)	96 (88–107)	< 0.0001
Blood glucose 120' (mg/dl)	105.5 (90.7–122.2)	162.5 (149–175.7)	< 0.0001
Fasting plasma insulin 0' (μU/ml)	17.6 (10.7–27.1)	27 (14.3–40)	0.001
Fasting plasma insulin 120' (μU/ml)	74.1 (42.3–147)	150.5 (88.7–200)	< 0.0001
$\mathrm{HOMA_{IR}}$	3.7 (2.2–5.8)	6.3 (3.5–10.2)	< 0.0001
HOMA %B	193 (140–251)	189 (136–236.5)	NS
Total cholesterol (mg/dl)	204.4 (182.3–234.9)	209.1 (190–236.9)	NS
Total triglycerides (mg/dl)	94.4 (68.8–136)	103.9 (84–148.2)	NS
HDL cholesterol (mg/dl)	52.7 (46–62.4)	48.8 (42.8–56.4)	0.01

Logarithmic transformation was used to normalize the distributions of BMI, blood pressure, $\mathrm{HOMA_{IR}}$, HOMA %B, plasma insulin, total and HDL-cholesterol and triglycerides. Data are given as median and the interquartile range. Continuous variables were compared by t-test and categorical variables by χ^2 test.

Table 2
Genotype distributions and allele frequencies for PGC1 gene SNPs in NGT subjects and IGT subjects

	n.	*Gly482Ser* SNP Genotypes*			Allele frequencies	
		Gly/Gly	*Gly/Ser*	*Ser/Ser*	T	G
NGT subjects	223	86 (38.6%)	96 (43%)	41 (18.4%)	0.60	0.40
IGT subjects	125	51 (40.8%)	56 (44.8%)	18 (14.4%)	0.63	0.37

Genotype distributions and allele frequencies were compared by chi-square analysis. Allele frequencies were in Hardy-Weinberg equilibrium.
*NGT subjects vs. IGT subjects: genotypes $\chi^2 = 0.91,\ df = 2,\ p = NS$; allele frequencies $\chi^2 = 1.98,\ df = 1,\ p = NS$.

explained the variance in $\mathrm{HOMA_{IR}}$ (regression model 1: $r^2 = 0.15$; model 2: $r^2 = 0.20$).

Multiple regression analysis was also employed to evaluate the proportion of variance of HOMA %B explained by the gene variant independently from the other variables (including sex, age, BMI, HDL, triglycerides). The analysis showed that the association of the gene variant with the HOMA %B index was just above marginal significance ($p = 0.052$) (data not shown).

4. Discussion

The present study provides evidence of association between the *Gly482Ser* SNP of the *PGC-1*α gene and reduced insulin sensitivity in obese subjects. This association was present in the whole population of obese subjects, and was most evident within NGT carriers of the *Ser* allele. IGT subjects showed a similar trend, with carriers of the *Ser* allele being more insulin resistant, but this difference did not reach significance. Possible explanation for this result could be that IGT subjects may already be the result of insulin-resistance, and therefore making it more difficult to discriminate between subjects within this group. Also, we should point out that the IGT group was smaller, possibly reducing the power of this stratified analysis. Genotype distributions did not differ between IGT and NGT groups, similarly to what reported by Stumvoll and coworkers [16]. It could be speculated that the *Gly482Ser* variant influences insulin sensitivity in a similar way in obese NGT and IGT subjects, predisposing obese subjects to become glucose intolerant when other genetic factors come into play.

The association between the *Gly482Ser* variant of the *PGC-1*α gene and reduced insulin sensitivity was also independent from all other known determinants of insulin resistance. Our results are consistent with previous studies reporting association with insulin resistance and related traits in various populations [2,4,10].

Table 3
Clinical characteristics of subjects according to *PGC-1a* gene *Gly482Ser* SNP carrier status

Variables	*PGC-1a* gene *Gly482Ser* genotypes		
	Gly/Gly	*Gly/Ser*	*Ser/Ser*
Age (yrs)	43 (31–53)	41.5 (30–52)	37 (31–50)
Body Mass Index (kg/m2)	37.5 (32–44.4)	39.3 (34.6–44.8)	39.4 (33.4–45.8)
Systolic blood pressure (mmHg)	130 (120–140)	130 (120–140)	130 (115–140)
Diastolic blood pressure (mmHg)	85 (80–90)	85 (80–90)	80 (70–90)
Blood glucose 0'(mg/dl)	89 (81–97.5)	88 (80–97)	88 (80–96)
Blood glucose 120' (mg/dl)	129 (96–151.2)	123 (100–155)	116 (94–148)
Fasting plasma insulin 0' (μU/ml)	17.7 (11.6–28.2)*	21.1 (12.9–33.2)	21.8 (13.6–31.8)
Fasting plasma insulin 120' (μU/ml)	108.5 (49.6–188.1)	95.5 (52.6–185.9)	82.7 (49.1–142.2)
$HOMA_{IR}$	4 (2.2–6.3)*	4.3 (2.6–7.6)	4.97 (3.1–7.4)
HOMA % B	177 (128–229)*	198 (141–250.7)	193 (156–270.5)
Total cholesterol (mg/dl)	208.1 (185.7–237.5)	204.4 (184–232.7)	201.8 (184.2–235.4)
Total triglycerides (mg/dl)	98.3 (72.8–138.3)	99.8 (73.3–142)	95 (63.7–138.7)
HDL cholesterol (mg/dl)	51 (46–60)	51 (43.9–61.1)	53.5 (44.1–61.7)

**Gly/Gly* carriers vs. *Gly/Ser* carriers: $p < 0.01$. *Gly/Gly* carriers vs. *Ser/Ser* carriers: $p < 0.01$ Logarithmic transformation was used to normalize the distributions of BMI, blood pressure, $HOMA_{IR}$, HOMA %B, plasma insulin, total and HDL-cholesterol and triglycerides. Data are given as median and the interquartile range. Continuous variables were compared by ANOVA and categorical variables by χ^2 test.

At variance with these results is the only negative study published so far [16], where no association was found in German and Dutch populations. As mentioned before, differences in ethnicity, in case selection, or in the clinical parameters under study are all possible explanations for this discrepancy It should be pointed out that our population included only obese subjects, and obesity may be a necessary cofactor for the *Gly482Ser* SNP of the *PGC-1*α gene to exert its effects on insulin sensitivity. In favour of this hypothesis is the study by Esterbauer and co-workers [3], where a significant association between the *Gly482Ser* variant and obesity -related indices in women has been reported.

Muller and co-workers have also shown association of the *Gly482Ser* variant with decreased early insulin release [10], and explained this observation with the hypothesis that this variant, by increasing free fatty acids and consequently inducing lipotoxicity, determines a reduction in beta-cell insulin secretion. We were not able to replicate this finding in the multiple regression analysis, where the association of the *PGC-1*α gene variant resulted not independent from other determinants of β-cell function. Nevertheless, our HOMA %B index may be less sensitive than the index used by Muller et al. [10], and further studies are needed to find conclusive evidences. In our study, however, non-carriers of the *Gly482Ser* variant showed a lower β-cell function than carriers, which were probably hyperinsulinemic to compensate the higher insulin-resistance.

The mechanism by which the *PGC-1*α gene *Gly482Ser* missense mutation may affect insulin sensitivity is not clearly understood. To our knowledge no functional studies have been performed with this variant. It could be speculated that defects in *PGC-1*α gene expression or activity may negatively influence any of the metabolic steps in which this gene is involved, such as hepatic gluconeogenesis, glucose transport in the muscle and thermogenesis [13]. Defects in any of these processes could then contribute to determine insulin-resistance.

One limitation of our study, but also a possible point of strength, is that the *Gly482Ser* variant was investigated only in obese subjects. Obesity *per se* is a strong risk factor for insulin resistance. The evidence of a significant association in the presence of obesity may indicate that this variant has an effect that is additive to obesity, where the clinical abnormalities are already the result of a few susceptibility genes interacting with environmental factors.

In conclusion, we have found significant association between the *Gly482Ser* missense mutation of the *PGC-1*α gene and reduced insulin sensitivity in obese subjects. This association resulted independent from all other known modulators of insulin resistance, and suggests a primary role for the *PGC-1*α gene on the genetic susceptibility to insulin resistance in obesity.

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