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Gly482Ser polymorphism in the peroxisome proliferator–activated receptor γ coactivator–1α gene is associated with oxidative stress and abdominal obesity

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Abstract

The objective of the study was to o investigate the relationship of the Gly482Ser (G482S) polymorphism in the peroxisome proliferator–activated receptor γ coactivator–1α (*PPARGC1A*) gene and type 2 diabetes mellitus (T2DM), obesity, and oxidative status in Chinese adults. We enrolled 276 T2DM patients and 1049 nondiabetic subjects aged at least 35 years. The G482S variant was detected using polymerase chain reaction and restriction enzyme digestion. The levels of thiobarbituric acid reactive substance, an indicator of lipid peroxidation, were measured in plasma samples. The homeostasis model assessment–estimated insulin resistance (HOMA-IR) index was determined for nondiabetic subjects. *P* values were adjusted for age, sex, and body mass index by using a generalized linear model. In this series, there was no association between G482S polymorphism and T2DM and obesity (body mass index >25 kg/m^2). However, the plasma fasting insulin levels and HOMA-IR indices were significantly higher in nondiabetic subjects harboring the variant (S/S) genotype than in those with the heterozygous (G/S) genotype. With regard to the effect of the different genotypes on body fat distribution, overweight nondiabetic subjects harboring the S/S or G/S genotype had a significantly higher waist-to-hip ratio than those with the wild-type (G/G) genotype. Furthermore, subjects with the S/S genotype had significantly higher serum thiobarbituric acid reactive substance levels than those with the G/G genotype; the diabetic group mainly contributed to this significant association ($P < .001$). In overweight, nondiabetic Chinese adults, G482S polymorphism in the *PPARGC1A* gene is associated with hyperinsulinemia, HOMA-IR indices, and abdominal obesity. Furthermore, in hyperglycemia, the S482 allele is related to increased oxidative stress.

1. Introduction

Peroxisome proliferator–activated receptor γ coactivator–1α (*PPARGC1A*) is a transcriptional coactivator of the peroxisome proliferator–activated receptor (PPAR) α and γ. The *PPARGC1A* genomic region on chromosome 4p15.1 has been linked to body mass index (BMI), abdominal fat, and fasting serum insulin levels [1-3]; therefore, *PPARGC1A* could serve as an indicator of susceptibility to obesity. Previous reports showed that haplotype combinations comprising Gly482Ser (G482S) polymorphism and an A to G substitution at position +2962 in the 3′-untranslated region of *PPARGC1A* were associated with obesity indices in middle-aged Austrian white women [4]. However, this association was not observed among Asian Indians [5]. The common G482S polymorphism in *PPARGC1A* has been reported to be associated with type 2 diabetes mellitus (T2DM) in Danish and Japanese [6,7], but not in French white, [8] populations. In Pima Indians, the G482S missense

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polymorphism was shown to affect lipid metabolism, thus influencing insulin secretion; but it was not found to be associated with T2DM [9].

Recent studies involving DNA microarray analysis suggested that coordinated changes occur in the genes involved in oxidative phosphorylation and in the *PPARGC1A* gene in T2DM patients and overweight nondiabetic individuals with a family history of diabetes [10,11]. However, it is not clear if the G482S missense polymorphism in *PPARGC1A* is associated with oxidative damage and antioxidative status, which are most likely responsible for diabetic complications.

In the present study, we investigated whether the G482S variant increases susceptibility to T2DM, oxidative damage, and obesity in the Chinese population.

2. Materials and methods

A total of 1325 unrelated Taiwanese individuals of ethnic Chinese backgrounds were enrolled in this study. The study group included 276 patients from the metabolism/endocrinology clinic and 1049 subjects from our health screening center. The subjects included in this study were of Han Chinese origin and were at least 35 years old. The exclusion criteria were type 1 diabetes mellitus, maturity-onset diabetes in youth, and secondary diabetes or hypertension caused by endocrinopathy or drug use. The 1325 participants were categorized into 2 groups: Group 1 (diabetes mellitus [DM] group) consisted of 276 subjects with known past histories of T2DM who were receiving regular follow-up care in our hospital. Individuals in whom the onset of diabetes occurred before the age of 30 years were not included in our study. Group 2 (non-DM group) comprised 1049 nondiabetic subjects randomly selected from the health screening center or outpatient department. Their nondiabetic status was determined by the patient's history and a fasting plasma glucose level of less than 100 mg/dL. The non-DM subjects were further categorized into 2 subgroups (obesity/nonobesity); *obesity* was defined by a BMI greater than 25 kg/m^2. *Hypertension* was defined by a systolic blood pressure greater than 140 mm Hg and/or a diastolic blood pressure greater than 90 mm Hg and/or whether the patient was receiving antihypertensive drug therapy. The waist circumference was measured midway between the crest of the ileum and the inferior margin of the last rib in a horizontal plane, as recommended by the World Health Organization. Insulin resistance and β-cell function were assessed by homeostasis model assessment–estimated insulin resistance (HOMA-IR) and HOMA-β, respectively. Informed prior consent was provided by all participants. The studies were conducted according to the guidelines of the Declaration of Helsinki, and the study protocols were approved by the Ethics Committee of the Chang Gung Memorial Hospital.

DNA was extracted from peripheral leukocytes and amplified using an adaptation of the polymerase chain reaction (PCR) and restriction enzyme digestion. The forward PCR primer used was 5′-CAAGTCCTCAGTCCTCAC-3′, and the reverse primer was 5′-GGGGTCTTTGAGAAAATAAGG-3′. The PCR reaction mixture (50 μL) contained 20 μmol/L of each primer, 200 μmol/L of each deoxyribonucleotide triphosphate, 200 ng of DNA, and 1 unit of *Taq* DNA polymerase. Samples of total cellular DNA were subjected to 30 cycles of PCR, and the presence of the G482S variant (rs8192678) in *PPARGC1A* was determined by PCR–restriction fragment length polymorphism analysis with the enzyme *Msp*I. The PCR products were digested overnight with 1 μL of the enzyme at 37°C and electrophoresed with both positive and negative controls on a 3% agarose gel for 60 minutes at 80 V.

Levels of free thiols and thiobarbituric acid reactive substance (TBARS) in the plasma were determined using previously described methods [12]. Free thiols were determined by directly reacting thiols with 5,5-dithiobis(2-nitro benzoic acid) to form 5-thio-2-nitrobenzoic acid. The amount of thiols was calculated from the absorbance determined using the extinction coefficient of 5-thio-2-nitrobenzoic acid (A_{412} = 13 600 mol L^{-1} cm^{-1}) [13]. The concentration of TBARS was assessed using the method of Ohkawa et al [14] After centrifugation, the plasma samples were stored at −80°C for future analysis. Results are expressed as micromoles TBARS per liter. A standard curve of TBARS was obtained by the hydrolysis of 1,1,3,3-tetraethoxypropane.

Results are expressed as the mean ± standard deviation. Group comparisons were performed using 1-way analysis of variance, the Student t test, or χ^2 test. Logistic regression was used to identify the independent risk factors. The P values obtained for TBARS, free thiols, waist circumference, fasting insulin, HOMA-IR indices, and HOMA-β values among the different G482S genotypes were adjusted for age, sex, and BMI by the generalized linear model. $P <$ .05 was considered statistically significant.

3. Results

3.1. Oxidative stress and phenotypic differences among the different G482S genotypes in all subjects

Frequencies of the wild-type (G/G), heterozygous (G/S), and variant (S/S) genotypes at the G482S site were 32.5% (n = 431), 47.6% (n = 631), and 19.8% (n = 263), respectively. Comparisons among the different genotypes are shown in Table 1. Subjects harboring the S/S genotype had a significantly higher serum levels of TBARS, a well-known secondary product of lipid peroxidation and an indicator of oxidative damage, than those with the G/G genotype (P = .044). This result was consistent even after adjusting for age, sex, and BMI (P = .043). In a model assuming dominant transmission, subjects harboring the 482S allele (G/S + S/S) had a significantly higher serum TBARS level than those without this allele (G/G) (P = .037 and P = .029 after adjusting for age, sex, and BMI). The

Table 1
Comparisons between the different Gly482Ser genotypes

	G/G (n = 431)	G/S (n = 631)	S/S (n = 263)	*P* (*P*1)	*P* (*P*1): S/S + G/S vs G/G
Age (y)	56.1 ± 10.4	55.3 ± 10.6	55.7 ± 10.7	NS (NS)	NS (NS)
Sex (% female)	44.1	44.2	44.1	NS (NS)	NS (NS)
DM (%)	21.1 (91/431)	20.4 (129/631)	21.3 (56/263)	NS (NS)	NS (NS)
TBARS (μmol/L)	1.31 ± 0.72[a]	1.40 ± 0.76	1.43 ± 0.82[a]	.044[a] (.043)	.037 (.029)
Thiol (μmol/L)	1.92 ± 0.47	1.94 ± 0.44[b]	1.87 ± 0.52[b]	.033[b](.041)	NS (NS)
BMI (kg/m^2)	24.9 ± 3.5	25.0 ± 3.5	25.2 ± 3.6	NS (NS)	NS (NS)
Waist (cm)	85.2 ± 9.7	85.5 ± 10.5	86.0 ± 10.4	NS (NS)	NS (NS)
WHR	0.90 ± 0.07	0.90 ± 0.08	0.91 ± 0.08	NS (NS)	NS (NS)

*P*1 indicates *P* value adjusted for age, sex, and BMI; NS, not significant.
[a] *P* value between S/S and G/G.
[b] *P* value between S/S and G/S.

antioxidant status, which was determined by the levels of free thiols in the plasma, was significantly lower in subjects with the S/S genotype than in those with the G/S genotype. ($P = .033$ and $P = .041$ after adjusting for age, sex, and BMI). However, there were no differences with regard to BMI and frequencies of T2DM among the different genotypes.

3.2. Differences in subgroups with or without diabetes

With regard to the differences between the diabetic and nondiabetic groups, subjects with diabetes had a higher BMI ($P < .001$), waist-to-hip ratio (WHR) ($P < .001$), and plasma levels of TBARS ($P = .001$) but had lower levels of free thiols ($P < .001$), indicating increased oxidative stress and a reduction of antioxidant capacity. However, the frequency of the 482S allele did not differ between subjects with or without diabetes (Table 2).

The differences in oxidative stress and metabolic disorders among the different genotypes within the DM and non-DM groups are shown in Table 2. In the DM group, subjects with the S/S genotype had significantly higher serum TBARS levels than those with the G/S or G/G genotype ($P = .023$ and $P = .001$, and $P = .019$ and $P < .001$ after adjusting for age, sex, and BMI, respectively). In a model assuming dominant transmission, carriers of the 482S allele (G/S + S/S) had significantly higher serum TBARS levels than those with the G/G genotype ($P = .004$ and $P = .009$ after adjusting for age, sex, and BMI). In response to higher oxidative stress, subjects harboring the S/S genotype had significantly lower levels of free thiols in serum than those with the G/G genotype ($P = .042$ after adjusting for age, sex, and BMI). However, there was no significant difference in serum TBARS and thiol levels among the different genotypes in the non-DM group.

3.3. The effect of obesity on metabolic phenotypes among the different genotypes

To investigate the impact of different genotypes on obesity (BMI >25 kg/m^2), we analyzed the WHRs and HOMA-IR indices only in the non-DM group, as the treatments and control status of diabetes may affect the HOMA-IR indices and obesity status. The comparisons among the different G482S genotypes in the non-DM group, with or without obesity, are shown in Table 3. Obese subjects harboring the S/S and G/S genotypes had significantly higher WHRs than those with the G/G genotype ($P = .017$ and $P = .025$ after adjusting for age, sex, and BMI, respectively). This association was dominant in carriers of the 482S allele (S/S + G/S vs G/G; $P = .009$ after adjusting

Table 2
Comparisons between carriers and noncarriers of the S482 allele in the DM and non-DM groups

	DM (n = 276)				non-DM (n = 1049)				*P* (*P*1)
	G/G (n = 91)	G/S (n = 129)	S/S (n = 56)	*P* (*P*1)	G/G (n = 340)	G/S (n = 502)	S/S (n = 207)	*P* (*P*1)	Group
Age (y)	60.9 ± 9.2	60.3 ± 8.8	59.6 ± 7.3	NS (NS)	54.8 ± 10.3	54.0 ± 10.7	54.6 ± 11.3	NS (NS)	<.001 (<.001)
Sex (% female)	47.3	42.6	51.8	NS (NS) .001[a] (<.001)	43.2	44.6	42.0	NS (NS)	NS (NS)
TBARS (μmol/L)	1.33 ± 0.63[a]	1.51 ± 0.78[b]	1.79 ± 0.92[ab]	.023[b] (.019)	1.31 ± 0.74	1.37 ± 0.74	1.34 ± 0.77	NS (NS)	.001 (.045)
Thiol (μmol/L)	1.88 ± 0.47[a]	1.84 ± 0.44	1.72 ± 0.63[a]	.052[a] (.042)	1.94 ± 0.47	1.97 ± 0.44	1.91 ± 0.47	NS (NS)	<.001 (.056)
BMI (kg/m^2)	25.6 ± 3.6	26.4 ± 3.5	26.2 ± 4.2	NS (NS)	24.7 ± 3.5	24.7 ± 3.4	24.9 ± 3.4	NS (NS)	<.001 (<.001)
WHR	0.923 ± 0.065	0.921 ± 0.068	0.913 ± 0.071	NS (NS)	0.897 ± 0.072	0.901 ± 0.077	0.905 ± 0.082	NS (NS)	<.001 (.050)
Insulin (μU/mL)					14.0 ± 8.5	13.1 ± 7.2	14.2 ± 9.1	NS (NS)	
HOMA-IR					3.32 ± 2.4	3.07 ± 2.0	3.35 ± 2.3	NS (NS)	

Group: DM vs non-DM group.
[a] *P* value between S/S and G/G.
[b] *P* value between S/S and G/S.

Table 3
Comparisons among different Gly482Ser genotypes in nondiabetic subjects with or without obesity

Obesity (BMI >25 kg/m^2)	G/G (n = 155)	G/S (n = 217)	S/S (n = 95)	*P* (*P*1)	*P* (*P*1): S/S + G/S vs G/G
Age (y)	55.9 ± 10.1	55.2 ± 10.4	56.3 ± 10.5	NS (NS)	NS (NS)
Sex (% female)	38.7	35.9	37.9	NS (NS)	NS (NS)
TBARS (μmol/L)	1.40 ± 0.75	1.46 ± 0.71	1.36 ± 0.83	NS (NS)	NS (NS)
Thiol (μmol/L)	1.94 ± 0.49	1.90 ± 0.43	1.90 ± 0.49	NS (NS)	NS (NS)
BMI (kg/m^2)	27.7 ± 2.5	27.8 ± 2.3	27.9 ± 2.4	NS (NS)	NS (NS)
WHR	0.915 ± 0.07[a]	0.931 ± 0.07[b]	0.936 ± 0.08[ab]	.029[a], .035[b] (.017[a], .025[b])	.014 (.009)
Insulin (μU/mL)	16.3 ± 9.8	15.5 ± 7.9[b]	18.2 ± 11.0[b]	.019[b] (.013)	NS (NS)
HOMA-IR	3.91 ± 2.92	3.68 ± 2.32[b]	4.34 ± 2.84[b]	.046[b] (.043)	NS (NS)
Nonobesity (BMI <25 kg/m^2)	G/G (n = 185)	G/S (n = 285)	S/S (n = 112)	*P* (*P*1)	*P* (*P*1): S/S + G/S vs G/G
Age (y)	53.8 ± 10.5	53.1 ± 10.8	53.2 ± 11.8	NS (NS)	NS (NS)
Sex (% female)	47.0	51.2	45.5	NS (NS)	NS (NS)
TBARS (μmol/L)	1.24 ± 0.72	1.30 ± 0.76	1.31 ± 0.71	NS (NS)	.038 (NS)
Thiol (μmol/L)	1.93 ± 0.45[c]	2.02 ± 0.43[bc]	1.92 ± 0.47[b]	.032[b], .044[c] (.043, .050)	NS (NS)
BMI (kg/m^2)	22.2 ± 1.8	22.4 ± 2.0	22.4 ± 1.7	NS (NS)	NS (NS)
WHR	0.881 ± 0.074	0.878 ± 0.075	0.879 ± 0.075	NS (NS)	NS (NS)
Insulin (μU/mL)	12.1 ± 6.6	11.3 ± 6.0	10.9 ± 5.3	NS (NS)	NS (NS)
HOMA-IR	2.81 ± 1.7	2.59 ± 1.5	2.53 ± 1.4	NS (NS)	NS (NS)

[a] *P* value between S/S and G/G.
[b] *P* value between S/S and G/S.
[c] *P* value between G/G and G/S.

for age, sex, and BMI). The plasma fasting insulin levels and HOMA-IR indices were significantly higher in subjects harboring the S/S genotype than in those with the G/S genotype ($P = .019$ and $P = .046$, respectively); and these results were consistent even after adjusting for age, sex, and BMI ($P = .013$ and $P = .043$, respectively). Furthermore, obese subjects had higher plasma levels of TBARS ($P = .003$ and $P = .034$ after adjusting for age, sex, and BMI), fasting plasma insulin ($P < .001$), and HOMA-IR indices ($P < .001$) than nonobese ones. However, there were no significant differences in the plasma insulin levels or HOMA-IR indices among the different G482S genotypes in nonobese subjects; and there were no differences in the serum TBARS and thiol levels among the different genotypes in the obese and nonobese individuals.

4. Discussion

Multiple lines of in vitro evidence support the hypothesis that the expression of *PPARGC1A* plays a crucial role in protecting the mitochondria from oxidative stress through reduced accumulation of reactive oxygen species (ROSs) and reduced apoptotic cell death [15,16]. Mootha and colleagues [10] identified coordinated changes that occur in genes involved in oxidative phosphorylation and in the *PPARGC1A* gene in T2DM patients and their first-degree relatives. These results prompted us to examine the relationship between G482S polymorphism and oxidative stress and metabolic disorders. In the present study, we report an association between the S allele of the G482S polymorphism in the *PPARGC1A* gene and oxidative stress in all subjects and T2DM patients, although the main effect was observed in the DM group. These associations were independent of age, sex, and BMI.

In diabetic patients, high blood glucose increases the metabolic flux in the mitochondria along with the reduction of nicotinamide adenine dinucleotide to nicotinamide adenine dinucleotide hydrogen and may result in increased ROS formation. Malondialdehyde is a well-known secondary product of lipid peroxidation and was measured in this study as TBARS, an indicator of oxidative damage. In our study, diabetic subjects harboring the S allele had significantly higher concentrations of TBARS ($P = .009$ after adjusting for age, sex, and BMI). This implies that the oxidative damage due to hyperglycemia was more severe in subjects harboring the S allele. On the other hand, similar plasma levels of TBARS were observed in both diabetic and nondiabetic subjects who harbored the G/G genotype (1.33 ± 0.63 vs 1.31 ± 0.74 μmol/L), indicating that the G/G genotype probably confers protection against oxidative damage.

Organisms have antioxidative defense systems to protect themselves from the adverse effects of increased oxidative damage. In our study, this response was assessed by measuring the levels of total free thiols in the plasma. In diabetic subjects harboring the S allele, it is expected that the level of thiols would increase to compensate for the increased oxidative stress; however, these subjects had significantly lower plasma levels of thiols. This result suggests that subjects with the S allele may not be able to respond appropriately to oxidative damage.

Some previous reports support these hypotheses. Cell models have provided evidence that *PPARGC1A* plays a crucial role in regulating ROS-detoxifying enzymes, including MnSOD, UCP-2, and GPx1. The overexpression of *PPARGC1A* reduced the accumulation of ROSs, preventing

endothelial dysfunction and apoptotic cell death in response to high-glucose–induced oxidative stress [16]. *PPARGC1A*-null mice are more sensitive to the neurodegenerative effects of oxidative stressors, and an increase in the levels of *PPARGC1A* offers neural cells considerable protection from oxidative stress–mediated death. Lower *PPARGC1A* messenger RNA levels have been reported in subjects harboring the S allele as compared with homozygous individuals [17]. The frequencies of the S allele were found to be similar in diabetic and nondiabetic subjects, which is in agreement with results of studies on French white and Pima Indian cohorts but was in contrast to the findings of Danish white and Japanese cohort studies [6-9]. Although the frequency of diabetes in subjects with the S/S genotype was not high, these subjects may be at a higher risk for diabetic complications due to increased oxidative stress. Further studies to determine the association between the S allele and vascular complications of diabetes would provide valuable insights.

PPARGC1A is associated with thermogenesis and energy homeostasis through the activities of the coactivator effectors PPAR-α and PPAR-γ [18,19]. In this study, we analyzed the WHR in the non-DM group, as the treatments and control status of diabetes may affect the obesity status. We found an association between subjects harboring the S482 allele and increased WHR, which was augmented because of obesity (BMI >25 kg/m^2). This result was consistent, even after adjusting for age, sex, and BMI. However, this association was not found to be significant in the nonobese subjects (BMI <25 kg/m^2). Furthermore, obese nondiabetic subjects harboring the S/S genotype may have a higher risk of developing diabetes because they have higher fasting insulin levels, HOMA-IR indices, and WHRs. This hypothesis is supported by previous reports on the association between the *PPARGC1A* S482 allele and hindered nonesterified fatty acid clearance in obese individuals but not in lean individuals [20]. Furthermore, the expression of *PPARGC1A* was reported to be reduced in the adipose tissue of obese subjects [21] and in the muscles of obese mice [22]. On the basis of these observations and data from previous studies, we hypothesize that subjects harboring the S482 allele tend to have abdominal obesity under the excessive energy intake, which may be related to reduced *PPARGC1A* expression.

In conclusion, in Chinese adults, G482S polymorphisms in the *PPARGC1A* gene are associated with hyperinsulinemia, HOMA-IR indices, and abdominal obesity in overweight nondiabetic subjects. Furthermore, in hyperglycemia, the S482 allele will enhance oxidative stress.

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