Clinical Chemistry 51:2
416–423 (2005)

Endocrinology and Metabolism

Effect of Adiponectin Gene Polymorphisms on Circulating Adiponectin and Insulin Resistance Indexes in Women with Polycystic Ovary Syndrome

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Background: **We examined the possible association of adiponectin gene polymorphisms with polycystic ovary syndrome (PCOS) and their influence on serum adiponectin and insulin resistance indexes in Greek women with PCOS.**

Methods: **We genotyped samples from 100 women with PCOS characterized with respect to body mass index (BMI), glucose and insulin concentrations during an oral glucose tolerance test (OGTT), lipid profile, and serum adiponectin concentrations and from 140 healthy controls for the 45T>G and 276G>T polymorphisms in the adiponectin gene.**

Results: **The distributions of genotypes and alleles of both polymorphisms were no different in women with PCOS and controls, indicating that the individual polymorphisms are not associated with increased risk for PCOS. However, the two polymorphisms were found to be associated with insulin resistance indexes among women with PCOS and to influence adiponectin production. In particular, carriers of the TG genotype at position +45 had greater hyperinsulinemia, as estimated by the area under the curve for insulin ($AUC_{insulin}$) during the OGTT, than those with the TT genotype ($P < 0.05$), and this was independent of age and BMI. In addition, women with PCOS with the GG or GT genotypes at position +276 had a higher BMI ($P = 0.01$) and greater $AUC_{insulin}$ ($P = 0.01$) than carriers of the TT genotype. The latter genotype was found less frequently among overweight/obese women with PCOS than in normal-weight individuals ($P = 0.002$). In addition, the presence of the GG or GT genotype was associated with lower serum adiponectin than the TT genotype, independent of age, BMI, and insulin concentrations ($P = 0.03$). Serum adiponectin was negatively correlated with serum triglycerides and insulin resistance indexes and positively with HDL-cholesterol.**

Conclusions: **Adiponectin gene polymorphisms at positions +45 and +276 are not associated with PCOS. However, these genomic variants may influence production of adiponectin and the metabolic variables related to insulin resistance/metabolic syndrome in patients with PCOS.**

The polycystic ovary syndrome (PCOS)[5] is a common endocrine/metabolic disorder in women of reproductive age and has a strong genetic component *(1)*. Insulin resistance with compensatory hyperinsulinemia, central adiposity, and a metabolic profile similar to insulin resistance/metabolic syndrome are frequent metabolic abnormalities in PCOS that may further worsen the endocrine

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Received September 16, 2004; accepted November 17, 2004.
Previously published online at DOI: 10.1373/clinchem.2004.043109

[5] Nonstandard abbreviations: PCOS, polycystic ovary syndrome; BMI, body mass index; LH, luteinizing hormone; FSH, follicle-stimulating hormone; SHBG, sex hormone-binding globulin; FAI, free androgen index; OGTT, oral glucose tolerance test; AUC, area under the curve; OR, odds ratio; and CI, confidence interval.

manifestations of hyperandrogenism and ovulatory dysfunction *(2)*. Central adiposity appears to play an important role in the insulin resistance of the metabolic syndrome through dysregulated production of various adipocyte-derived cytokines and proteins (adipocytokines), including tumor necrosis factor-α, plasminogen activator inhibitor-1, leptin, resistin, and adiponectin *(3)*.

Adiponectin, a newly discovered protein, is secreted exclusively by differentiated adipocytes and circulates in abundant amounts in humans *(4)*. In contrast to other adipocytokines that are up-regulated in obesity, a paradoxic decrease in circulating adiponectin has been reported in persons with obesity, insulin resistance, and type 2 diabetes and, more recently, in obese women with PCOS *(5–8)*. Furthermore, administration of adiponectin improves insulin sensitivity in animal models of obesity, and insulin-sensitizing peroxisome proliferator-activated receptor-γ agonists increase adiponectin concentrations in humans with type 2 diabetes *(9, 10)*. Recent studies using knockout mice confirmed the insulin-sensitizing and antiatherogenic properties of adiponectin *(11, 12)*.

The above findings point to an important role of adiponectin in the pathophysiology of insulin resistance associated with the metabolic syndrome and related disorders, such as PCOS. Furthermore, the fact that recent genome scans have mapped a susceptibility locus for type 2 diabetes and the metabolic syndrome to chromosome 3q27, the region where the gene encoding adiponectin is located, suggests that genetic variability in the adiponectin gene may be a determinant of the phenotypic expression of the metabolic syndrome and also of PCOS *(13)*.

The adiponectin gene consists of three exons and two introns spanning a 17-kb region *(14)*. Sequence polymorphisms have been identified in humans and have been examined for their possible association with insulin resistance indexes and circulating adiponectin concentrations *(15–18)*. Most studies have focused on two polymorphisms, a silent T-to-G substitution in exon 2 (45T>G) and a G-to-T substitution in intron 2 (276G>T), that have been associated with obesity, insulin resistance, and the risk of type 2 diabetes *(19–22)*. Furthermore, these two polymorphisms were selected because of their high frequencies in all populations tested, whereas other reported polymorphisms were rare. In a recent study, the 45T>G polymorphism was also studied in women with PCOS and was related to Δ^4-androstenedione concentrations *(23)*.

The aim of the present study was twofold: (*a*) to investigate the possible association of polymorphisms at positions +45 and +276 in the adiponectin gene with the risk of PCOS; and (*b*) to examine the contribution of these two polymorphisms to insulin resistance indexes in women with PCOS.

Participants and Methods

STUDY PARTICIPANTS

The study population consisted of 100 Greek women [age range, 16–37 years; mean (SD) age, 23.7 (6.4) years] with PCOS. Diagnosis of PCOS was based on the criteria proposed by the 1990 NIH-National Institute of Child Health and Human Development conference on PCOS. These criteria are ovulatory dysfunction, clinical evidence of hyperandrogenism and/or hyperandrogenemia, and exclusion of related disorders such as congenital adrenal hyperplasia, hyperprolactinemia, or Cushing syndrome *(24)*. Hyperandrogenism was defined by the clinical presence of hirsutism (Ferriman–Gallwey score >8), acne or alopecia, and/or increased androgen concentrations. Menstrual dysfunction was defined by the presence of oligomenorrhea or amenorrhea. In those patients who were on medication, treatment was discontinued at least 6 months before their inclusion in the study. Women with PCOS were further divided in two subgroups based on their body mass index (BMI) values. Group 1 consisted of 33 normal-weight (BMI <25 kg/m^2) women, and group 2 consisted of 67 overweight/obese women (BMI ≥25 kg/m^2) with PCOS. A third group of 140 healthy normal-weight women with regular menstrual cycles (28–30 days) and no signs of hyperandrogenism were also used as controls for the distribution of the various adiponectin genotypes. The control group consisted of medical school students and staff of our hospital [mean (SD) age, 24.8 (6.9) years].

All women with PCOS were studied in the early follicular phase (days 3–5) of a spontaneous or progestin-induced menstrual cycle. The BMI of each patient was calculated as weight (kg)/height (m)^2. Blood samples were drawn after overnight fasting for the measurement of fasting serum glucose and insulin, lipid profile, serum gonadotropins [luteinizing hormone (LH) and follicle-stimulating hormone (FSH)], total testosterone, and sex hormone-binding globulin (SHBG). The free androgen index (FAI) was calculated using the formula: [total testosterone (nmol/L)/SHBG (nmol/L)] × 100.

All patients underwent a 75-g oral glucose tolerance test (OGTT). Blood was sampled for serum glucose and insulin concentrations before and at 30, 60, 90, and 120 min after glucose load. The fasting glucose-to-insulin ratio was estimated. The glucose and insulin responses to the OGTT were analyzed by calculating the area under the curve (AUC). The AUCs for glucose ($AUC_{glucose}$) and insulin ($AUC_{insulin}$) were determined according to the Tai procedure for the metabolic curves *(25)*. Adiponectin concentrations were also measured in women with PCOS after overnight fasting.

The study protocol was approved by the Hospital Ethics Committee, and all women studied gave informed consent.

HORMONE ASSAYS

Serum glucose was measured by the hexokinase method on a glucose analyzer (Olympus 600 Clinical Chemistry Analyzer; Olympus Diagnostica GmbH). Insulin was measured by a microparticle enzyme immunoassay on an AxSYM Immunoanalyzer (Abbott Laboratories). The CV

of this method was 5%. Total testosterone and serum gonadotropins (LH and FSH) were measured by chemiluminescent microparticle immunoassays on an Abbott-ARCHITECT Immunoanalyzer (Abbott Laboratories). The CVs were 4% for total testosterone, 3.5% for LH, and 4% for FSH. SHBG was measured by a chemiluminescent immunometric method (IMMULITE 2000 Immunoanalyzer; DPC), and the CV was 5.5%. Total cholesterol, HDL-cholesterol, and triglycerides were measured by enzymatic methods (Olympus 600 Clinical Chemistry Analyzer). LDL-cholesterol was calculated by the Friedewald equation [LDL = total cholesterol (mg/L) − HDL-cholesterol (mg/L) − triglycerides (mg/L)/5 *(26)*]. Serum adiponectin was measured by a sensitive ELISA (R&D Systems Inc.). The intraassay CVs ranged from 2.5% to 4.7%, and the interassay CVs ranged from 5.8% to 6.5%.

GENOTYPE ANALYSIS

Genomic DNA was isolated from peripheral blood leukocytes of women with PCOS and the controls. The adiponectin 45T>G polymorphism was genotyped by amplification of genomic DNA using the following primers: forward, 5′-GAAGTAGACTCTGCTGAGATGG-3′; reverse, 5′-TATCAGTGTAGGAGGTCTGTGATG-3′. The product was digested with *Sma*I (New England BioLabs Inc.), and the digestion products were resolved by electrophoresis in a 2% agarose gel.

The adiponectin 276G>T polymorphism was genotyped by amplification of genomic DNA using the following primers: forward, 5′-GGCCTCTTTCATCACAGACC-3′; reverse, 5′-AGATGCAGCAAAGCCAAAGT-3′. The product was digested with *Bsm*I (New England BioLabs), and the digestion products were resolved by electrophoresis in a 2% agarose gel.

STATISTICAL ANALYSES

Genotype and allele frequencies were compared among study groups by use of the χ^2 test. Hardy–Weinberg equilibrium for each polymorphism was also tested, comparing the observed genotype frequencies with those expected (χ^2 test). For the genotypes present in statistically significant different frequencies, the odds ratios (ORs) and 95% confidence intervals (CIs) were also estimated.

Gaussian distribution of continuous variables was tested by the Kolmogorov–Smirnov test. Logarithmic transformations were applied to insulin, triglyceride, and adiponectin concentrations to ensure gaussian distribution of these variables, and the values presented were back-transformed. Biochemical differences between two continuous variables were estimated with the Mann–Whitney *U*-test or *t*-test as appropriate. Analysis of covariance was also performed with age, BMI, and insulin resistance indexes. Simple and partial Pearson correlations were used to establish associations between adiponectin concentrations and features of PCOS alone and after adjustment for age and BMI. Continuous variables are expressed as the mean (SD). $P < 0.05$ was set as statistically significant. All analyses were performed with the Statistica Software Package (Ver. 5.1; Statsoft Inc.).

Results

ASSOCIATION BETWEEN ADIPONECTIN GENE POLYMORPHISMS AND PCOS

The clinical and endocrine characteristics of PCOS women are presented on Table 1. The genotype distributions of the 45T>G and 276G>T polymorphisms in the adiponectin gene were in Hardy–Weinberg equilibrium ($P > 0.05$) in both the PCOS and control groups, and the two polymorphisms were in linkage disequilibrium ($\chi^2 = 20.2$; $P < 0.001$; $D' = 0.732$). Overall, there was no statistically significant difference in the distributions of genotypes and alleles for both polymorphisms between PCOS women and controls, indicating that the individual polymorphisms at position +45 and +276 were not associated with increased risk for PCOS (Table 2).

METABOLIC PROFILE AND ADIPONECTIN CONCENTRATIONS IN PCOS WOMEN ACCORDING TO BMI

The biochemical and metabolic markers related to hyperandrogenism, insulin resistance, the lipid profile, and adiponectin concentrations according to BMI (group 1, BMI <25 kg/m^2; group 2, BMI ≥ 25 kg/m^2) are compared in Table 3. As expected, overweight/obese women had higher rates of hyperandrogenemia, as indicated by the FAI, and higher values for markers of insulin resistance, as indicated by fasting and post-glucose load hyperinsulinemia, than normal-weight PCOS women. This PCOS subgroup also had higher serum triglyceride concentrations and lower HDL-cholesterol concentrations than normal-weight women, but there were no differences in total cholesterol and LDL-cholesterol concentrations. In addition, overweight/obese women with PCOS had lower serum adiponectin concentrations than did normal-weight women ($P = 0.004$).

Table 1. Anthropometric and endocrine data of women with PCOS.[a]

Number	100
Age, years	23.7 (6.4)
BMI, kg/m^2	29.5 (7.7)
FAI	14.3 (13.3)
Fasting glucose/insulin ratio	9.6 (8.3)
$AUC_{glucose}$	14 803 (3142)
$AUC_{insulin}$	11 345 (9836)
Total cholesterol, mg/L	1781 (349)
Triglycerides, mg/L	922 (600)
HDL-cholesterol, mg/L	458 (144)
LDL-cholesterol, mg/L	1597 (302)
Adiponectin, mg/L	11.4 (5.2)

[a] Values are the mean (SD).

Table 2. Genotype and allele frequencies of 45T>G and 276G>T polymorphisms of the adiponectin gene in women with PCOS and controls.[a]

Polymorphism	PCOS (n = 100)	Controls (n = 140)
45T>G		
Genotypes, n (%)		
TT	77 (77)	106 (75.7)
TG	23 (23)	30 (21.4)
GG	0	4 (2.9)
Alleles, n (%)		
T	177 (88.5)	242 (86.4)
G	23 (11.5)	38 (13.6)
276G>T		
Genotypes, n (%)		
GG	39 (39)	52 (37.2)
GT	49 (49)	73 (52.1)
TT	12 (12)	15 (10.7)
Alleles, n (%)		
G	127 (63.5)	177 (63.2)
T	73 (36.5)	103 (36.8)

[a] There were no statistical differences in genotype and allele distributions among the two study groups.

DISTRIBUTION OF ADIPONECTIN GENE POLYMORPHISMS IN PCOS WOMEN ACCORDING TO BMI

The genotype and allele frequencies of adiponectin polymorphisms at positions +45 and +276 in the two subgroups of PCOS women are shown in Table 4. The homozygous TT genotype and T allele at position +45 were less frequent, and the TG genotype and G allele were more frequent among overweight/obese PCOS women than in normal-weight women, but the differences were not statistically significant. However, distribution of the genotypes at position +276 was significantly different between the two subgroups. Thus, the homozygous TT genotype of the 276G>T polymorphism was less frequent in the overweight/obese group ($P = 0.002$; OR = 8; 95% CI, 1.99–32.06), and the GT genotype was more frequent in this group ($P = 0.003$; OR = 0.25; 95% CI, 0.10–0.63) than in normal-weight PCOS women.

Table 3. Anthropometric and metabolic profiles in normal-weight (group 1) and overweight/obese (group 2) women with PCOS.[a]

	Group 1	Group 2	*P*
Number of women	33	67	
Age, years	20.8 (4.0)	25.2 (6.8)	0.001
BMI, kg/m^2	21.9 (1.7)	33.3 (6.7)	<0.001
LH/FSH	1.4 (0.8)	1.3 (1.1)	NS[b]
SHBG, nmol/L	36.1 (11.5)	31.8 (20.3)	0.01
Total testosterone, μg/L	0.97 (0.53)	1.04 (0.45)	NS
FAI	10.6 (7.6)	16.2 (15.0)	0.03
Fasting glucose, mg/L	881 (57)	931 (85)	0.003
Fasting insulin,[c] mIU/L	83 (41)	189 (122)	<0.001
Fasting glucose/insulin ratio[c]	14.73 (10.65)	7.23 (5.7)	<0.001
$AUC_{glucose}$	13 320 (2326)	15 556 (3248)	<0.001
$AUC_{insulin}$[c]	7267 (3809)	13 384 (11 234)	<0.001
Total cholesterol, mg/L	1706 (313)	1815 (361)	NS
Triglycerides,[c] mg/L	615 (208)	1059 (666)	<0.001
HDL-cholesterol, mg/L	511 (106)	433 (152)	<0.001
LDL-cholesterol, mg/L	1583 (298)	1603 (306)	NS
Adiponectin,[c] mg/L	12.7 (4.7)	10.2 (5.5)	0.004

[a] Values are the mean (SD).
[b] NS, not significant.
[c] Significance was tested on log-transformed values.

EFFECT OF ADIPONECTIN GENE POLYMORPHISMS ON INSULIN RESISTANCE INDEXES AND SERUM ADIPONECTIN IN WOMEN WITH PCOS

The clinical and metabolic characteristics of the PCOS women according to genotypes at positions +45 and +276 are shown in Table 5. With regard to the 45T>G polymorphism, there were no differences in BMI, hormone concentrations, and lipid profiles between the different genotypes. However, in PCOS women with the TG genotype compared with those with the TT genotype, insulin concentrations were higher at 90 min ($P < 0.003$) and 120 min ($P < 0.03$) after glucose load, whereas the basal insulin concentrations and $AUC_{glucose}$ were similar. As a result, women with the TG genotype had significantly higher $AUC_{insulin}$ values than women with the TT genotype (Fig. 1). These associations remained significant after adjustment for the confounding factors BMI, age, and $AUC_{glucose}$ ($P = 0.003$ for insulin concentrations at 90 min; $P < 0.05$ for insulin concentrations at 120 min; $P = 0.05$ for $AUC_{insulin}$). Overall, serum adiponectin concentrations tended to be lower in the women with TG genotype, the group with higher insulin resistance, but the difference did not reach statistical significance (Fig. 2).

Regarding the 276G>T polymorphism, patients homozygous and heterozygous for the G allele (GG and GT) were grouped together because they had similar metabolic profiles. Women with these genotypes had a higher BMI ($P = 0.01$), $AUC_{glucose}$ ($P = 0.005$), and $AUC_{insulin}$ ($P = 0.01$), and there was a tendency for higher triglyceride concentrations ($P = 0.07$) than in women with the TT genotype. In addition, serum adiponectin concentrations were significantly lower in carriers of the GG or GT genotype than in TT homozygous women, and the difference remained even after controlling for age, BMI, and insulin concentrations ($P = 0.03$; Table 5 and Figs. 1 and 2).

RELATIONSHIP BETWEEN SERUM ADIPONECTIN AND METABOLIC VARIABLES IN PCOS WOMEN

There was an inverse correlation between adiponectin concentrations and BMI ($r = -0.354$; $P = 0.002$), fasting glucose-to-insulin ratio ($r = -0.364$; $P = 0.002$), $AUC_{glucose}$ ($r = -0.355$; $P = 0.01$), fasting insulin ($r = -0.397$; $P = 0.001$), $AUC_{insulin}$ ($r = -0.337$; $P = 0.03$), and triglyceride concentrations ($r = -0.452$; $P < 0.001$), and a positive relationship with SHBG ($r = 0.246$; $P = 0.03$) and HDL-

Table 4. Genotype and allele frequencies of 45T>G and 276G>T polymorphisms in the two PCOS subgroups.

Polymorphism	Group 1 (n = 33)	Group 2 (n = 67)	*P*	OR (95% CI)
45T>G				
Genotypes, n (%)				
TT	28 (84.8)	49 (73.1)	NS[b]	
TG	5 (15.2)	18 (26.9)	NS	
GG	0	0	NS	
Alleles, n (%)				
T	61 (92.4)	116 (86.6)	NS	
G	5 (7.6)	18 (13.4)	NS	
276G>T				
Genotypes, n (%)				
GG	15 (45.4)	24 (35.8)	NS	
GT	9 (27.3)	40 (59.7)	0.003	0.25 (0.10–0.63)
TT	9 (27.3)	3 (4.5)	0.002	8 (1.99–32.06)
Alleles, n (%)				
G	39 (59.1)	88 (65.7)	NS	
T	27 (40.9)	46 (34.3)	NS	

[a] Group 1, normal-weight patients; group 2, overweight/obese patients.
[b] NS, not significant.

cholesterol ($r = 0.335$; $P = 0.008$). There was no correlation between adiponectin and total cholesterol and LDL-cholesterol, whereas there was a negative correlation of borderline significance with FAI ($r = -0.209$; $P = 0.06$). These correlations, with the exception of $AUC_{glucose}$ and SHBG, remained significant even after adjustment for age and BMI.

Discussion

In the present study we assessed whether adiponectin gene polymorphisms at positions +45 and +276 are associated with the risk for PCOS among Greek women and whether the two polymorphisms are associated with variations in serum adiponectin concentrations and insulin resistance indexes in a cohort of PCOS women.

Although the individual 45T>G and 276G>T adiponectin polymorphisms were not associated with increased risk for PCOS, this is the first study that evaluates the influence of these two polymorphisms on metabolic variables of PCOS, and they were found to be related to insulin resistance indexes and obesity among women with PCOS. Furthermore, these genomic variants were associated with circulating adiponectin concentrations, suggesting a role of adiponectin in the metabolic phenotype of PCOS.

More precisely, carriers of the TG genotype at position +45 were more insulin resistant as judged by the greater post-glucose load hyperinsulinemia compared with the women with the TT genotype, whereas there were no differences in glucose concentrations between the two

Table 5. Clinical and metabolic characteristics in women with PCOS according to adiponectin genotypes at positions +45 and +276.[a]

	45T>G polymorphism			276G>T polymorphism		
	TT genotype	TG genotype	*P*	GG + GT genotypes	TT genotype	*P*
Number of women	77	23		88	12	
Age, years	23.7 (6.4)	23.7 (6.6)	NS[b]	24.0 (6.5)	22.1 (4.9)	NS
BMI, kg/m^2	29.4 (8.0)	29.9 (6.8)	NS	30.2 (7.8)	24.6 (4.8)	0.01
FAI	15.0 (13.9)	11.7 (10.2)	NS	15.0 (13.0)	9 (4.6)	NS
Fasting glucose/insulin ratio[c]	10.1 (8.7)	8.2 (7.0)	NS	9.5 (8.5)	11.3 (6.9)	NS
$AUC_{glucose}$	14 563 (3108)	15 588 (3193)	NS	15 105 (3075)	12 492 (2492)	0.005
$AUC_{insulin}$[c]	10 095 (7478)	15 098 (14 439)	0.045	11 924 (10 134)	5846 (2950)	0.01
Total cholesterol, mg/L	1790 (353)	1751 (341)	NS	1780 (355)	1789 (310)	NS
Triglycerides,[c] mg/L	915 (655)	945 (375)	NS	955 (621)	626 (206)	0.07
HDL-cholesterol, mg/L	451 (103)	480 (233)	NS	452 (143)	511 (147)	NS
LDL-cholesterol, mg/L	1607 (296)	1562 (333)	NS	1590 (304)	1664 (282)	NS
Adiponectin,[c] mg/L	11.5 (5.5)	10.8 (3.6)	NS	10.9 (5.2)	13.6 (4.9)	0.03

[a] Values are the mean (SD).
[b] NS, not significant.
[c] Significance was tested on log-transformed values.

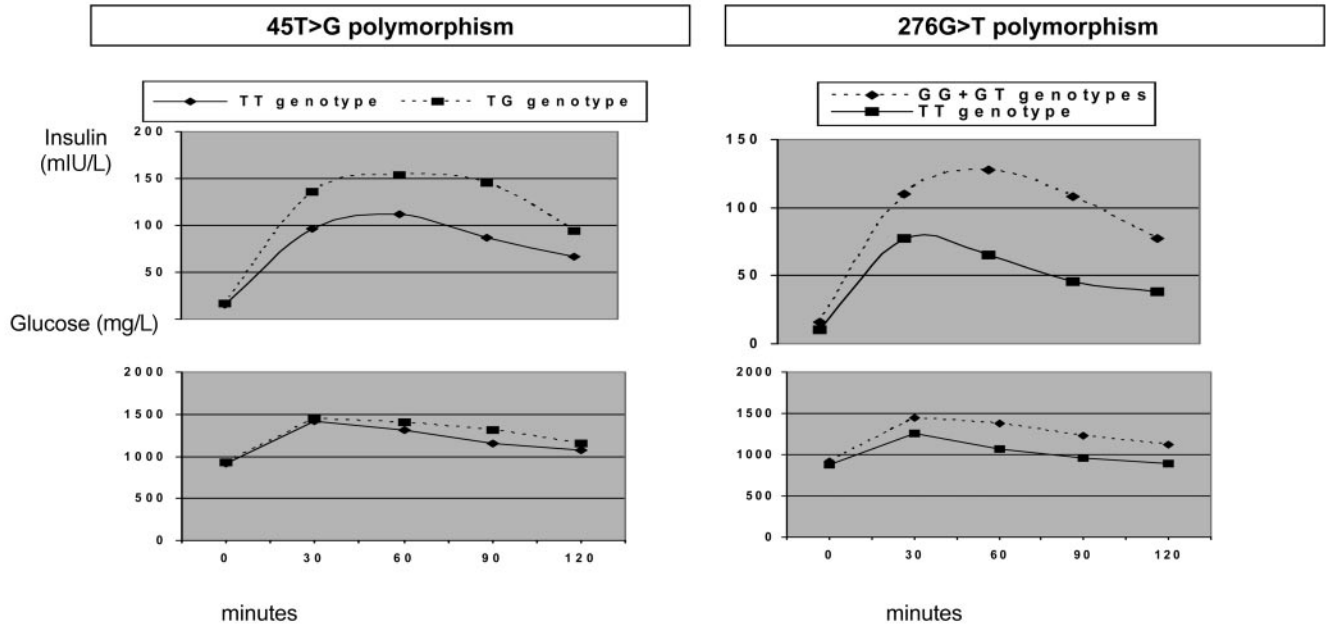

Fig. 1. Comparison of mean insulin and glucose values during OGTT in PCOS patients according to adiponectin genotype at positions +45 and +276.

The $AUC_{insulin}$ was higher ($P < 0.05$) in carriers of the TG genotype than in those with the TT genotype at position +45, whereas glucose concentrations were similar in the two genotype groups. Both $AUC_{insulin}$ and $AUC_{glucose}$ values were higher ($P = 0.01$ and 0.005 respectively) in carriers of the GG + GT genotypes than in those with the TT genotype at position +276.

genotype groups. This association was also independent of the degree of adiposity. In addition, the presence of the TG genotype was associated with lower serum adiponectin concentrations than the TT genotype, although the difference was not statistically significant, probably because of the small number of PCOS women for whom serum adiponectin values were available.

On the other hand, PCOS women with the GG or GT genotype at position +276 of the adiponectin gene were more obese and more insulin resistant than carriers of the TT genotype. The latter genotype was found less frequently among overweight/obese women with PCOS than in normal-weight women. Furthermore, the presence of the G allele, in either the homozygous or heterozygous state, was associated with lower serum adiponectin concentrations than the TT genotype, and this association was independent of age, BMI, and insulin concentrations.

It has been suggested that hypoadiponectinemia associated with obesity and the metabolic syndrome might be a consequence of increased adiposity and/or insulin resistance. The present study suggests that hypoadiponectinemia may also be a primary genetically determined defect contributing to an insulin resistance phenotype in women with PCOS. This notion is in agreement with a recent genome scan analysis that identified two major and four potential loci for serum variations in adiponectin. One of these was on chromosome 3, which harbors the adiponectin gene *(27)*.

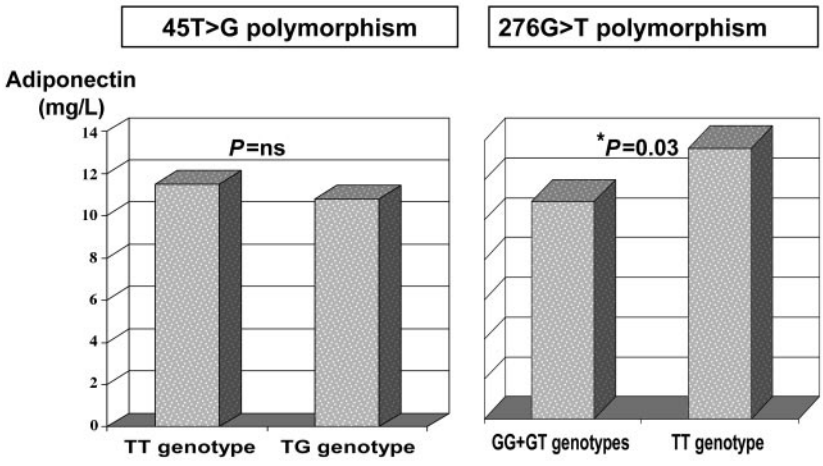

Fig. 2. Comparison of mean adiponectin concentrations according to adiponectin genotypes at positions +45 and +276.

*, the difference remained significant after controlling for age, BMI, and insulin concentrations.

The exact molecular mechanisms through which these two polymorphisms influence adiponectin gene expression or biological function related to insulin sensitivity are not known at present because the 45T>G polymorphism is a synonymous mutation and the 276G>T polymorphism is an intronic one. However, it is plausible that these polymorphisms are in linkage disequilibrium with some other functional genetic loci responsible for an alteration in production of adiponectin or for the ability of adiponectin to polymerize, which affects its biological action *(28)*.

Although these two polymorphisms are linked, they were found to be differentially associated with specific features of PCOS. This could be attributable either to the evolutionary stage of acquisition of the two polymorphisms or to their strong or weak linkage to adiponectin gene regulatory elements. Between the two polymorphisms, 276G>T probably has a stronger impact on PCOS because it is directly associated with adiponectin concentrations.

Previous studies have examined the association of these and other adiponectin gene variations with type 2 diabetes and other components of the metabolic syndrome. Both the 45T>G and 276G>T polymorphisms were associated with risk of type 2 diabetes in a Japanese study *(19)*. In a German study, the 45T>G polymorphism was associated with obesity and insulin resistance only among individuals without a family history of diabetes *(20)*. The 276G>T polymorphism was also associated with increased risk for insulin resistance, in particular among lean individuals *(21)*. In a recent Italian study, a haplotype defined by the 45T>G and 276G>T polymorphisms was significantly associated with obesity and features of insulin resistance *(22)*. However, the association of these two polymorphisms with type 2 diabetes and insulin resistance or adiponectin concentrations was not documented in other studies *(14, 15, 17)*.

Recently, San Millan et al. *(29)* investigated the potential association of PCOS with genomic variants, including 45T>G and 276G>T adiponectin gene polymorphisms, related to insulin resistance, type 2 diabetes mellitus, and obesity. In agreement with our results, they found no differences in the distributions of these two polymorphisms between women with PCOS and controls. In a previous study of a different group of Greek women with PCOS, the TG and GG genotypes of the 45T>G polymorphism were slightly more frequent in women with PCOS than in controls, and these particular genotypes were associated with higher Δ^4-androstenedione concentrations *(23)*. In the present study, we focused on insulin resistance/metabolic variables in association with both the 45T>G and 276G>T adiponectin gene polymorphisms. However, the association of the G allele with high androgen concentrations reported in the previous study *(23)* may be attributable to the fact that this particular allele is also associated with higher rates of insulinemia, as is shown in the present study. This, in turn, may exaggerate Δ^4-androstenedione production in this group of individuals with PCOS.

Finally, the present study suggests a role of hypoadiponectinemia in the pathophysiology of the metabolic and lipid abnormalities of PCOS. Women with PCOS who have low adiponectin concentrations had a higher BMI and triglyceride concentrations, higher insulin resistance indexes, and lower HDL-cholesterol and SHBG concentrations than did women with higher adiponectin concentrations. It is already known that adiponectin acts through binding to adiponectin receptors *(30)*. In skeletal muscle, adiponectin increases phosphorylation of AMP-activated kinase *(31)*. In addition, adiponectin appears to increase activity of peroxisome proliferator-activated receptor-α, leading to increased fatty acid oxidation in muscle, thereby decreasing the circulating triglycerides and intramyocellular lipid content *(9)*.

It is generally accepted that women with PCOS have increased risk for developing type 2 diabetes and coronary artery disease *(32, 33)*. Taking into consideration that individuals with low adiponectin concentrations are more likely to develop type 2 diabetes and coronary artery disease *(34, 35)* and that there may be a genetic contribution to the development of hypoadiponectinemia, perhaps adiponectin polymorphisms could be used for the stratification of PCOS patients at risk.

In conclusion, the association found between the 45T>G and 276G>T polymorphisms in the adiponectin gene and insulin resistance indexes/obesity seems to reflect general associations not unique to PCOS. This study demonstrates that the adiponectin gene does not appear to play a causative role in the development of PCOS per se but that the polymorphisms of this gene may influence the phenotypic expression of PCOS, in part through variation in adiponectin concentrations.

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