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ORIGINAL ARTICLE

The plasminogen activator inhibitor-1 (PAI-1) gene −844 A/G and −675 4G/5G promoter polymorphism significantly influences plasma PAI-1 levels in women with polycystic ovary syndrome

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Abstract Mutations in the plasminogen activator inhibitor-1 (PAI-1) gene, along with increased PAI-1 levels, have been implicated in the pathogenesis of polycystic ovarian syndrome (PCOS). We investigated a possible influence of the promoter polymorphism (−844 A/G and −675 4G/5G) in the PAI-1 gene on plasma PAI-1 levels in 126 PCOS patients and 97 healthy controls. Levels of total testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH), fasting plasma glucose (FPG), fasting insulin, and PAI-1 were measured, and body mass index (BMI), waist-to-hip ratio (WHR), LH/FSH ratio, and homeostasis model assessment for insulin resistance (HOMA-IR) were calculated. PAI-1 −675 4G/5G and −844 A/G gene polymorphisms were also performed. Total testosterone, fasting insulin, and PAI-1 levels; BMI, LH/FSH, and HOMA-IR were significantly higher in PCOS patients than controls ($P < 0.05$). The odds ratio of 4G/4G genotype, 4G allele, and the combination genotype of 4G/4G and −844 A/A were 2.49 (95% confidence interval (CI), 1.4–4.44), 2.1 (95% CI, 1.43–3.08), and 2.9 (95% CI, 1.41–5.98), respectively, ($P < 0.001$). In the PCOS group, the PAI-1 level of the A/A was significantly higher than that of the A/G or G/G genotype, similarly was 4G/4G genotype compared with 4G/5G or 5G/5G genotype. The plasma PAI-1 levels of the combination of the PAI-1 −844 A/A and −675 4G/4G or 4G/5G genotypes, or the coadunation of 4G/4G and −844 non-G/G (A/A + A/G) genotypes were significantly high in PCOS women compared with controls. A trend to a positive interaction between PAI-1 −675 4G/5G and −844 A/G gene polymorphism may elevate plasma PAI-1 levels and hypofibrinolysis, which is probably an important hereditary risk factor in PCOS.

Keywords Polycystic ovarian syndrome · Plasminogen activator inhibitor-1 · Gene polymorphism

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Introduction

Polycystic ovarian syndrome (PCOS), the most commonly reproductive endocrinopathy characterized by unexplained chronic anovulation and hyperandrogenism, is associated with many cardiovascular risk factor like insulin resistance (IR), central obesity, hypertension, and unfavorable lipid profile [1, 2]. Both genes and the environment contribute to the etiology of PCOS [3]. Familial clustering of patients suggests that genetic factors play a considerable role in the development of PCOS and the disease process itself might be highly polygenic although the mechanism remains unclear [4].

Among the cardiovascular risk factors, the presence of increased activity of plasminogen activator inhibitor-1 (PAI-1) has been well documented [5, 6]. Elevated PAI-1 levels have been demonstrated in previous studies of women with PCOS [7, 8]. The overproduction of PAI-1 leads to distortions in the ovarian plasminogen–plasmin

pathway and anovulation in women with PCOS [9]. Several polymorphisms within the PAI-1 gene have been described that influence PAI-1 level, including the two common polymorphisms (−675 4G/5G insertion–deletion mutation and −844 A/G single nucleotide polymorphism (SNP)) [10]. The 4G allele of the −675 (4G/5G) variation has been associated with elevated PAI-1 concentrations and on some occasions with an increased risk of cardiovascular disease [11]. The genotypic subtypes 4G/4G and 4G/5G, in PCOS, were present with statistically higher frequency compared with controls, and the presence of the 4G allele in PAI-1 promoter region of the further, increased the PAI-1 levels [7]. Although both PAI-1 gene 4G/5G and −844 A/G variants are in strong linkage disequilibrium, they exert different effects on PAI-1 levels, with a strong elevation reported in PAI-1 levels were correlated with the 4G/5G mutation, but not with the −844 A/G mutation [12, 13].

So far, there were very few data available on the relations between −844 A/G polymorphism and the risk of PCOS patients, and no studies are available on the linkage of the polymorphisms of the PAI-1 gene −675 4G/5G and −844 A/G genotype with the risk of PCOS. The basic purpose of this study, therefore, was to prospectively investigate the role and co-existence effect of −675 4G/5G and −844 A/G polymorphisms in the PAI-1 promoter as risk factors of PCOS, and also explored the interaction between the genotype and PAI-1 level in Chinese women.

Materials and methods

Subjects

All patients seeing a doctor in reproduction centre of the first affiliated hospital of Harbin medical university were enrolled as subjects. The protocol was approved by local Ethics Committee for Human Studies and written informed consent was obtained from each subject before entry into the study. 126 PCOS patient fulfilling the revised 2003 diagnostic criteria of the Rotterdam consensus for PCOS [14] met two of the following three manifestations: (1) oligo- and/or anovulation, (2) clinical and/or biochemical signs of hyperandrogenism, and (3) polycystic ovaries with exclusion of other etiologies (congenital adrenal hyperplasias, androgen secreting neoplasia or Cushing's syndrome). Ultrasound criteria used for diagnosis were an enlarged ovary with ≥10 peripherally arranged small follicular cysts and a hyperechogenic central stroma. The 97 healthy volunteers matched for age with proven fertility (seen in our clinic for health examination), no menstrual cycle irregularities, with no clinical or biochemical hyperandrogenism and without PCO.

All subjects, homogenous women, had normal chromosome, thyroid, renal, and hepatic functions. Oral contraceptives or other drugs which could affect the hormonal levels were discontinued for at least 3 months before the study. To gain entry to the study, body weight had to remain stable for at least 2 months. Exclusion criteria for all subjects included delivery or pregnancy occurring within the preceding 3 months, cigarette smoking, known hypertension, cardiovascular disease, diabetes or other chronic diseases. Either in automatic menstrual cycle or post-progesterone withdrawal bleeding, all people submitted were during the follicular phase.

Methods

The blood samples were anticoagulated with sodium citrate, and genomic DNA (gDNA) was isolated routinely from peripheral blood of all individuals by the standard phenol–chloroform method. For the PAI-1 activity assay, the samples were centrifuged to obtain plasma, which was then stored at −70°C until analysis. The levels of testosterone (T) and PAI-1 were determined by enzyme-linked immunosorbent assay (ELISA). The COALIZA kits (DR Lab, California, USA) were used. Fasting insulin levels in the sera of all patients were determined by enhanced chemiluminescence (ECL) (Bayer automated chemiluminescence system). Fasting plasma glucose (FPG) was examined by the glucose oxidase method (Shanghai Kehua Bio-Engineering Co., Ltd). We calculated body mass index (BMI) [mass (kg)/height^2 (m^2)] to estimate obesity and the waist-to-hip ratio (WHR) to assess body fat distribution. Insulin sensitivity indices (homeostasis model assessment for insulin resistance (HOMA-IR) were also calculated [15]. To analyze the −675 4G/5G and −844 A/G polymorphism in the promotor region of the PAI-1 gene, genomic DNA was extracted from leukocytes of PCOS patients and amplified by polymerase chain reaction (PCR) using gene-specific primers.

PAI-1−844 A/G and −675 4G/5G genotyping

The −844 A/G and 4G/5G polymorphism were screened by the chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The −844 A/G PCR primers were provided by the Shanghai GeneCore Bio Technologies Company as the following: 5′-CAG GCT CCC ACT GAT TCT AC-3′ (Forward) and 5′-GAG GGC TCT CTT GTG TCA AC-3′ (Reverse) [16]. The PCR system was carried out in a final volume of 25 μl containing gDNA 0.8 μl (0.25 μg/μl), each primer 0.75 μl (12.5 pmol), supplied 10× buffer enzyme 2.5 μl, 4× dNTP 2 μl (2.5 mmol/l), Taq DNA polymerase 0.6 μl (1.25 U/μl). The PCR was performed by initial denaturation at 94°C for

3 min, followed by 30 amplification cycles at: 94°C during 30 s for denaturation, 60°C during 30 s for annealing, and 72°C during 30 s for extension, and finally, 72°C during 5 min for ending extension. The PCR products were incubated with 3 U of *Xho* I restriction enzyme for an hour in a heat bath at 37°C. The mixture was 10× buffer 3.0 μl, PCR products 27 μl, and *Xho* I restriction enzyme 1.5 μl (1 U/μl). The digested products were then electrophoresed on a 2% agarose gel stained with ethidium bromide. A fragment of 510 bp was amplified by PCR. Digestion fragments of 364 and 146 bp represent the wild type genotype (G/G); fragments of 510, 364, and 146 bp represent the heterozygote (A/G); and 510 bp represent the homozygote genotype (A/A).

This single allele deletion/insertion 4G/5G polymorphism is situated at −675 bp in the promoter region of the PAI-1 gene. The PCR primer was also provided by the Shanghai GeneCore Bio Technologies Company. The primers used in PCR were: insertion 5G allele: 5′-AGA GTC TGG ACA CGT GGG GG-3′, deletion 4G allele: 5′-AGA GTC TGG ACA CGT GGG GA-3′, each in a separate PCR reaction together with the common downstream primer 5′-TGC AGC CAG CCA CGT GAT TGT CTA G-3′. The reaction mixture had a total volume of 25 μl and contained: 10× reaction buffer 2.5 μl, 4× dNTP (dATP/dGTP/dCTP/dTTP) 2 μl, each primer 0.6 μl, gDNA 1.0 μl, and Taq DNA polymerase 0.35 μl, and the remaining was PCR H_2O. Two PCR reactions were run per sample (one for each allele). The 5G allele PCR conditions were: 94°C 5 min, 94°C 30 s, 67°C 30 s, 72°C 30 s, for 30 cycles and 72°C 5 min for extension at the end. The 4G allele PCR conditions were: 94°C 5 min, 94°C 30 s, 64°C 30 s, 72°C 30 s, for 30 cycles and 72°C 5 min for extension at the end. The PCR products were electrophoresed on a 2% agarose gel in order to analysis the 4G/5G polymorphism genotypes. 4G allele-specific primer and downstream primer amplified 139 bp bands, and 5G allele-specific primer and downstream primer amplified 149 bp bands. 4G/4G homozygotes only yielded a 139 bp band for the 4G allele-specific reaction, 5G/5G homozygotes only demonstrated a 140 bp band for the 5G allele-specific reaction, whereas 4G/5G heterozygotes demonstrated 139 and 140 bp bands for both reactions. All genotyping was assessed independently by two individuals.

Statistical analysis

For statistical analysis, results are reported as mean value ± SD. The Student's *t*-test was used to compare the clinical features between the PCOS patients and controls. The observed numbers of each PAI-1 −844 A/G and −675 4G/5G genotype were compared with those expected for population in Hardy–Weinberg equilibrium by using a χ^2 test. The Pearson's χ^2-test was used to analyze the distribution of genotypes frequencies between groups. The criterion of the association between factors and disease is using odds ratio (OR) and 95% confidence interval (CI). We performed a Kolmogorov–Smirnov test and the hypothesis that the data are normally distributed was not rejected. P value <0.05 was considered statistically significant. Statistical analyses were performed with the statistical package for social sciences (SPSS for Windows XP (Microsoft Corp.) version 13.0).

Results

The main characteristics of all subjects are shown in Table 1. There was no significant difference between PCOS and control group in the terms of age, WHR, and FPG. The following parameters in PCOS group were statistically different compared with controls: BMI ((23.68 ± 1.97) vs. (22.84 ± 1.8) kg/m^2, $P = 0.0012$), LH ((9.13 ± 3.42) vs. (4.46 ± 2.06) (IU/L), $P < 0.0001$), LH/FSH ((2.85 ± 1.12) vs. (1.38 ± 0.35), $P < 0.0001$), total testosterone ((3.38 ± 0.62) vs. (1.85 ± 0.46) nmol/l, $P < 0.0001$), fasting insulin

Table 1 Anthropometric characteristics, metabolic and hormonal profile in PCOS patients

	PCOS ($n = 126$)	Controls ($n = 97$)	P value
Age (years)	27.68 ± 2.89	27.44 ± 2.72	0.5299
BMI (kg/m^2)	23.68 ± 1.97	22.84 ± 1.80	0.0012
WHR	0.76 ± 0.03	0.75 ± 0.02	0.0673
Total testosterone (nmol/l)	3.38 ± 0.62	1.85 ± 0.46	<0.0001
LH (IU/L)	9.13 ± 3.42	4.46 ± 2.06	<0.0001
FSH (IU/L)	3.35 ± 1.02	3.17 ± 0.99	0.190
LH/FSH ratio	2.85 ± 1.12	1.38 ± 0.35	<0.0001
PAI-1 (ng/ml)	57.18 ± 8.44	52.62 ± 3.59	<0.0001
FPG (mmol/l)	4.36 ± 0.74	4.25 ± 0.73	0.271
Fasting insulin (μIU/ml)	14.29 ± 3.35	10.13 ± 2.38	<0.0001
HOMA-IR	2.75 ± 0.72	1.9 ± 0.5	<0.0001

Values are means ± SD; *PCOS* polycystic ovarian syndrome, *BMI* body mass index, *WHR* waist-to-hip ratio, *FPG* fasting plasma glucose, *PAI-1* plasminogen activator inhibitor-1, *HOMA-IR* homeostasis model assessment for insulin resistance

Table 2 PAI-1 −675 4G/5G and −844 A/G genotype and allele frequencies in subjects and relative risk for PCOS compared with controls

Position	Genotype	PCOS *n* (%)	Controls *n* (%)	χ^2	OR	95% CI	*P*
−844	A/A	50 (39.7)	27 (27.8)	3.403	1.71	1.03–3.02	0.0651
	A/G	59 (46.8)	51 (52.6)	0.7254	1 (reference value)		0.3944
	G/G	17 (13.5)	19 (19.6)	1.5043	0.64	0.31–1.31	0.22
	A allele	159 (63.1)	105 (54.1)	3.6525	1.45	0.99–2.12	0.056
	G allele	93 (36.9)	89 (45.9)		0.69	0.47–1.01	
−675	4G/4G	55 (43.7)	23 (23.7)	9.581	2.49	1.40–4.44	**<0.01**
	4G/5G	56 (44.4)	47 (48.5)	0.3544	1 (reference value)		0.5516
	5G/5G	15 (11.9)	27 (27.8)	9.0984	0.35	0.18–0.69	**<0.01**
	4G allele	166 (65.9)	93 (47.9)	14.4808	2.10	1.43–3.08	**<0.001**
	5G allele	86 (34.1)	101 (52.1)		0.48	0.33–0.70	

OR odds ratio, *95% CI* 95% confidence interval

Bold values denote $P < 0.05$ was considered statistically significant

levels ((14.29 ± 3.35) vs. (10.13 ± 2.38) μIU/ml, $P < 0.0001$), and HOMA-IR (2.75 ± 0.72 vs. 1.9 ± 0.5, $P < 0.0001$).

The distribution of PAI-1 −844 A/G and −675 4G/5G genotypes observed showed no significant differences when compared with those predicted from the Hardy–Weinberg equilibrium for either PCOS patients ($P > 0.05$), which means the distributions achieve genetic equilibrium and have group representativeness.

The data concerning the PAI-1 gene promoter −844 A/G and −675 4G/5G polymorphism were shown in Table 2. The frequencies of A/A, A/G, and G/G genotypes were 39.68, 46.83, and 13.49%, respectively, in the PCOS patients. The A/A genotype and A allelic frequency in PCOS group were higher than controls without statistical difference (39.68% vs. 27.84%, $P = 0.0651$; 63.1% vs. 54.12%, $P = 0.056$, respectively), and G/G genotype and G allelic frequency were more prevalent in control group than in PCOS women also without significant difference (19.59% vs. 13.49%, $P = 0.22$; 45.88% vs. 36.9%, $P = 0.056$, respectively).

The prevalence of the −675 4G/4G genotype was significantly higher in PCOS patients than in controls (43.65% vs. 23.71%, $P = 0.002$), leading to an OR of 2.49 (95% CI, 1.40–4.44). Similarly, the PAI-1 4G allele frequency was statistically high in PCOS patients compared with controls (65.87% vs. 47.94%, $P = 0.0001$), leading to an OR of 2.1 (95% CI, 1.43–3.08). The 5G/5G genotype and 5G allele were more prevalent in healthy controls than in PCOS patients (27.84% vs. 11.91%, $P = 0.0026$; 52.06% vs. 34.13%, $P = 0.0001$, respectively).

The −844 A/G and 4G/5G polymorphisms of the PAI-1 gene were found to be in strong linkage disequilibrium in both groups of subjects investigated. PCOS women carrying the combinations of A/A genotype and PAI-1 4G/4G genotype were significantly higher than controls (26.98% vs. 11.34%, $P = 0.0039$), leading to an OR of 2.9 (95% CI, 1.41–5.98). At the same time, the proportion of individuals who had the 5G/5G and −844 A/G genotype was statistically lower in the PCOS group (6.35% vs. 17.53%, $P = 0.0087$, Table 3).

Table 4 showed the association of PAI-1 4G/5G and −844 A/G genotypes, respectively, with plasma PAI-1 levels in the two groups. PCOS women had significantly higher PAI-1 levels than control group ((57.18 ± 8.44) vs.

Table 3 The combination of PAI-1 −675 4G/5G and −844 A/G genotype in subjects and relative risk for PCOS compared with controls

−675	−844	PCOS *n* (%)	Controls *n* (%)	χ^2	OR	95% CI	*P*
4G/4G	A/A	34 (27.0)	11 (11.3)	8.3273	2.9	1.41–5.98	**<0.01**
4G/4G	A/G	19 (15.1)	10 (10.3)	1.1023	1.55	0.68–3.51	0.2938
4G/4G	G/G	2 (1.6)	2 (2.1)	0.0596	0.77	0.09–6.28	0.8071
4G/5G	A/A	15 (11.9)	14 (14.4)	0.3097	0.8	0.37–1.76	0.5779
4G/5G	A/G	10 (7.9)	9 (9.3)	0.1266	–		0.7220
4G/5G	G/G	2 (1.6)	2 (2.1)	0.0596	0.77	0.09–6.28	0.8071
5G/5G	A/A	31(24.6)	24 (24.7)	0.0006	–		0.9809
5G/5G	A/G	8 (6.3)	17 (17.5)	6.8779	0.32	0.14–0.75	**<0.01**
5G/5G	G/G	5 (4.0)	8 (8.3)	1.8281	0.5	0.18–1.37	0.1764

Bold values denote $P < 0.05$ was considered statistically significant

Table 4 The plasma PAI-1 levels (ng/ml) of PCOS women and controls in each genotype of PAI-1 gene promoter −675 4G/5G −844 A/G polymorphism

Position	Genotype	PCOS	Controls	*P* value
−844	A/A	64.47 ± 7.16	52.66 ± 4.25	<0.0001
	A/G	52.78 ± 5.16	53.18 ± 3.39	0.642
	G/G	50.31 ± 3.36	51.08 ± 2.72	0.452
−675	4G/4G	63.42 ± 7.37	52.91 ± 3.79	<0.0001
	4G/5G	52.85 ± 5.92	53.20 ± 3.84	0.726
	5G/5G	50.47 ± 3.45	51.37 ± 2.65	0.348

Values are means ± SD

(52.62 ± 3.59) ng/ml, $P < 0.0001$) (Table 1). The plasma PAI-1 levels of PCOS patients were 64.47 ± 7.16, 52.78 ± 5.16, and 50.31 ± 3.36 ng/ml for the A/A, A/G, and G/G genotypes, 63.42 ± 7.37, 52.85 ± 5.92, 50.47 ± 3.45 ng/ml for the 4G/4G, 4G/5G, and 5G/5G genotypes, respectively. The results showed that in PCOS patients, the plasma PAI-1 level was significantly higher in subjects with the 4G/4G or A/A genotype than those with the non-4G/4G or non-A/A genotypes, respectively, ($P < 0.001$). In PCOS group, subjects with A/A or 4G/4G genotype had significantly higher plasma PAI-1 levels than those carrying same genotype in controls ($P < 0.001$).

Table 5 showed that the plasma PAI-1 levels were measured simultaneously with the combinative distribution of PAI-1 −844 A/G and 4G/5G genotypes in the study population. PCOS women carrying the combination of the −844 A/A and 4G/4G or 4G/5G genotypes had significantly higher plasma PAI-1 levels than controls ((68.03 ± 4.64) vs. (52.28 ± 4.84) ng/ml, $P < 0.0001$; (57.95 ± 5.83) vs. (53.39 ± 3.68) ng/ml, $P = 0.0188$, respectively). At the same time, the plasma PAI-1 levels in the coadunation of PAI-1 4G/4G and −844 non-G/G (A/A + A/G) genotypes of PCOS group were significantly high compared with healthy subjects ((56.45 ± 3.69) vs. (53.5 ± 2.49) ng/ml, $P = 0.0321$).

Table 5 The combination of PAI-1 −675 4G/5G and −844 A/G genotype with plasma PAI-1 levels (ng/ml) in PCOS subjects and controls

	PCOS ($n = 126$)	Controls ($n = 97$)	*P* value
4G/4G + A/A	68.03 ± 4.64	52.28 ± 4.84	**<0.0001**
4G/4G + A/G	56.45 ± 3.69	53.50 ± 2.49	**<0.05**
4G/4G + G/G	51.35 ± 6.29	53.45 ± 4.17	0.7320
4G/5G + A/A	57.95 ± 5.83	53.39 ± 3.68	**<0.05**
5G/5G + A/A	52.78 ± 6.25	49.63 ± 5.55	0.6469
4G/5G + A/G	51.26 ± 5.17	53.75 ± 4.18	0.0602
4G/5G + G/G	50.11 ± 3.46	51.45 ± 2.84	0.3718
5G/5G + A/G	49.99 ± 3.47	52.19 ± 2.39	0.0766
5G/5G + G/G	50.31 ± 2.74	50.08 ± 2.09	0.8661

Values are means ± SD

Bold values denote $P < 0.05$ was considered statistically significant

Discussion

The PAI-1 4G/5G polymorphism could be one of the factors representing a genetic link to the developmental origin hypothesis for PCOS. Homozygosity for the 4G allele of the PAI-1 gene represents a severe risk for pregnancies, predisposing to prematurity, intrauterine growth retardation, miscarriage, and stillbirth [9, 17, 18]. In contrast, an earlier study in PCOS patients with or without miscarriages did not reveal a significant difference with regard to the 4G/5G polymorphism of the PAI-1 gene [19]. Our findings confirm a tendency of homozygous 4G allele carriers to be bound for increased risk of PCOS patients, consistent with previous studies suggesting a protective nature of PAI-1 5G allele in PCOS pathogenesis. In view of differences in ethnic backgrounds, sample size, and other factors, the agreement reached in these and similar studies confirm the association of the 4G variant with increased PCOS risk.

Although several studies have suggested a contribution from the PAI-1 −844 A/G variant in some diseases development, no data are available on the relations between this polymorphism and the risk of PCOS patients. We found that patients with PCOS had an increased prevalence of the −844 A/A genotype and A allele frequencies, a lower frequency of the 5G/−844G haplotype, compared with the normal controls, although these were not significantly different ($P > 0.05$). Similar associations of the −844 A/G polymorphism with other several disease including cardiovascular diseases, deep vein thrombosis, stroke and colorectal cancer [12, 13, 20–22] were found in previous study. In contrast, recently has been associated with a high risk of venous thrombosis in factor V Leiden carriers and with a mild preeclampsia [23, 24]. The fact that the 4G/5G polymorphism was more closely associated with the risk of PCOS than was the −844 A/G polymorphism in the present study might be due to random fluctuations.

To the best of our knowledge, this is the first study to describe an association between combined homozygosity for the PAI-1 −844 A/G and 4G/5G polymorphisms with the cumulatively increased risk occurrence of PCOS. PCOS women carrying the combinations of A/A genotype and PAI-1 4G/4G genotype were significantly higher than controls, which may result in hypofibrinolysis and further increase the PCOS risks. Moreover, the proportion of individuals who had the 5G/5G and −844 A/G genotype was statistically lower in the PCOS group, which may be useful for the risk of PCOS.

The level of plasma PAI-1 activity is responsible for the active regulation of the whole fibrinolytic process, and

increased PAI-1 activity in the plasma may promote thrombosis formation by inhibiting production of fibrinolytic enzyme plasmin [25]. PAI-1 antigen levels are influenced by environmental factors and by genetic factors. The PAI-1 promoter of the PAI-1 gene contains two common polymorphisms (−844 A/G and −675 4G/5G). Due to its antifibrinolytic properties, PAI-1 was previously described as harmful in PCOS etiology. Compared with the other PAI-1 variants, the most significant variation in PAI-1 expression is in the PAI-1 4G/5G alleles. As an inherited risk factor, PAI-1 4G/5G polymorphism was found to play a key role in PCOS pathogenesis, presumably through elevation of PAI-1 levels [7]. Unlike the 5G allele, the 4G allele of the 4G/5G polymorphism does not bind a transcription repressor protein, which confers a high PAI-1 expressor nature to the 4G allele and a low PAI-1 expressor nature to the 5G allele [12]. In this study, we found that plasma PAI-1 levels of PCOS phenotypic group were statistically higher than those of controls, which are in accordance with previous studies [7], and significant changes in PAI-1 levels were seen in homozygous PAI-1 4G/4G carriers compared with other 4G/5G genotype carriers in PCOS patients.

This study is also the first report on the association between −844 A/G polymorphism and plasma PAI-1 levels in patients with PCOS, and contrary previous finding on no such associations in patient deep vein thrombosis [13] and in patients with stroke [21]. We also found that the genotypes appear to contribute to the increased PAI-1 levels, and the homozygosity for the −844 A/A genotype or the 4G/4G genotype carrier in PCOS group had the higher PAI-1 levels than controls. In PCOS patients, with the highest values in the A/A genotype, median values in the A/G genotype and the lowest values in the G/G genotype, suggesting functional importance of the A/G polymorphism in regulating the expression of PAI-1 gene.

In the study herein, the −844 A/G and 4G/5G polymorphisms of the PAI-1 gene are in strong linkage disequilibrium, as was previously shown in other studies [13]. The linkage disequilibrium coefficient (D′) between the two PAI-1 polymorphisms was estimated by using log-linear model analysis [26]. PCOS women carrying the combination of the −844 A/A and non-5G/5G genotypes, or the coadunation of PAI-1 4G/4G and −844 non-G/G genotype, had significantly higher plasma PAI-1 levels than normal controls. It is possible from these results to confirm a functional role for any of the two polymorphisms on the etiopathogenesis of PCOS. They might also be markers of an as-yet-unidentified functional mutation. Despite the fact that the two polymorphisms studied might be involved in the promoter activity of the PAI-1 gene [27], genetic polymorphisms explain only a small part of PAI-1 plasma level variations in healthy individuals compared with the effect of environmental factors [28]. Although the explanation for this role remains speculative at this stage, our findings suggest that the contribution of PAI-1 to PCOS may involve pathways other than fibrinolysis.

In conclusion, in the present study, a trend to a positive interaction between PAI-1 −675 4G/5G and −844 A/G gene polymorphism may elevate plasma PAI-1 levels and hypofibrinolysis, which is probably an important hereditary risk factors in PCOS. Our study provides evidence of an association of −844 A/G polymorphism with plasma PAI-1 levels in PCOS women. Moreover, the genotype and allele frequencies have not been reported previously in PCOS. In order to confirm this observation, it is mandatory to perform larger studies in PCOS patients. This finding emphasized that the genetic background should be taken into account in the PCOS population. Based on our findings and those of others, it could be important to assess more accurately the risk of PCOS and to better manage prophylactic and diagnostic measures. Further prospective studies and further explanation are necessary to confirm our results.

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