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The 4G/5G polymorphism of the plasminogen activator inhibitor-1 gene is associated with severe preeclampsia

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Abstract Preeclampsia is associated with thrombosis of the intervillous or spiral artery. A deletion/insertion polymorphism (4G or 5G) in the promoter of the plasminogen activator inhibitor type 1 (*PAI-1*) gene is suggested to be involved in regulating the synthesis of the inhibitor, 4G allele, being associated with the enhanced gene expression and plasma PAI-1 levels. We assessed the association between preeclampsia and the 4G/5G polymorphism of the *PAI-1* gene in 115 preeclamptic patients, 210 pregnant controls, and 298 healthy volunteer controls. The frequency of the homozygotes for the 4G allele was significantly higher in the patients than in the control pregnant women ($P = 0.04$) or in the healthy volunteers ($P = 0.02$). The 4G allele frequency was also significantly higher in the patients than in the control group of pregnant women ($P = 0.03$) and in the healthy volunteers ($P = 0.02$). These results suggest that the presence of the 4G/4G genotype of the *PAI-1* gene is one of the risk factors for preeclampsia.

Key words Preeclampsia · Plasminogen activator inhibitor type 1 · Gene · Polymorphism

Introduction

Severe preeclampsia contributes to maternal and fetal morbidity and mortality. Causal factors include environmental and inherited components. Although the pathogenesis of preeclampsia is not well understood, intervillous or spiral artery thrombosis and inadequate placental perfusion are associated with severe preeclampsia (Dekker and Sibai 1998).

Plasminogen activator inhibitor-1 (PAI-1) is a major inhibitor of fibrinolysis. A central step in the fibrinolytic process is the conversion of plasminogen to plasmin, which is regulated by such activators as tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA). PAI-1, which is a fast-acting inhibitor of t-PA, regulates the rate of clot dissolution (Pannekoek et al. 1986). A significant elevation of plasma and placental PAI-1 levels has been found in pregnant woman with severe preeclampsia as compared with levels in normal pregnant women (Estelles et al. 1989; Reith et al. 1993). A decrease in plasma fibrolytic activity caused by increased PAI-1 levels has also been reported in preeclampsia (Estelles et al. 1989; Caron et al. 1991; Estelles et al. 1994).

The *PAI-1* gene, which contains nine exons and is distributed over approximately 12.3 kb, has been localized to q21.3–q22 of chromosome 7 (Klinger et al. 1987; Follo and Ginsburg 1989; Bosma et al. 1988). A common single guanosine insertion/deletion polymorphism (4G/5G polymorphism) in the promoter region of the *PAI-1* gene, situated 675 base pairs upstream from the transcriptional start site, was recently identified. In-vitro assays of promoter activity demonstrated that the 4G allele had a significantly higher activity than the 5G allele (Dawson et al. 1993). The polymorphism was associated with plasma PAI-1 activity in vivo. Plasma PAI-1 activity is higher in individuals homozygous for the 4G allele than in those who are heterozygous or homozygous for the 5G allele (Dawson et al. 1993; Eriksson et al. 1995; Ye et al. 1995; Ossei-Gerning et al. 1997). An increase in the frequency of the 4G/4G genotype has been reported in young men with myocardial infarction (Eriksson et al. 1995), in patients with non-insulin dependent diabetes mellitus who have a history of ischemic heart disease (Mansfield et al. 1995), and in patients with coronary atheroma and a previous myocardial infarction (Ossei-Gerning et al. 1997). In contrast, no difference in genotype distribution was observed between the patients and the control subjects in the ECTIM (Etude Cas-Temoins de I'Infarctus du Myocarde) study (Ye et al. 1995).

We have hypothesized that the 4G allele of the 4G/5G

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polymorphism in the promoter region of the *PAI-1* gene is a genetic risk factor for preeclampsia. In the present study, we examined the association between preeclampsia and the 4G allele of the polymorphism in a retrospective case-control design.

Subjects and methods

Subjects

All subjects studied were unrelated Japanese individuals. There were 115 preeclamptic patients, 210 pregnant controls, and 298 healthy volunteer controls. The preeclamptic patients ($n = 115$; aged 20–43 years) were those who were managed at the University of Tsukuba Hospital. The diagnosis of preeclampsia was based on the criteria of the American College of Obstetricians and Gynecologists (ACOG 1990). Severe preeclampsia was defined as a systolic blood pressure of ≧160 mmHg and/or a diastolic blood pressure of ≧110 mmHg, with proteinuria. None of the subjects had a history of hypertension, of diabetic nephropathy, or of other renal disease. The pregnant control women (aged 20–43 years), who had delivered at greater than 22 weeks of gestation at the University of Tsukuba Hospital, were 126 primiparous and 84 multiparous women. They had not had hypertension or proteinuria during the pregnancy. None of the control multiparous women had had a history of preeclampsia in a previous pregnancy. The profiles of these subjects are shown in Table 1. The patients with preeclampsia had a higher mean body mass index (BMI) than the pregnant controls at admission for delivery. A greater percentage of the patients with preeclampsia had a family history of hypertension compared with the pregnant controls. Obesity and a family history of hypertension are well known risk factors for preeclampsia.

In addition, to assess the frequencies of the allele of the 4G/5G polymorphism of the *PAI-1* gene in Japanese individuals, we genotyped a panel of 298 healthy volunteers who were recruited at their annual health examinations, performed at the Tsuchiura Kyodo General Hospital. They were unrelated normotensive Japanese adults (79% male; mean age, 51.4 ± 7.7 years). The Ethics Committee of Tsukuba University approved the protocol of this study, and written informed consent for participation was obtained from each subject before blood was sampled.

Table 1. Profiles of cases and pregnant controls

	Patients with preeclampsia $n = 115$	Controls $n = 210$	P
Age (years)	31.2 ± 5.2	31.2 ± 4.8	NS
Primiparous/multiparous	66/39	126/84	NS
BMI before conception	22.1 ± 3.1	21.4 ± 3.5	NS
BMI at admisson for delivery	26.5 ± 3.6	25.5 ± 3.0	0.01
Family history (%)[a]	49.6	21.9	0.000006

BMI, Body mass index
[a] Percentage of subjects whose parents or siblings had a history of hypertension

Methods

Genomic DNA was isolated from peripheral blood leukocytes by the phenol-extraction method. Genotypes for the 4G/5G polymorphism of the *PAI-1* gene were determined using the polymerase chain reaction-restricted fragment length polymorphism (PCR-RFLP) method, according to Margaglione et al. (1997).

Statistical analysis

Hardy-Weinberg equilibrium was tested by a χ^2 test with one degree of freedom (DF) separately in cases and controls. Allele frequencies were deduced from the genotype frequencies, and the difference between cases and controls was tested by a χ^2 test with one DF. Genotype distributions for 4G/4G vs 4G/5G + 5G/5G were also compared between cases and controls by a χ^2 test. A level of $P < 0.05$ was accepted as significant.

Results

The distribution of genotypes of the 4G/5G polymorphism of the *PAI-1* gene is shown in Table 2. The frequency of the 4G/4G, 4G/5G, 5G/5G genotypes was 0.60, 0.32, and 0.08, respectively, in the patients with preeclampsia; 0.48, 0.40, and 0.12, respectively, in the pregnant control subjects; and 0.47, 0.41, and 0.12, respectively, in the healthy volunteers. The observed number of genotypes in the three groups did not differ significantly from those that would be expected from the Hardy-Weinberg equilibrium. The frequency of individuals homozygous for the 4G allele was significantly higher in the patients than in the pregnant control group ($P = 0.04$) or in the healthy volunteers ($P = 0.02$). Compared with the pregnant controls, the odds ratio for the risk of preeclampsia associated with the 4G/4G genotype was 1.62 (95% confidence interval [CI], 1.02–2.57). The frequency of the 4G/5G polymorphism of the *PAI-1* gene is shown in Table 3. The frequency of the 4G allele was significantly higher in the patients with preeclampsia than in either the control group of pregnant women ($P = 0.03$) or the healthy volunteers ($P = 0.02$). The odds ratio for the risk of preeclampsia associated with the 4G allele was 1.49 (95% CI, 1.03–2.15). The armitage linearity tendency test showed the gene dose effect of the 4G allele ($P = 0.02$).

Discussion

The present study suggests an association between the 4G allele of the 4G/5G polymorphism of the *PAI-1* gene and the development of preeclampsia. To our knowledge, this is the first such report. The 4G allele frequency was higher in

Table 2. Genotype distribution of the 4G/5G polymorphism of the *PAI*-1 gene

	n	4G/4G	4G/5G	5G/5G	*P**	OR (95% CI)*
Patients with preeclampsia	115	69 (0.60)[a]	37 (0.32)[a]	9 (0.08)[a]	—	—
Pregnant controls	210	101 (0.48)	84 (0.40)	25 (0.12)	0.04	1.62 (1.02–2.57)
Volunteer controls	298	140 (0.47)	122 (0.41)	36 (0.12)	0.02	1.67 (1.08–2.59)
Total controls	508	241 (0.47)	206 (0.41)	61 (0.12)	0.02	1.66 (1.10–2.51)

*4G/4G vs 4G/5G + 5G/5G
OR, Odds ratio; CI, confidence interval
[a]Frequency is shown in parentheses

Table 3. Allele frequency of the 4G/5G polymorphism of the PAI-1 gene

	n	4G	5G	*P*	OR (95% CI)
Patients with preeclampsia	115	0.76	0.24	—	—
Pregnant controls	210	0.68	0.32	0.03	1.49 (1.03–2.15)
Volunteer controls	298	0.67	0.33	0.02	1.53 (1.08–2.17)
Total controls	508	0.68	0.32	0.01	1.51 (1.09–2.11)

the Japanese control population than in Caucasians (Ye et al. 1995; Ossei-Gerning et al. 1997; Burzotta et al. 1998; Mannucci et al. 1997). This discrepancy may be due to fundamental genetic differences between Japanese and Caucasians.

Increased plasma levels of total plasminogen activator inhibitor (PAI) activity in preeclampsia have been noted for more than a decade (Wiman et al. 1984; de Boer et al. 1988; Gilabert et al. 1990). Plasma total PAI activity is mostly PAI-1 activity. Estelles et al. (1989) and Reith et al. (1993) reported increased plasma PAI-1 activity in hypertensive pregnant women. Although we did not assess PAI-1 activity in our subjects, our observation suggests that the increased plasma levels of PAI-1 in the patient with preeclampsia is, at least in part, genetically influenced by the *PAI-1* gene polymorphism, and that the 4G allele of the *PAI-1* gene may be one of the genetic risk factors for preeclampsia. However, as the genotypic and allelic differences between cases and controls were marginally significant in this study, confirming the association in independent populations is warranted.

To date, two thrombophilic mutations have been reported to be associated with an increased risk of preeclampsia. The resistance to activated protein C caused by an adenine-to-guanine mutation at nucleotide 506 in the factor V gene (so-called Leiden mutation) is associated with an increased risk of preeclampsia (Dizon-Townson et al. 1996). Homozygosity for the T allele of the C677T polymorphism in the methylentetrahydrofolate reductase (*MTHFR*) gene was also reported to be associated with an increased risk of preeclampsia (Sohda et al. 1997). The present study reports the third thrombophilic mutation to be associated with an increased risk of preeclampsia. Therefore, it is hypothesized that the presence of thrombophilic mutations constitutes a genetic predisposition to preeclampsia.

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