

Correlation Between Preeclampsia and Prevalence of Polymorphism of Angiotensinogen, Methylenetetrahydrofolate Reductase and Factor β , Prothrombin Genes Among Japanese Women

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OBJECTIVE: To determine the genotypes of four candidate genes in Japanese women with a history of preeclampsia, and in a control group of parous woman.

STUDY DESIGN: Fifty-two pregnant women with a history of preeclampsia in their first pregnancy and 113 normotensive gravid women were studied. All subjects were Japanese women with singleton gestations. Genomic DNA was extracted, and genotypes of angiotensinogen (AGT), methylenetetrahydrofolate reductase (MTHFR), factor β Leiden, and prothrombin genes were analyzed.

RESULTS: The frequencies of homozygous AGT gene mutation and homozygous MTHFR gene mutation in preeclampsia were significantly higher than that in control. The calculated risk associated with the presence of both mutations did not exceed the risk with polymorphism of each gene. None of the examined cases showed polymorphism of factor β Leiden and prothrombin G20210A genes.

CONCLUSION: In Japanese patients with preeclampsia, the angiotensinogen gene and particularly MTHFR gene may play a role in the pathogenesis of preeclampsia

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Introduction

Preeclampsia is a progressive, multisystem disorder unique to pregnant women and is a leading cause of maternal death and contributes significantly to premature deliveries. It is characterized by hypertension and proteinuria, and its incidence is influenced by various factors, such as parity, race, and environmental factors. It is estimated to be about 5~10% in European countries and at 5.8% in first pregnancies and 0.4% in second pregnancies.¹ The etiology of preeclampsia is still unknown, but genetic factors have been implicated since the syndrome shows a familial tendency.² Published reports of pedigree analysis suggest that development of preeclampsia may be based on a single recessive gene or dominant gene with incomplete penetrance.³ However, more recent studies have suggested that the pattern of inheritance is multifactorial and depends on several genetic loci with greater or smaller contributions from environmental factors. In addition, not only maternal gene but also

fetal gene may be implicated, and maternal-fetal interaction could not be ignored.

It is unlikely that a particular genotype is involved in the development of preeclampsia. Rather, many loci confer genetic liability that predisposes individuals to the disease. During the last decade, a growing number of genetic variants have been implicated in the development of preeclampsia. To date, reported preeclampsia-related genes are classified in following categories,

- ① Renin-Angiotensin system association genes (AGT, Angiotensin Type 1 Receptor (AGTR1), AGTR1 Agonistic Antibodies, Angiotensin Type 2 Receptor),
- ② Endothelial Nitric Oxide Synthase (eNOS),
- ③ Coagulopathy and Vascular injury genes (MTHFR, Factor Leiden, Prothrombin),
- ④ Oxidative Stress Candidate Genes (Lipoprotein Lipase, Apolipoprotein E), and
- ⑤ Immunoregulatory Candidate Genes (HLA, TNF- α).

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Among them, mutations of angiotensinogen (*AGT*), methylenetetrahydrofolate reductase (*MTHFR*), *Factor Leiden*, and prothrombin genes seem to be the most likely candidate in Japanese although no mutation in single gene can correctly predict the disease. The detection rate of *AGT* and *MTHFR* gene polymorphisms are high in Japanese, and relationship between preeclampsia and DNA polymorphism in these two genes were also reported. And, in European countries, strong relationship between preeclampsia and *Factor Leiden*, prothrombin genes polymorphisms were reported.

In the present study, we determined the composite genetic risk for preeclampsia by simultaneous genotyping of these loci.

Material And Methods

Patients participating in this study were recruited from the obstetric population followed at Nagasaki University Hospital between December 1997 and March 2000. Informed consent was obtained from all patients, and the ethics committee of our university approved the study protocol. Fifty-two pregnant women with preeclampsia in their first pregnancy and 113 normotensive gravid women were recruited for this retrospective study. All subjects were Japanese women with singleton gestations. According to the working group of the National High Blood Pressure Education Program (NHBPEP 2000), preeclampsia is defined by the de novo appearance of hypertension after 20 weeks of gestation, systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg, accompanied by new-onset proteinuria, defined as ≥ 300 mg per 24 h. Genomic DNA was extracted from peripheral blood leukocytes by standard procedures (Quiagen DNA extract kit), and the genotype was determined by a PCR restriction fragment length polymorphism method (RFLP) as described below.

Angiotensinogen M235T polymorphism

A 165-base-pair DNA fragment of the angiotensinogen gene was amplified by polymerase chain reaction (PCR) using the sense primer 5' CAG GGT GCT CAC ACT GGA CCC C-3' and antisense primer 5' CCG TTT GTG CAG GGC CTG GCT CTC T-3', according to the method described by Kamitani et al.⁴ After endonuclease digestion with *Thi111-I*, PCR fragments were electrophoresed in 10% polyacrylamide gels and stained with ethidium bromide.

MTHFR C677T polymorphism

A 189-base-pair DNA fragment of the *MTHFR* gene was amplified by the PCR using the sense primer 5' TGA AGG AGA AGG TGT CTG CGG GA-3' and antisense primer 5' AGG ACG GTG CGG TGA GAG TG-3', according to the method described by Morita et al.⁵ After endonuclease digestion with *HinfI*, PCR fragments were electrophoresed in 10% polyacrylamide gels and stained with ethidium bromide.

Factor gene analysis

A 287-base-pair DNA fragment of *Factor* gene was amplified by the PCR using the sense primer 5' GGA ACA ACA CCA TGA TCA CAG CA-3' and antisense primer 5' TAG CCA GGA GAC CTA ACA TGT TC-3', according to the method described by Zoller et al.⁶ After endonuclease digestion with *MnlI*, PCR fragments were electrophoresed in 8% polyacrylamide gels and stained with ethidium bromide.

Prothrombin G20210A polymorphism

A 640-base-pair DNA fragment of the prothrombin gene was amplified by PCR using the sense primer 5' CGG TGT GTG TGT AGG AAC TCC-3' and antisense primer 5' CAA TGT CAG ATG CTG GGG ACT-3', according to the method described by Poort et al.⁷ After endonuclease digestion with *Alw 44I*, PCR fragments were electrophoresed in 1% agarose gels and stained with ethidium bromide.

Statistical analysis

Differences between groups were examined for statistical significance using the Fisher's exact test or chi-square test. Odds ratio and 95 percent confidence intervals (95%CI) were calculated. A *p* value less than 0.05 denoted the presence of a statistically significant difference.

RESULTS

We studied 52 pregnant women with preeclampsia in their first pregnancy and 113 normotensive gravid women (Table 1).

Angiotensinogen

Table 2 shows the M235T genotyping results. M235T homozygosity (genotype TT) was detected in 33 of 52 (63.5%) patients with preeclampsia and 51 of 113 (45.1%) control subjects. The frequency of each genotype in the control group was consistent with those reported previously by other investigators.^{4,8,9} Compared with the other genotypes (TM and MM), the frequency of aa was significantly higher in preeclampsia than in controls ($p < 0.05$). The observed genotype frequencies were not different from those predicted from Hardy-Weinberg equilibrium.

MTHFR

Table 3 shows the results of C677T genotyping. C677T homozygosity (TT) was identified 18 of 52 (34.6%) in control subjects. The frequency of each genotype in the control was consistent with those reported previously by other investigators.^{5,10} Compared with the other genotypes (CT and CC), the frequency of

Table 1. Clinical Characteristics

Variable	Preeclampsia (n=52)	Control (n=113)	p
	Mean (95%CI)	Mean (95%CI)	
Age	30.1 (28.3, 32.0)	30.4 (29.5, 31.3)	NS
BMI (kg/m ²)	21.1 (18.4, 23.8)	20.7 (21.0, 22.4)	NS
Systolic BP (mmHg)	164 (153, 172)	116 (113, 119)	< .001
Diastolic BP (mmHg)	102 (95, 109)	71 (69, 73)	< .001
Birth Weight (g)	2,244 (1,857, 2,598)	2,994 (2,687, 3,298)	< .001
Weeks of gestation	35.0 (32.6, 37.2)	39.1 (38.2, 40.0)	< .001

BMI: Body mass index

CI : confidence interval; BP : blood pressure, NS : not significant

Table 2. AGT genotype distribution in patients with pre-eclampsia and control subjects

	MM (%)	MT (%)	TT (%)
PE (n=52)	4 (7.7)	15 (28.9)	33 (63.5)
Controls (n=113)	11 (9.7)	51 (45.1)	51 (45.1)

PE : preeclampsia

Table 3. MTHFR genotype distribution in patients with pre-eclampsia and control subjects.

	CC (%)	CT (%)	TT (%)
PE (n=52)	17 (32.7)	17 (32.7)	18 (34.6)
Controls (n=113)	48 (42.4)	54 (47.8)	11 (9.7)

PE : Preeclampsia

Table 4. Odds ratios of preeclampsia in the presence of AGT, MTHFR and combined AGT + MTHFR gene polymorphisms.

	Odds ratio	95%CI
AGT homozygous mutation (TT) (n=33)	2.11	1.08-4.15
MTHFR homozygous mutation (TT) (n=18)	4.91	2.11-11.4
Both homozygous mutation (TT/TT) (n=8)	4.79	1.66-13.8

TT was significantly higher in preeclampsia than in control subjects ($p < 0.01$). The observed genotype frequencies were not different from those predicted from Hardy-Weinberg equilibrium.

Analysis of the effect of modification was performed to evaluate the estimates of risk associated with AGT gene mutation (genotype aa) and MTHFR gene mutation (genotype aa), and their combination (Table 4). The frequency of double homozygous (TT/TT) in the Preeclampsia is 13.4% (8/52), and 3.5% (4/113) in control. The calculated risk associated with the presence of both mutations (odds ratio 4.79, 95%CI: 1.66-13.8) did not exceed the risk of MTHFR single gene mutation (odds ratio 4.91, 95%CI: 2.11-11.4).

Factor and prothrombin gene

Previous studies have shown that *factor Leiden* mutation and prothrombin G20210A mutation are very rare in Asia.¹¹ In our population sample, no Leiden or prothrombin G20210A mutations were detected in all 165 women.

Discussion

Preeclampsia is one of the most serious complications during pregnancy and a syndrome that affects virtually all-maternal organ systems. The condition is still the leading cause of maternal and infant morbidity and mortality.¹² Several studies have examined the etiology and pathophysiology of preeclampsia, but the exact mechanism remains unknown. Furthermore, several studies reported the possible association between genetic factors and preeclampsia.^{13,14}

In the present study, we investigated the genotypes of four candidate genes, AGT, MTHFR, *factor Leiden*, and prothrombin gene in the same patients with history of preeclampsia. Our results demonstrated that pregnant woman with both homozygous for AGT gene mutations and MTHFR gene mutations have a higher risk for preeclampsia comparing to the controls with other genotypes.

Jeunemaitre et al.¹⁵ were the first to report the genetic association between the AGT gene and hypertension. Ward et al.⁸ proposed that preeclampsia could be viewed as a pregnancy-induced proteinuric hypertension, and thus the condition could be associated with AGT gene mutation. They were able to demonstrate a significant association between preeclampsia and a molecular variant of AGT, M235T. Based on this finding they suggested that increased concentration of angiotensinogen in individuals carrying variants of AGT, such as M235T, might be associated with increased production of angiotensinogen. They also argued that chronic stimulation in M235T carriers could increase vascular tone and promote vascular hypertrophy, consequently causing preeclampsia during pregnancy. In Japanese, the frequency of M235T is higher than in Caucasians, but as in Caucasians, several reports confirmed the association between M235T mutation and preeclampsia,^{8,15} but

others could not confirm these findings.^{16,17}

Hyperhomocysteinemia has been identified as an important risk factor for occlusive vascular disease,¹⁸ and some reports suggested a relationship between repeated fetal loss or abruptio placentae and hyperhomocysteinemia.¹⁹ *MTHFR* is one of the key enzymes essential for normal homocystein metabolism, and abnormalities in this enzyme can lead to hyperhomocysteinemia. Frosst et al.²⁰ were the first group to identify a common mutation in *MTHFR* gene (C677T) in hyperhomocysteinemia, and significantly higher concentrations of plasma homocystein in individuals homozygous for this mutation. Based on these findings, they suggested that this mutation is an important genetic factor in vascular disease. Other groups later supported these results.⁴ Vascular damage consisting of thrombosis of small uterine arteries may be involved in the pathophysiology of preeclampsia.^{21,22} Souda et al.⁹ reported the association between preeclampsia and *MTHFR* gene mutation, C677T. However, other investigators could not confirm such relationship.²³⁻²⁷

Our results showed a significant correlation between mutations of these two genes and preeclampsia, and that the presence of *MTHFR* gene mutation has a strong impact on the incidence of preeclampsia. Our analysis also demonstrated that *AGT* and *MTHFR* gene mutations play a role in the pathogenesis of preeclampsia. Our results indicate that combination analysis of genetic mutations is potentially clinically useful for genetic screening of patients at risk for the development of preeclampsia. However, in our combination study we could not find additive effects both mutations; i.e., the calculated risk associated with the presence of both mutations was not higher than that of *MTHFR* mutations only. The reason for the lack of the additive effects may be simply due to the small sample size. To examine this issue, we are currently attempting to analyze more patients for mutations of these genes. It is probable that a group of genes could accurately predict the risk of preeclampsia, while individual genes may have only a limited predictive power. Therefore, combination analysis of only two genes may not establish significant predictive power. However, available data suggest that a very common allele is necessary to explain the observed inheritance of preeclampsia. Thus, we believe in the existence of a few "major loci", and many "minor loci", and postulate that *MTHFR* gene may be at the front and *AGT* gene at the back in such a scheme.

Our results showed the lack of mutations of *factor Leiden* and prothrombin genes in our 165 patients with preeclampsia. These results are different from those of previous studies.²⁸ In Caucasian, several reports suggested the presence of a significant correlation between mutation of *factor Leiden* gene and preeclampsia.^{29,30} However, the majority of these studies were from European countries and to our knowledge, no such correlation has been previously reported in Asian-based studies. In this regard, Rees et al. reported the world distribution of *factor Leiden* mutation, and demonstrated confinement of *factor Leiden* mutation to European countries with the exception of two cases.¹¹ These genodemographic findings are in agreement with our results.

In conclusion, based on the results of the present study, we speculate that *AGT* and *MTHFR* are likely to be implicated in preeclampsia in Japanese women. We suggest that preeclampsia is a heterogeneous disease. To identify the underlying genetic factors associated with preeclampsia, one has to consider the variability in the incidence of preeclampsia among different countries and races, and that such variability probably reflects different prevalence of mutations of candidate genes.

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