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Association analysis of nine missense polymorphisms in the coagulation factor V gene with severe preeclampsia in pregnant Japanese women

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Abstract The Leiden mutation in the coagulation factor V (*F5*) gene associated with preeclampsia in Caucasians has not been found in Japanese populations. We examined the association of 20 missense polymorphisms in the *F5* gene in 133 pregnant Japanese women with preeclampsia and in 224 unrelated, healthy, pregnant Japanese women. Among nine polymorphisms identified in the subjects, the M385T and R485K polymorphisms were associated with preeclampsia ($P = 0.05$ and $P = 0.02$, respectively). Haplotype analysis indicated that the R485K polymorphism is truly associated with preeclampsia, whereas the association of the M385T polymorphism is due to linkage disequilibrium. Taken together with reports that the R485 allele yields poor factor V function in comparison with that of the K485 allele and that the *F5* Leiden mutation is associated with preeclampsia in Caucasian populations, the findings of the present study suggest that the *F5* gene is associated with preeclampsia in pregnant Japanese women.

Key words Preeclampsia · Polymorphism · Coagulation factor V · Haplotype · Japanese

Introduction

Preeclampsia, which is diagnosed by increased blood pressure together with proteinuria, is a pregnancy-specific syndrome that occurs in 3%–5% of pregnancies (Roberts and Cooper 2001) and is a leading cause of maternal and perinatal mortality. Associations of preeclampsia with polymorphisms in the angiotensinogen (*AGT*) (Kobashi et al. 1999a; Ward et al. 1993), coagulation factor V (*F5*) (Dizon-Townson et al. 1996; Grandone et al. 1997), lipoprotein lipase (*LPL*) (Hubel et al. 1999), glutathione S-transferase (*GSTP1*) (Zusterzeel et al. 2000), apolipoprotein E (*APOE*) (Nagy et al. 1998a), and endothelial nitric oxide synthase (*eNOS*) (Yoshimura et al. 2000) genes have been reported. We previously reported that preeclampsia is associated with polymorphisms in the 5,10-methylenetetrahydrofolate reductase (*MTHFR*) (Sohda et al. 1997) and plasminogen activator inhibitor 1 (*PAI1*) (Yamada et al. 2000) genes.

Human coagulation factor V (FV) circulates in the plasma as a 330-kDa single chain glycoprotein. Organization of the functional domains (A1-A2-B-A3-C1-C2) is similar to that of factor VIII. FV is activated by selective proteolytic cleavage that removes the large B domain, and FVa is inactivated by activated protein C (APC) (Rosing and Tans 1997). Association between preeclampsia and the Leiden mutation in *F5* has been reported several times (Dizon-Townson et al. 1996; Grandone et al. 1997; Mimuro et al. 1998; Nagy et al. 1998b; Rigo et al. 2000). The Leiden mutation is a single base mutation in the *F5* gene that results in an amino acid substitution (R506Q) at a predominant APC-cleavage site and is a risk factor for familial venous thrombosis (Rosing and Tans 1997). Because the Leiden mutation has not been identified in Japanese individuals (Kobashi et al. 1999b; Ohnishi et al. 2000; Ozawa et al. 1996; Rees et al. 1995; Ro et al. 1999), possible association between preeclampsia and the Leiden mutation in Japanese women cannot be investigated. However, we previously found an association between the R485K polymorphism in *F5* and preeclampsia in a Japanese population (Watanabe et al. 2001). Although our findings support association between the *F5* gene and preeclampsia, it is possible that the observed association reflects linkage disequilibrium between the polymorphism and the true susceptibility locus. To investigate this possibility, we examined possible associations between 20 nonsynonymous polymorphisms in the *F5* gene and preeclampsia.

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Subjects and methods

Samples

Peripheral blood samples were obtained from 133 women with preeclampsia and from 224 healthy pregnant volunteers. The subjects were unrelated Japanese. Samples were collected at the University of Tsukuba Hospital, Japan. The diagnosis of preeclampsia was based on the criteria of the American College of Obstetricians and Gynecologists (1996). None of the subjects had a history of hypertension or renal disease. The age-matched pregnant controls were apparently healthy and without hypertension or proteinuria. None of the control subjects had experienced preeclampsia during a pregnancy. All patients and controls were described in a previous report (Watanabe et al. 2001). The Ethics Committee of Tsukuba University approved the research protocol, and written informed consent was obtained from each subject.

Methods

Genomic DNA was isolated from peripheral blood leukocytes by phenol extraction. Missense mutations in the *F5* gene were identified from three databases (HGBASE, http://www.hgbase.de/; dbSNP, http://www.ncbi.nlm.nih.gov/SNP/; and the JSNP database, http://snp.ims.u-tokyo.ac.jp/index.html) (Table 1). The genotypes of the missense mutations were determined by restriction fragment length polymorphism (RFLP) analysis and direct sequence analysis after polymerase chain reaction (PCR) amplification (Table 2).

Statistical analysis

Deviation from Hardy-Weinberg equilibrium and differences in genotype and allele distributions between groups were evaluated by χ-squared test and Fisher's exact test. We

Table 1. Missense polymorphisms in the coagulation factor V (*F5*) gene in the dbSNP, HGBASE, and JSNP databases

dbSNP No.	HGBASE No.	JSNP No.	Exon	Polymorphism
rs6019	6014		3	D79H
rs6033	6116		8	M385T
rs6020	3439		10	R485K
rs6025	27		10	R506Q
rs6031	6647		13	P781S
rs6018	6670		13	N789T
rs4524	7038	IMS-JST011184	13	K830R
rs4525	7916	IMS-JST011183	13	H837R
rs6032	7946	IMS-JST011182	13	E897K
rs6005	7759		13	H1118Q
rs1800595			13	H1254R
rs1046712			13	I1257L
rs1800595	5003		13	H1299R
rs1046712	5002		13	L1302I
rs6007	7538		13	E1502A
rs6011	7559		15	T1657S
rs6034	7605		16	L1721V
rs6030	2465		16	V1736M
	7679		17	M1792I
rs6027	7024		25	D2194G

Table 2. PCR primers and genotyping method

Polymorphism	Primers	Method (enzyme)
D79H	5′-CTCAGGACCAGGAGGAATGG 5′-ATGGATGC TCAAGGGCATAT	RFLP (Nde I)
M385T	5′-ACATACAGTGAATCCCCGTA[a] 5′-ATGAGCATCTTTTTCTTTTA	RFLP (Rsa I)
R485K	5′-ACCCACAGAAAATGATGCCCAG 5′-TGCCCCATTATTTAGCCAGGAG	RFLP (Bsm AI)
P781S, N789T, K830R, H837R, E897K	5′-TCACCAACAAGCCACCACAG 5′-CCATCTCCCAACCAAAATCT	direct sequence
H1118Q	5′-TCTATGGATTTTGGCTGGAT 5′-CACTGAGCTCTGGAGAAGAGGATAT[a]	RFLP (EcoR V)
H1254R, I1257L, H1299R, L1302I	5′-CTTCAGACCCCAGTCACAGA 5′-TCATAGGGCACATAATCAAT	direct sequence
E1502A	5′-CCACTGAGACACCTCATTGGCGAGA[a] 5′-ACCTTGGGTCCCTTACGCTTAGCAT	RFLP (Hha I)
T1657S	5′-TATGAAAAATCATCAGAGGGAGAGA[a] 5′-CAATGAAAGTACCTACTGGGTTCAC	RFLP (Bsm AI)
V1721L	5′-ATGAGTGATTATCAGAAGAGCAAGG 5′-TGTAGTATTCCTTTTTGGCAGAGTA[a]	RFLP (Rsa I)
V1736M	5′-ACTACATAAGGACAGCAACATGCAT[a] 5′-CAAAGTCTGAGGAAAATACCGTGAA	RFLP (Acc I)
M1792I	5′-TCTGCTTTAGCCTCTCACTA 5′-CTCACCCACTCTTGCTCGTA[a]	RFLP (Rsa I)
D2194G	5′-ATTTGGCTTTCAGATTTTTG 5′-ATGTTCAATTCTAGTAGATA[a]	RFLP (EcoR V)

PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism
[a] Mismatch primers; mismatch is underlined

investigated linkage disequilibrium for all possible two-way comparisons of the polymorphisms with standard methods (D′ and r) (Akey et al. 2001). The following statistical values were determined with the Arlequin ver. 2.0 computer program (http://anthropologie.unige.ch/arlequin/): linkage disequilibrium (D), standardized disequilibrium (D′ = D/Dmax if D > 0; D′ = D/Dmin if D < 0), significance of linkage disequilibrium (χ-squared test), and differences in allele and haplotype distributions between patients and controls. Haplotype counts were estimated on the basis of Expectation-Maximization estimates of haplotype frequency. *P* values were obtained after more than 300,000 Markov chain steps. *P* values of less than 0.05 were considered statistically significant.

Results

There were 20 missense mutations in the *F5* gene in the three databases searched in the present study (Table 1). Nine of these polymorphisms were found among the study participants. In addition, we detected one novel silent mutation (4028C/T, S1285S). Genotype and allele distributions in patients and controls are shown in Table 3. The K830R, H837R, and E897K polymorphisms were in complete linkage disequilibrium; therefore, these polymorphisms are shown together in Table 3. The results of linkage disequilibrium tests are shown in Table 4. The H837R and E897K polymorphisms were excluded because of complete linkage disequilibrium with the K830R polymorphism. With the exception of the L1302I polymorphism, all the polymorphisms were in significant linkage disequilibrium ($P < 0.05$).

Genotype frequencies of polymorphisms in the control subjects did not differ significantly from those predicted by Hardy-Weinberg equilibrium. A statistically significant difference between the patients and the control subjects was observed in the allele frequencies of the R485K polymorphism ($P = 0.02$), and a difference between the patients and the control subjects in the allele frequencies of the M385T polymorphism was marginally significant ($P = 0.05$).

Because the M385T and R485K polymorphisms were in linkage disequilibrium, an association of preeclampsia with one polymorphism could actually reflect association with the other. To evaluate this possibility, we carried out haplotype analysis of these two polymorphisms (Table 5). Overall haplotype distributions differed significantly between the patients and the control subjects ($P = 0.003$). Both the T-K and M-K haplotypes were observed more frequently in the patient group than in the control group. However, opposite directions were observed between the M-K and M-R haplotypes, indicating that the M allele of the M385T polymorphism is not likely to be associated with preeclampsia.

Discussion

In the present study, we examined the association with preeclampsia of all nonsynonymous polymorphisms in the *F5* gene listed in public single-nucleotide polymorphism databases and found potential associations for the M385T and R485K polymorphisms. Because these two polymorphisms are in linkage disequilibrium with each other, it is possible that the association of one polymorphism really

Table 3. Genotypic and allelic distributions of *F5* gene polymorphisms

Polymorphism	Population	Genotype count			P^a	Allele count		P^a
M385T		MM	MT	TT		M	T	
	Patients	123	10	0	0.13	256	10	0.05
	Controls	192	30	2		414	34	
R485K[b]		KK	RK	RR		K	R	
	Patients	80	42	11	0.03	202	64	0.02
	Controls	102	98	24		302	146	
N789T		NN	NT	TT		N	T	
	Patients	117	16	0	0.48	250	16	0.30
	Controls	191	31	2		385	35	
K830R		KK	KR	RR		K	R	
H837R	Patients	82	42	9	0.77	206	60	0.78
E897K	Controls	139	74	11		352	96	
S1285S		TT	TC	CC		T	C	
(4028C/T)	Patients	70	51	12	0.97	191	75	0.86
	Controls	114	90	20		318	130	
L1302I		LL	LI	II		L	I	
	Patients	117	16	0	0.84	250	16	0.64
	Controls	193	30	1		416	32	
V1736M		MM	VM	VV		M	V	
	Patients	63	57	13	0.93	183	83	1.00
	Controls	104	100	20		308	140	
D2194G		DD	DG	GG		D	G	
	Patients	119	14	0	0.29	252	14	0.18
	Controls	189	33	2		411	37	

[a] *P* calculated by Fisher's exact test
[b] *P* previously reported by Watanabe et al. 2001

Table 4. Analysis of linkage disequilibrium for all possible two-way comparisons among eight polymorphisms (n = 357)

SNPs[b]	M385T	R485K (2.7 kb)[a]	N789T (10.0 kb)[a]	K830R (10.1 kb)[a]	S1285S (11.3 kb)[a]	L1302I (11.5 kb)[a]	V1736M (22.9 kb)[a]	D2194G (38.3 kb)[a]
M385T (0.06)		D′ = 0.835 r = 0.327 $P < 0.001$	D′ = 0.875 r = 0.799 $P < 0.001$	D′ = 0.574 r = 0.077 $P = 0.040$	D′ = 0.967 r = 0.386 $P < 0.001$	D′ = 0.654 r = 0.045 $P = 0.235$	D′ = 0.966 r = 0.362 $P < 0.001$	D′ = 0.875 r = 0.799 $P < 0.001$
R485K (0.29)			D′ = 0.611 r = 0.263 $P < 0.001$	D′ = 0.637 r = 0.522 $P < 0.001$	D′ = 0.648 r = 0.636 $P < 0.001$	D′ = 0.764 r = 0.318 $P < 0.001$	D′ = 0.605 r = 0.577 $P < 0.001$	D′ = 0.639 r = 0.274 $P < 0.001$
N789T (0.07)				D′ = 1.000 r = 0.148 $P < 0.001$	D′ = 0.973 r = 0.425 $P < 0.001$	D′ = 0.417 r = 0.032 $P = 0.407$	D′ = 0.971 r = 0.399 $P < 0.001$	D′ = 0.937 r = 0.936 $P < 0.001$
K830R (0.22)					D′ = 0.973 r = 0.811 $P < 0.001$	D′ = 0.905 r = 0.126 $P < 0.001$	D′ = 0.860 r = 0.672 $P < 0.001$	D′ = 0.910 r = 0.134 $P < 0.001$
S1285S (0.29)						D′ = 0.855 r = 0.145 $P < 0.001$	D′ = 0.915 r = 0.858 $P < 0.001$	D′ = 0.945 r = 0.414 $P < 0.001$
L1302I (0.07)							D′ = 0.734 r = 0.134 $P < 0.001$	D′ = 0.125 r = 0.009 $P = 0.804$
V1736M (0.31)								D′ = 0.971 r = 0.399 $P < 0.001$
D2194G (0.07)								

The H837R and E897K polymorphisms were excluded because of complete linkage disequilibrium with the K830R polymorphism
[a] distance from M385T polymorphism
[b] frequency of minor allele in parentheses

Table 5. Estimated haplotype frequencies of the M385T and R485K polymorphisms of the *F5* gene

Haplotype (M385T-R485K)	Patients (n = 266)	Controls (n = 448)
T-K	74.9%	66.8%
T-R	21.5%	25.9%
M-K	1.5%	0.5%
M-R	2.3%	7.0%

$P = 0.0029$ (global test of differentiation; 300,000 Markov steps)

reflects that of the other. With haplotype analysis, we observed the T-K and M-K haplotypes more frequently in preeclamptic patients than in control subjects. Our findings suggest that the association of the M385T polymorphism most likely reflects the true association of the R485K polymorphism, and, therefore, we concluded that the R485K polymorphism is associated with preeclampsia in our patients.

The M385T polymorphism is located in the region encoding the heavy chain of FV, which is involved directly in the generation of thrombin and the inactivation of FVa by APC (de Visser et al. 2000), and it was first reported by de Visser et al. (2000). They reported that the T385 allele is not associated with increased risk of venous thrombosis and that this variation is not conserved across species. These findings support our conclusion that the M385T polymorphism is not associated directly with preeclampsia.

The *F5* R485K polymorphism was first reported by Gandrille et al. (1995). There are differences in the frequencies of the R485K alleles among races (Helley et al. 1997); the frequency of the K485 allele is low in Caucasians and high in Asians (Helley et al. 1997; Hiyoshi et al. 1998). An association of the R485K polymorphism with thrombotic and cardiovascular diseases has been reported (Hiyoshi et al. 1998; Le et al. 2000). Hiyoshi et al. (1998) reported that the K485 allele is a risk factor for thrombosis in a Thai population, and Le et al. (2000) reported that the K485 allele confers increased risk for coronary artery disease in a Chinese population. In the present study, the frequency of the K485 allele was higher in the preeclampsia patients than in the control group (75.9% vs. 67.4%, $P = 0.02$). The R485K polymorphism is located in the alpha-loop of the A2 domain, which is close to the Leiden mutation (Le et al. 2000). The R485 allele is conserved in cows, pigs, and mice. A significant association between poor response to normalized APC and the R485K polymorphisms in normal populations has not been found (Gandrille et al. 1995; Helley et al. 1997). However, Le et al. (2000) reported that the K485 allele is associated with reduced response to normalized APC in patients with coronary artery disease but not in control subjects. Thus, it is possible that the R485K polymorphism differs functionally with respect to pathological conditions of the cardiovascular system. In the present study, the K485 allele was associated with preeclampsia, indicating that activation of intravascular coagulation is associated with preeclampsia.

It is possible that other polymorphism(s) in linkage disequilibrium with R485K may be associated directly with preeclampsia. One limitation of our study was that we did not examine polymorphisms not listed in the public databases.

In conclusion, our data, taken together with reports of association between the *F5* Leiden mutation and preec-

lampsia in Caucasian populations suggest that the *F5* gene is associated with preeclampsia.

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