Endocrine (2012) 42:700–707
DOI 10.1007/s12020-012-9699-7

ORIGINAL ARTICLE

Estrogen synthesis genes *CYP19A1*, *HSD3B1*, and *HSD3B2* in hypertensive disorders of pregnancy

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Received: 1 March 2012 / Accepted: 8 May 2012 / Published online: 26 May 2012
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Abstract Hypertension in pregnancy is a multifactorial disorder caused by a complex combination of environmental factors and several predisposing genes. Since estrogen modulates placental vascular development, estrogen synthases are considered plausible candidate genes. The aim of this haplotype-based case–control study was to estimate whether polymorphisms of the maternal estrogen synthesis genes (*CYP19A1*, *HSD3B1* and *HSD3B2*) are associated with preeclampsia (PE) and gestational hypertension (GH). To examine the genetic markers in 69 PE and 62 GH patients and in 155 age-matched, primiparous, healthy control subjects, genotyping of 5 SNPs for the *CYP19A1* gene (rs1870049, rs936306, rs700518, rs700519, and rs4646), 3 SNPs for the *HSD3B1* gene (rs3765945, rs6203, and rs1047303), and 2 SNPs for the *HSD3B2* gene (rs2854964 and rs1819698) was performed. For rs700158 of *CYP19A1*, the frequencies of the AG+GG genotype and the G allele were significantly higher in PE as compared to controls ($P = 0.037$, $P = 0.033$, respectively). Logistic regression analyses indicated that the AG+GG genotype of rs700158 was a PE risk factor (odds ratio = 2.15, $P = 0.026$). In addition, the frequency of the G–G haplotype established by rs700518–rs4646 was also significantly higher for PE ($P = 0.017$). These data suggest that the estrogen synthesis gene, *CYP19A1* is associated with PE in the Japanese population.

Keywords CYP19A1 · HSD3B1 · HSD3B2 · Preeclampsia · SNP

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Introduction

Hypertension in pregnancy (HP), which comprises preeclampsia (PE) and gestational hypertension (GH), is one of the most common and serious complications of pregnancy and can cause hypertensive cerebropathy, deep vein thrombosis, pulmonary embolism, lung edema, intrauterine growth retardation, and premature delivery. It is therefore considered to be a serious disease that affects both the mother and fetus. Although it occurs in about 5–10 % of pregnancies, the mechanism through which the disorder can induce its clinical symptoms is unknown. Recently, it has been reported that HP is indeed associated with the same pathophysiology as hypertension, and thus it has become an important topic in the field of gynecology worldwide [1, 2]. Accumulating evidence derived from

clinical, epidemiological, and experimental studies suggest that an estrogen deficiency plays a major role in the pathogenesis of hypertension in postmenopausal women [3]. Women with PE, particularly severe PE, have been reported to have lower estrogen levels during pregnancy [4, 5]. We previously investigated the relationship of PE with the estrogen receptor alpha (*ESR1*) and the estrogen receptor beta (*ESR2*) genes by examining single nucleotide polymorphisms (SNPs) in a Japanese population [6, 7]. Our findings demonstrated an association between the *ESR1* and *ESR2* gene polymorphisms and PE.

Estrogen synthesis is catalyzed by two 3β-HSD isoenzymes in the first stage. These isoenzymes are 93.5 % homologous and encoded by two different genes. The type 1 gene (*HSD3B1*) is almost exclusively expressed as 3β-HSD in the placenta and peripheral tissues, including in the mammary gland, prostate, and the skin. In contrast, the type 2 gene (*HSD3B2*) is predominantly expressed as 3β-HSD in the adrenal gland, ovary, and testis [8]. In the final stage, estrogen is catalyzed by aromatase (encoded by *CYP19A1*). We have previously shown that the *CYP19A1* is a susceptibility gene for essential hypertension [9]. It has already been reported that, in humans, SNPs of *CYP19A1* are associated with differences in the estrogen level [10]. Therefore, we hypothesized that the estrogen synthesis genes, *CYP19A1*, *HSD3B1*, and *HSD3B2* are the susceptibility genes of HP. The aim of the present study was to perform a case–control study in Japanese subjects to investigate relationships between the SNPs in the estrogen synthesis genes and HP.

Materials and methods

Subjects

The present study group consisted of 69 PE and 62 GH patients along with 155 age-matched, primiparous, healthy control subjects. All subjects had singleton pregnancies and lived in the Kanto district in Japan. They were recruited from patients and healthy volunteers visiting the Nihon University Hospital in Tokyo, and informed consent was obtained from each individual as per the protocol approved by the Human Studies Committee of Nihon University. PE was defined as hypertension with proteinuria occurring after the 20th week of gestation, but resolving by the 12th week postpartum. GH was defined as hypertension without proteinuria occurring after the 20th week of gestation, but resolving by the 12th week postpartum. Subjects were diagnosed with hypertension if they had a systolic blood pressure (SBP) above 140 mmHg and/or a diastolic blood pressure (DBP) above 90 mmHg on repeated prelabor measurements. We ascertained that all PE and GH patients were normotensive before 20 weeks of gestation and that they also had blood pressures that returned to normal in the puerperium. The proteinuria criteria was greater than 1+ on a dipstick test, greater than 2+ protein on a voided urine sample, and greater than 300 mg on a 24 h urine specimen or a protein/creatinine ratio of 0.3. These criteria are compatible with the fundamental characteristics of HP established by the International Society of the Study of Hypertension in Pregnancy (ISSHP) [11].

Genotyping

We selected 5 SNPs for the *CYP19A1* gene (rs1870049, rs936306, rs700518, rs700519, and rs4646), 3 SNPs for the *HSD3B1* gene (rs3765945, rs6203, and rs1047303), and 2 SNPs for the *HSD3B2* gene (rs2854964 and rs1819698) as the genetic markers (Figs. 1, 2). All SNPs were selected from the public databases that are available at the NCBI dbSNP and at the International HapMap Project websites (http://www.ncbi.nlm.nih.gov and http://www.hapmap.org, respectively). Since the linkage disequilibrium (LD) analysis, which was based on the JPT HapMap, found that the SNPs tagged for each locus were on the same block ($r^2 > 0.8$), this made it possible to perform a complete linkage block analysis. Genotyping for these SNPs was done using a kit from Applied Biosystems Inc., (Foster City, CA, USA). Blood samples were collected from all participants, and genomic DNA was extracted from the peripheral blood leukocytes by a phenol and chloroform extraction [12]. Genotyping was performed using TaqMan® SNP Genotyping Assays (Applied Biosystems Inc.). These were performed by the method of *Taq* amplification as has been previously described [13, 14]. All those involved with carrying out the genotyping were blinded to the phenotypic information.

Statistical analysis

All continuous variables were expressed as the mean ± SD. Differences in continuous variables between HP patients and non-HP subjects were analyzed by the Mann–Whitney *U* test. Differences in categorical variables were analyzed by Fisher's exact test. Differences in distributions of genotypes and alleles between HP patients and non-HP subjects were analyzed by Fisher's exact test. Based on the genotype data of the genetic variations, we performed LD analysis and haplotype-based case–control analysis by means of the expectation maximization (EM) algorithm and the software SNPAlyze version 3.2 (Dynacom Co., Ltd., Yokohama, Japan) [15, 16]. The pairwise LD analysis was performed using 3 SNP pairs. We used |D′| values of >0.5 to assign the SNP locations to 1 haplotype block. SNPs with an r^2 value of <0.5 were selected

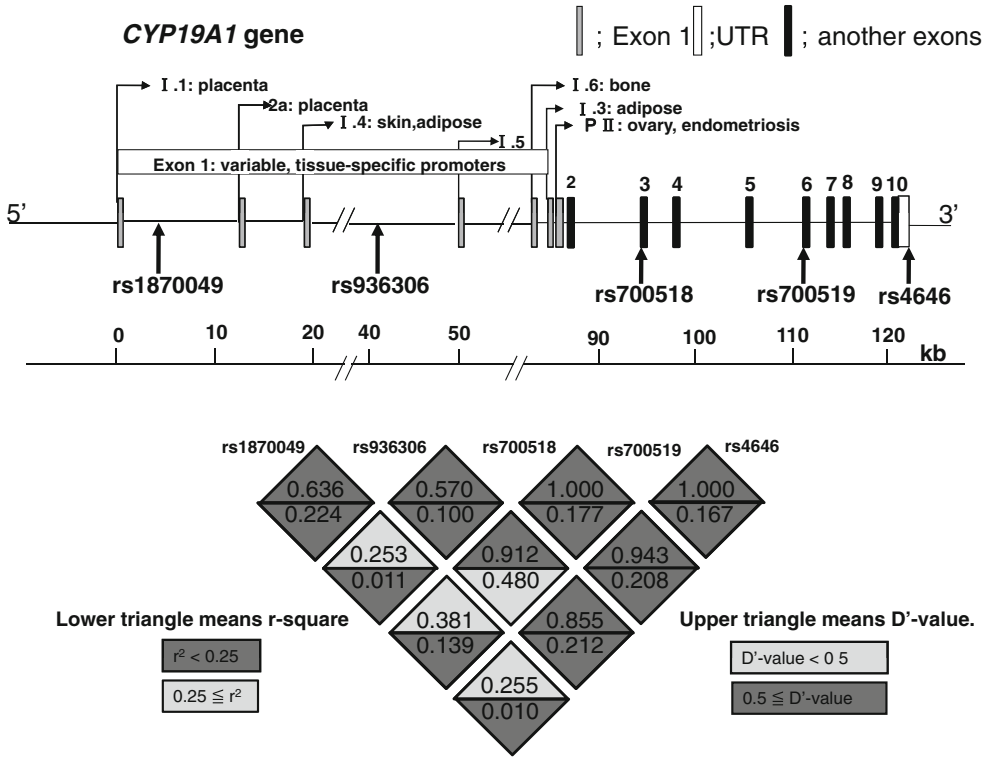

Fig. 1 Genomic structure and linkage disequilibrium patterns of *CYP19A1*

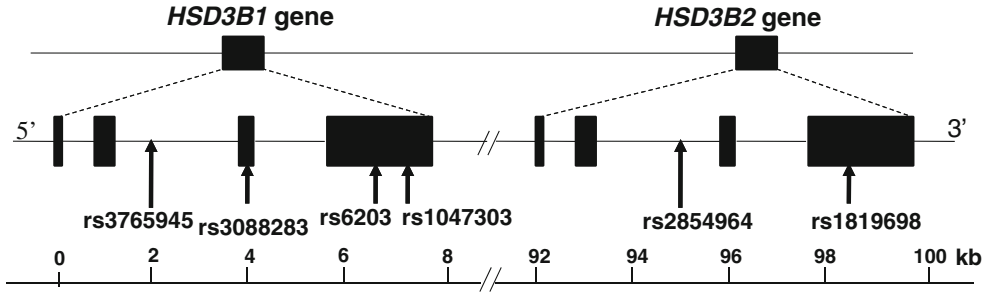

Fig. 2 Genomic structures of *HSD3B1* and *HSD3B2*

as tagged. In the haplotype-based case–control analysis, haplotypes with a frequency of <0.02 were excluded (Fig. 1). The frequency distribution of the haplotypes was calculated by performing a permutation test by the bootstrap method. In addition, logistic regression analysis was performed to assess the contribution of the major risk factors. Statistical significance was established at $P < 0.05$. Statistical analyses were performed by means of SPSS software for Windows, version 12 (SPSS Inc., Chicago, IL, USA).

Results

The clinical characteristics of the PE and GH patients and the control subjects are shown in Table 1. Table 2 shows the distributions of the genotype and allele frequencies for all the polymorphisms in the three groups. All the groups were within the Hardy–Weinberg equilibrium ($P > 0.05$). For all the polymorphisms in the GH subjects, no significant variation was observed for the individual genotype and allele distributions. However, in PE patients, the frequency of the AG+GG genotype and G allele of rs700518 of *CYP19A1* was significantly higher in the PE patients as compared to the controls ($P = 0.037$ and $P = 0.032$, respectively). To investigate the effect of these gene polymorphisms on PE, we performed a logistic regression analysis that examined BMI, age, and familial history of hypertension. As shown in Table 3, the AG+GG genotype of rs700518 was a major risk factor for PE (OR 2.15; 95 % CI 1.09–4.22; $P = 0.026$). Subsequently, we then established the haplotype using 5 polymorphisms in the *CYP19A1* gene. As shown in Fig. 1, all five polymorphisms were located in one haplotype block as all the |D′| values of the adjoining paired SNPs were beyond 0.5. Table 4 lists all the SNP combinations that exhibited significant

Table 1 Characteristics of study participants

	Control ($n = 122$)	PE ($n = 69$)	P values compared with control	GH ($n = 62$)	P values compared with control
Age (years)	29.48 ± 5.03	30.00 ± 5.63	0.524	31.01 ± 5.63	0.128
SBP (mmHg)	117.31 ± 15.69	165.58 ± 23.09	<0.001	151.77 ± 22.00	<0.001
DBP (mmHg)	72.59 ± 9.33	100.15 ± 17.26	<0.001	92.45 ± 17.49	<0.001
BMI before pregnancy (kg/m^2)	20.60 ± 2.92	22.92 ± 4.13	<0.001	24.71 ± 4.49	<0.001
BMI during pregnancy (kg/m^2)	24.69 ± 2.60	26.76 ± 3.99	<0.001	24.23 ± 4.11	<0.001
Body weight gain during pregnancy (kg)	10.42 ± 4.21	9.82 ± 6.32	0.483	8.74 ± 5.94	0.151
Gestational age at delivery (weeks)	38.67 ± 2.02	34.54 ± 4.14	0.000	37.03 ± 2.75	0.008
Birth weight of neonates (g)	3004.5 ± 454.8	1991.1 ± 112.2	<0.001	2660.3 ± 120.7	0.011
Apgar score	8.67 ± 0.61	7.11 ± 2.51	<0.001	8.21 ± 1.24	0.140
Frequency of primigravidas (%)	0.0	52.1	N.C.	49.2	NC
Family history of HT (%)	19.1	49.2	<0.001	21.3	0.708

Continuous variables are expressed as mean ± standard deviation. Categorical variable are expressed as percentage. The P values for the continuous variables were calculated by the Mann–Whitney U test. The P values for the categorical variables were calculated by Fisher's exact test

NC indicates a statistical result with no χ^2 calculation in the contingency table

PE preeclampsia, *GH* gestational hypertension, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *BMI* body mass index, *HT* hypertension

differences for the overall P value. The frequency of the G–G haplotype established by rs700518–rs4646 in the PE patients was significantly higher as compared to that in the control subjects ($P = 0.017$).

Discussion

Epidemiological research performed throughout the past decades has demonstrated that HP has a familial association. Based on these findings, it has been postulated that genetic control and inheritance play a major role in the pathology of HP [17]. Although genetic factors have been suggested to be responsible for more than 50 % of the liability to PE, the pattern of inheritance remains unclear [18, 19]. Moreover, there is still controversy with regard to whether PE and GH have the same pathophysiology.

This study was the first to evaluate the association of polymorphisms localized in aromatase with GH or PE. The main finding of the present study is that there is an association between the *CYP19A1* polymorphism and PE. In addition, the G–G haplotype established by rs700517–rs4646 of *CYP19A1* was more commonly observed in women in the PE group than in women of the control group. Conversely, our results suggested there were no significant effects of the *CYP19A1* polymorphisms on the susceptibility to GH. Our analyses also showed that the polymorphisms of the *HSD3B1* and *HSD3B2* genes were not statistically different in the controls when compared to the PE and GE groups. In this study, we applied strict criteria to the control group by only selecting healthy primigravida subjects who had no evidence of any medical or obstetrical complications. These strict criteria for the control group insured increased sample confidence for our study data.

Although we found significant associations between the *CYP19A1* gene polymorphism and PE in the present study, there was no significant association found between the polymorphism and GH. This is consistent with the supposition that there may be a different genetic basis for GH and PE, at least in terms of the *CYP19A1*-related inherited components. Similar findings have been found for the vascular endothelial growth factor (VEGF) gene and the angiotensin converting enzyme (ACE) gene polymorphisms, which have been shown to be associated with PE, but not with GH [20, 21]. Proteinuria is a major feature and a marker of severity in PE, but not in GH. In animal models, estradiol inhibits podocyte damage independent of the α-estrogen receptor [22]. In humans, with the onset of menopause and the reduction in estradiol synthesis, the progression of renal disease accelerates [23]. Therefore, on the basis that estrogen signaling is critical for the establishment and maintenance of the glomerular filtration barrier, it is reasonable to expect that *CYP19A1* polymorphisms would affect the development of PE, and not GH as we have found in the present study.

The placenta is the main source of pregnancy hormones; PE is associated with placental malfunction including altered levels of hormones such as estrogen and human chorionic gonadotropin. It has been shown that the PE

Table 2 Genotype and allele distributions in control, PE, and GH patients

Gene	Variants	Function			Control ($n = 122$)	PE ($n = 69$)	P values compared with control	GH ($n = 62$)	P values compared with control
CYP19A1	rs1870049	Intron SNP5	Genotype	AA	66 (0.54)	38 (0.55)		37 (0.60)	
				AG	50 (0.41)	29 (0.42)		21 (0.34)	
				GG	6 (0.05)	2 (0.03)	0.7991	4 (0.06)	0.6233
			Dominant model	AA	66 (0.54)	38 (0.55)		37 (0.60)	
				AG+GG	56 (0.46)	31 (0.45)	0.8967	25 (0.40)	0.4712
			Recessive model	GG	6 (0.05)	2 (0.03)		4 (0.06)	
				AG+AA	116 (0.95)	67 (0.97)	0.5033	58 (0.94)	0.6645
			Allele	A	182 (0.75)	105 (0.76)		95 (0.77)	
				G	62 (0.25)	33 (0.24)	0.7451	29 (0.23)	0.6708
	rs936306	Intron SNP4	Genotype	GG	47 (0.39)	21 (0.30)		27 (0.43)	
				GA	57 (0.47)	39 (0.57)		27 (0.43)	
				AA	18 (0.15)	9 (0.13)	0.1707	9 (0.14)	0.7689
			Dominant model	GG	47 (0.39)	21 (0.30)		27 (0.43)	
				GA+AA	75 (0.61)	48 (0.70)	0.2620	36 (0.57)	0.5687
			Recessive model	AA	18 (0.15)	9 (0.13)		9 (0.14)	
				GA+GG	104 (0.85)	60 (0.87)	0.7445	54 (0.86)	0.9319
			Allele	G	151 (0.62)	81 (0.59)		81 (0.65)	
				A	93 (0.38)	57 (0.41)	0.5397	45 (0.36)	0.6509
	rs700518	Silent mutation (Val 80 Val) SNP3	Genotype	AA	54 (0.44)	20 (0.29)		27 (0.44)	
				AG	55 (0.45)	37 (0.54)		26 (0.42)	
				GG	13 (0.11)	12 (0.17)	0.0885	9 (0.15)	0.610
			Dominant model	AA	54 (0.44)	20 (0.29)		27 (0.44)	
				AG+GG	68 (0.56)	49 (0.71)	0.0374	35 (0.56)	0.9265
			Recessive model	GG	13 (0.11)	12 (0.17)		9 (0.15)	
				AG+AA	109 (0.89)	57 (0.83)	0.1849	53 (0.85)	0.4455
			Allele	A	163 (0.67)	77 (0.56)		80 (0.65)	
				G	81 (0.33)	61 (0.44)	0.0325	44 (0.35)	0.6615
	rs700519	Missense mutation (Arg 264 Cys) SNP6	Genotype	CC	63 (0.52)	41 (0.59)		32 (0.52)	
				CT	54 (0.44)	24 (0.35)		23 (0.37)	
				TT	5 (0.04)	4 (0.06)	0.4211	7 (0.11)	0.1522
			Dominant model	CC	63 (0.52)	41 (0.59)		32 (0.52)	
				CT+TT	59 (0.48)	28 (0.41)	0.2996	30 (0.48)	0.9973
			Recessive model	TT	5 (0.04)	4 (0.06)		7 (0.11)	
				CT+CC	117 (0.96)	65 (0.94)	0.5946	55 (0.89)	0.0618
			Allele	C	180 (0.74)	106 (0.77)		87 (0.70)	
				T	64 (0.26)	32 (0.23)	0.5104	37 (0.30)	0.4633
	rs4646	UTR-3 SNP1	Genotype	GG	56 (0.46)	38 (0.55)		34 (0.55)	
				GT	54 (0.44)	29 (0.42)		23 (0.37)	
				TT	12 (0.10)	2 (0.03)	0.1574	5 (0.08)	0.5181
			Dominant model	GG	56 (0.46)	38 (0.55)		34 (0.55)	
				GT+TT	66 (0.54)	31 (0.45)	0.2233	28 (0.45)	0.2517
			Recessive model	TT	12 (0.10)	2 (0.03)		5 (0.08)	
				GT+GG	110 (0.90)	67 (0.97)	0.0772	57 (0.92)	0.6919
			Allele	G	166 (0.68)	105 (0.76)		91 (0.73)	
				T	78 (0.32)	33 (0.24)	0.0958	33 (0.27)	0.2901

Table 2 continued

Gene	Variants	Function			Control ($n = 122$)	PE ($n = 69$)	P values compared with control	GH ($n = 62$)	P values compared with control
HSD3B1	rs3765945	Intron	Genotype	TT	108 (0.89)	60 (0.87)		58 (0.94)	
				TC	12 (0.10)	9 (0.13)		4 (0.06)	
				CC	2 (0.02)	0 (0.00)	0.4594	0 (0.00)	0.4332
			Dominant model	TT	108 (0.89)	60 (0.87)		58 (0.94)	
				TC+CC	14 (0.11)	9 (0.13)	0.7491	4 (0.06)	0.2783
			Recessive model	CC	2 (0.02)	0 (0.00)		0 (0.00)	
				TC+TT	120 (0.98)	69 (1.00)	0.2850	62 (1.00)	0.3107
			Allele	T	228 (0.93)	129 (0.93)		120 (0.97)	
				C	16 (0.07)	9 (0.07)	0.9892	4 (0.03)	0.1827
	rs6203	Silent mutation (Leu 338 Leu)	Genotype	TT	64 (0.52)	36 (0.52)		33 (0.53)	
				TC	42 (0.34)	29 (0.42)		21 (0.34)	
				CC	16 (0.13)	4 (0.06)	0.2299	8 (0.13)	0.9952
			Dominant model	TT	64 (0.52)	36 (0.52)		33 (0.53)	
				TC+CC	58 (0.48)	33 (0.48)	0.9698	29 (0.47)	0.9216
			Recessive model	CC	16 (0.13)	4 (0.06)		8 (0.13)	
				TC+TT	106 (0.87)	65 (0.94)	0.1126	54 (0.87)	0.9679
			Allele	T	170 (0.70)	101 (0.73)		87 (0.70)	
				C	74 (0.30)	37 (0.27)	0.4672	37 (0.30)	0.9230
	rs1047303	Missense mutation (Thr 367 Asn)	Genotype	AA	114 (0.93)	67 (0.97)		59 (0.95)	
				AC	8 (0.07)	2 (0.03)		2 (0.03)	
				CC	0 (0.00)	0 (0.00)	NC	1 (0.02)	0.2441
			Dominant model	AA	114 (0.93)	67 (0.97)		59 (0.95)	
				AC+CC	8 (0.07)	2 (0.03)	0.2755	3 (0.05)	0.6421
			Recessive model	CC	0 (0.00)	0 (0.00)		1 (0.02)	
				AC+AA	122 (1.00)	69 (1.00)	NC	61 (0.98)	0.1595
			Allele	A	236 (0.97)	136 (0.99)		120 (0.97)	
				C	8 (0.03)	2 (0.01)	0.2820	4 (0.03)	0.9785
HSD3B2	rs2854964	Intron	Genotype	AA	82 (0.67)	47 (0.68)		40 (0.65)	
				AT	34 (0.28)	19 (0.28)		20 (0.32)	
				TT	6 (0.05)	3 (0.04)	0.9814	2 (0.03)	0.7461
			Dominant model	AA	82 (0.67)	47 (0.68)		40 (0.65)	
				AT+TT	40 (0.33)	22 (0.32)	0.8981	22 (0.35)	0.7145
			Recessive model	TT	6 (0.05)	3 (0.04)		2 (0.03)	
				AT + AA	116 (0.95)	66 (0.96)	0.8582	60 (0.97)	0.5947
			Allele	A	198 (0.81)	113 (0.82)		100 (0.81)	
				T	46 (0.19)	25 (0.18)	0.8589	24 (0.19)	0.9076
	rs1819698	UTR-3	Genotype	GG	46 (0.38)	26 (0.38)		21 (0.34)	
				GA	59 (0.48)	28 (0.41)		28 (0.45)	
				AA	17 (0.14)	15 (0.22)	0.3348	13 (0.21)	0.4719
			Dominant model	GG	46 (0.38)	26 (0.38)		21 (0.34)	
				GA+AA	76 (0.62)	43 (0.62)	0.9974	41 (0.66)	0.6094
			Recessive model	AA	17 (0.14)	15 (0.22)		13 (0.21)	
				GA+GG	105 (0.86)	54 (0.78)	0.1653	49 (0.79)	0.0990
			Allele	G	151 (0.62)	80 (0.58)		70 (0.56)	
				A	93 (0.38)	58 (0.42)	0.4523	54 (0.44)	0.3145

PE preeclampsia, *GE* gestational hypertension

NC indicates a statistical result with no Chi square calculation in the contingency table due to inclusion of a cell with no DNA sample

Table 3 Association between PE and AG+GG genotypes of rs700518

Risk factors	Crude OR (95 % CI)	*P*	Adjusted OR (95 % CI)[a]	*P*
AG+GG genotype	2.15 (1.09–4.22)	0.026	3.14 (1.34–7.36)	0.008
BMI before pregnancy	1.06 (1.02–1.10)	0.001	–	–
Family history of HT	1.75 (0.88–3.51)	0.112	–	–

OR odds ratio, *CI* confidence interval, *HT* hypertension, *BMI* body mass index

[a] Adjusted for BMI before pregnancy and family history of HT

Table 4 Haplotype-based case–control studies between control and PE using SNPs in CYP19A1 gene

Haplotype			Overall *P* value	Frequency (%)		*P* value
				Control	PE	
	rs700518	rs4646	0.047			
H1	A	G		35.5	31.9	0.431
H2	G	G		32.6	44.2	0.017
H3	A	T		31.8	23.9	0.094

Haplotypes with frequencies >0.02 were estimated using SNPAlyze software

P values were based on a permutation test using the bootstrap method

PE preeclampsia

estradiol content decreased by 37 % as compared to the levels found in women with a physiological pregnancy [24]. More recently, it has been demonstrated that the biological pathway responsible for the production of estradiol and the activity of placental aromatase is altered during PE leading to a deficiency of the placental aromatase [25]. As aromatase is an essential enzyme in the estrogen pathway, it is possible that variations in the aromatase gene (*CYP19A1*) can in some way give rise to different conditions in the endocrine environment that ultimately lead to impaired fertility. In fact, it has already been reported that the aromatase RNA levels in fat tissue are lower in individuals who are homozygotes for the G allele of rs700518 in *CYP191A* as compared to homozygotes for the C allele [26]. Our present results indicated that the G allele of rs700518 in *CYP19A1* was more common in PE patients than control subjects. In addition, the AG+GG genotype of rs700518 proved to be a major risk factor for PE. Since we could not measure the aromatase activity or aromatase RNA levels in the placenta samples, there is a possibility that the G allele of rs700518 is responsible for decreasing the aromatase expression in the placenta, thereby leading to PE.

It is worth noting that inherited liability to essential hypertension is well recognized, which raises the possibility that PE may share susceptibility genes with this condition [27]. In clinical practice, a substantial proportion of women affected by PE have been shown to have predisposing medical conditions, which include chronic hypertension [28]. We have previously reported that the *CYP19A1* gene polymorphisms, rs700518 and rs10046, as well as the haplotype constructed with rs1870049 and rs10046 in the *CYP19A1* gene are associated with essential hypertension in Japanese males [9]. Although there is a possibility that the results could be skewed due to the small sample numbers in the current study, our findings do suggest that the genetic backgrounds of PE and essential hypertension may very well be similar.

In summary, we have demonstrated an association between the maternal genotype of the *CYP19A1* gene and PE in a Japanese population. To definitively prove this, it will be necessary to replicate these findings in other study populations and ethnic groups. In addition, further studies will also need to be undertaken that examine the effects of the *CYP19A1* genotypes in infants along with studies that examine possible interactions between the maternal and infant genotypes. If our findings are confirmed, further studies to identify the relationships of the functional variants in these genes and the underlying biological mechanisms will be warranted.

Acknowledgments We would like to thank Ms. K. Sugama for her excellent technical assistance. This work was supported by a grant from the Toray Co., Ltd. and the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT)-Supported Program for the Strategic Research Foundation at Private Universities, 2008–2012.

Conflict of interest We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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