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GENETIC INFERTILITY

Single nucleotide polymorphisms and haplotypes of the genes encoding the *CYP1B1* in Korean women: No association with advanced endometriosis

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Abstract *Objective*: To investigate whether single nucleotide polymorphisms and its haplotypes of gene encoding CYP1B1 are associated with the risk of advanced endometriosis in Korean women.

Methods: We investigated 221 patients with histopathologically confirmed endometriosis rAFS stage III/IV and 188 control group women who were surgically proven to have no endometriosis. The genetic distribution of four different CYP1B1 polymorphisms at Ala119Ser, Leu432Val, $Asp^{449}(C > T)$, Asn453Ser were analyzed by polymerase chain reaction (PCR) and restriction fragment length polymorphism of PCR products. Haplotype analysis was also performed.

Results: We found no overall association between each individual CYP1B1 genotype or haplotype and the risk of endometriosis. Also, the odds ratio of each haplotypes of CYP1B1 showed no association with the risk of endometriosis.

Conclusions: These results suggest that CYP1B1 genetic polymorphism may not be associated with development of advanced endometriosis in Korean women.

Keywords Endometriosis · CYP1B1 · Genetic polymorphism · Haplotype

CYP1B1 genetic polymorphism may not be associated with development of advanced endometriosis.

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Introduction

Endometriosis may affect 7–10% of women of reproductive age, causing chronic pelvic pain and contributing to infertility [1]. Susceptibility to endometriosis is thought to depend on the complex interaction of genetic, immunologic, hormonal and environmental factors. Local and systemic release of estrogen regulates the growth of endometriotic tissues, and pro-estrogenic and anti-estrogenic hormonal stimuli appear to have important effects on the pathogenesis and clinical course of this disease [2, 3]. Endometriosis is also associated with a high degree of exposure to organic pollutants such as dioxins and polychlorinated biphenyls [4, 5]. The inter-individual differences in the inactivation of estrogen and its metabolites, or in the metabolism of diverse carcinogens,

such as dioxin, may result in higher lifetime exposures to hormone-dependent growth promotion or to cellular damage that may lead to the development of endometriosis.

Various cytochrome P450 (CYP) enzymes are involved in the hydroxylation of estrogen, among which CYP1A1 and CYP1B1 are two of the most important enzymes. CYP1B1 is a phase I enzyme that catalyzes the conversion of 17β-E2 to the catecholestrogens, 4-hydroxyoestradiol (4-OH-E2) and 2-hydroxyoestradiol (2-OH-E2) [6]. CYP1B1 has also been shown to be involved in the metabolic activation of certain environmental carcinogens, including polycyclic aromatic hydrocarbons and aryl amines [7]. Dioxins may cause endometriosis by transactivating the promoters of the genes that encode CYP1A1, CYP1A2 and CYP1B1 genes, thus increasing the amounts of CYP1A1, CYP1A2 and CYP1B1 in endometrial tissues that reach pelvic peritoneal surfaces due to retrograde menstruation, which might play an important role in the pathogenesis of endometriosis [8]. The linkage of endometriosis and genetic polymorphisms in the genes that encode CYP1A1 and CYP1A2 have already been addressed by several studies [8, 9].

The human CYP1B1 gene is located on chromosome 2 at 2p21–22 and comprises three exons and two introns [6]. To date, more than 50 single nucleotide polymorphisms (SNP) of this gene have been reported [10, 11] with several of them having been studied, including Ala119Ser, Leu432Val, Asp^{449}(C > T) and Asn453Ser. Some of the CYP1B1 polymorphisms have been shown to be associated with various cancers such as prostate, lung, endometrium and breast cancer [10, 12–15]. Many epidemiological studies support a link between endometriosis and cancers, specifically to estrogen-dependent breast cancers [16–18]. Thus, CYP1B1 is a candidate susceptibility gene of endometriosis because it is involved not only in the hydroxylation of E2 but also in the activation of polycyclic aromatic hydrocarbons and heterocyclic aromatic amines to form mutagenic intermediates. Therefore, CYP1B1 polymorphisms may contribute to interindividual differences in lifetime exposure levels to E2 metabolites and environmental carcinogens.

There has been only one association study between endometriosis and CYP1B1 genetic polymorphisms which found that Leu432Val and Asn453Ser were not associated with endometriosis [19]. But the association between endometriosis and the CYP1B1 haplotypes and the Ala119Ser and Asp^{449}(C > T) genetic polymorphisms has not been studied. In the present study we hypothesized that CYP1B1 polymorphisms are associated with the risk of endometriosis, and assessed whether CYP1B1 Ala119Ser, Leu432Val, Asp^{449}(C > T) and Asn453Ser polymorphism and their haplotypes are associated with the risk of endometriosis in Koreans.

Materials and methods

Materials

The study subjects were recruited between September 1996 and December 2004 at the Department of Obstetrics and Gynecology of Ewha Womans University Hospital. The endometriosis patients had undergone laparotomy or laparoscopy, and the disease was confirmed histologically from biopsies. Because no samples of other ethnic groups in Korea were recruited, this study was limited to a native Korean population in the same hospital. The patient group consisted of 221 unrelated Korean women who were diagnosed as advanced stage endometriosis (stage III and IV) according to the revised American Fertility Society classification [20]. All of the women participated in this study were non-familial. The indications for surgery in the 188 patient of the control group were benign ovarian cyst ($n = 177$) and paratubal cysts ($n = 11$). Patients with leiomyoma, adenomyosis, invasive carcinoma of the uterine cervix or ovarian cancer were excluded from the control group. Blood samples were collected for DNA extraction in both groups. The study protocol was approved by the Institutional Review Board on the Use of Human Subjects in Research at Ewha Womans University and informed consent was obtained from each patient.

Methods

DNA extraction

The blood samples were collected in vacutainer tubes containing EDTA, and stored at -20°C. Genomic DNA was extracted from 200 μl of blood using a QIA amp Blood kit (QIAGEN Inc., USA).

Analysis of CYP1B1 polymorphisms

For the analysis of CYP1B1 polymorphisms, polymerase chain reaction (PCR) procedure and restriction fragment length polymorphism of PCR products were designed. The primers of the four polymorphic sites and PCR conditions are summarized in Table 1. Each 20 μl of PCR mixture contained 0.1 μg of genomic DNA, 10 nmol/ml of each primers, 5 mmol/L of dNTP, 0.5 Units of Taq polymerase (Promega, Madison, WI), 200 mmol/L of Tris-HCl (pH8.3), 500 mmol/L of KCl, and 30 mmol/L of $MgCl_2$. In order, each PCR products were digested with 10 U/μl of *Ngo*MIV, 5 U/μl of *Fok*I, 10 U/μl of *Acu*I and 10 U/μl of *Mwo*I restriction enzymes (New England Biolabs Inc.). The DNA fragments were then seperated and visualized by electrophoresis on 2% agarose gel containing ethidium bromide.

Table 1 Summary of PCR primer sets and conditions for CYP1B1 polymorphisms

Polymorphisms	PCR primers	Initial denature	Denature	Annealing	Extension	Cycle	Final incubation
Ala119Ser	5′-TAA ACC CGC TGT CCA TCC A-3′(F) 5′-GAG TAG TGG CCG AAA GCC AT-3′(R)	94°C, 120s	94°C, 45s	60°C, 45s	72°C, 45s	35	72°C, 7 min
Leu432Val	5′-CAC TGC CAA CAC CTC TGT CT-3′(F) 5′-GCA GGC TCA TTT GGG TTG-3′(R)	95°C, 120s	95°C, 30s	60°C, 30s	72°C, 120s	35	72°C, 7 min
Asp^{449}(C > T)	5′-CAC TGC CAA CAC CTC TGT CT-3′(F) 5′-GCA GGC TCA TTT GGG TTG-3′(R)	95°C, 120s	95°C, 30s	60°C, 30s	72°C, 120s	35	72°C, 7 min
Asn453Ser	5′-CAC TGC CAA CAC CTC TGT CT-3′(F) 5′-GCA GGC TCA TTT GGG TTG-3′(R)	95°C, 120s	95°C, 30s	60°C, 30s	72°C, 120s	35	72°C, 7 min

Statistical analysis

Among control subjects, genotype frequencies for each CYP1B1 marker were examined for deviation from Hardy-Weinberg equilibrium (HWE) using the χ^2-test. The haplotype combination at CYP1B1 Ala119Ser, Leu432Val, Asp^{449}(C > T), Asn453Ser in the individuals was inferred using the maximum likelihood estimation method with the Haplotype program (http://www.people.fas.harvard.edu/∼junliu/Haplo) [21–22]. The differences in the distribution of the genotypes, haplotypes, and diplotype distributions between the groups were assessed by a chi-square test. Logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for CYP1B1 genotypes and to evaluate interaction of CYP1B1 genotypes with estrogen and dioxin exposures. The potential confounding factors that affect endometriosis risk, such as age, body mass index (BMI) was adjusted for using logistic models in the estimation of ORs. Adjustments for these factors did not produce substantial changes in the results. We reported the results with and without adjustments for these factors. All analyses were conducted using the Statistical Package for Social Science version 12.0 (SPSS Inc., Chicago, IL). Pairwise linkage disequilibrium (LD) between four CYP1B1 polymorphic loci was assessed using Haploview program (Version 3.2, available at http://www.broad.mit.edu/mpg/haploview/index.php).

Table 2 The odds ratio of Ala119Ser, Leu432Val, Asp^{449}(C > T), Asn453Ser polymorphisms of the *CYP1B1* genotypes

CYP1B1 polymorphism	Genotype/allele	Number (%) Control	Number (%) Endometriosis	OR (95% CI)	*p*-value	Adj OR (95% CI) Age, BMI	*p*-value
Ala119Ser	Ala/Ala	113 (60.1)	146 (66.1)	1	–	1	–
	Ala/Ser	68 (36.2)	69 (31.2)	0.785 (0.518–1.190)	0.254	0.760 (0.497–1.162)	0.206
	Ser/Ser	7 (3.7)	6 (2.7)	0.663 (0.217–2.029)	0.472	0.630 (0.203–1.949)	0.422
	Ala/Ser + Ser/Ser	75 (39.9)	75 (33.9)	0.774 (0.517–1.159)	0.213	0.748 (0.495–1.131)	0.169
	Ala allele	294 (78.2)	361 (81.7)	1	–	1	–
	Ser allele	82 (21.8)	81 (18.3)	0.804 (0.571–1.134)	0.214	0.784 (0.553–1.112)	0.172
Leu432Val	Leu/Leu	160 (85.1)	178 (80.5)	1		1	
	Leu/Val	25 (13.3)	41 (18.6)	1.474 (0.858–2.533)	0.160	1.447 (0.839–2.497)	0.184
	Val/Val	3 (1.6)	2 (0.9)	0.599 (0.099–3.632)	0.578	0.571 (0.094–3.468)	0.542
	Leu/Val + Val/Val	28 (14.9)	43 (19.5)	1.380 (0.819–2.326)	0.226	1.352 (0.799–2.286)	0.261
	Leu allele	345 (91.8)	397 (89.8)	1	–	1	
	Val allele	31 (8.2)	45 (10.2)	1.261 (0.781–2.038)	0.292	1.233 (0.761–1.998)	0.394
Asp^{449}(C > T)	CC	161 (85.6)	181 (81.9)	1	–	1	–
	TC	24 (12.8)	38 (17.2)	1.408 (0.810–2.449)	0.225	1.400 (0.810–2.449)	0.225
	TT	3 (1.6)	2 (0.9)	0.593 (0.098–3.594)	0.570	0.565 (0.093–3.435)	0.536
	TC + TT	27 (14.4)	41 (18.1)	1.318 (0.774–2.244)	0.310	1.305 (0.763–2.231)	0.330
	C allele	346 (92.0)	400 (90.5)	1		1	
	T allele	30 (8.0)	42 (9.5)	1.211 (0.742–1.977)	0.444	1.195 (0.730–1.957)	0.478[a]
Asn453Ser	Asn/Asn	185 (98.4)	217 (98.2)	1	–	1	–
	Asn/Ser	3 (1.6)	4 (1.8)	1.137 (0.251–5.144)	0.868	1	
	Ser/Ser	0 (0.0)	0 (0.0)	NA	NA	1.137 (0.251–5.144)	0.868
	Asn allele	370 (98.4)	434 (98.2)	1		1	
	Ser allele	6 (1.6)	8 (1.8)	1.137 (0.391–3.306)	0.814	1.137 (0.391–3.306)	0.814

Table 3 Frequencies of diplotype and haplotype with the risk of emdometriosis among Ala119Ser, Leu432Val, Asp^{449}(C > T) and Asn453Ser polymorphisms of the *CYP1B1* genotype

	Number (%)				Adj OR (95% CI)	
	Control	Endometriosis	OR (95% CI)	*p*-value	Age, BMI	*p*-value
Diplotype 355G > T,4326C > G, 4379C > T,4390A > G						
GCCA/GCCA-00	88 (47.1)	107 (48.9)	1	–	–	–
GCCA/GGCA-02	0 (0)	2 (0.9)	NA	0.999	NA	0.999
GCCA/GGTA-03	12 (6.4)	26 (11.9)	1.782 (0.850–3.734)	0.126	1.789 (0.849–3.770)	0.126
GCCA/TCCA-04	51 (27.3)	54 (24.7)	0.871 (0.541–1.401)	0.568	0.852 (0.524–1.387)	0.520
GCCA/GCCG-06	15 (8.0)	10 (4.6)	0.658 (0.293–1.479)	0.311	0.519 (0.220–1.225)	0.134
GTTA/GGTA-13	2 (1.1)	2 (0.9)	0.822 (0.114–5.958)	0.847	0.767 (0.105–5.589)	0.794
GGCA/TTCA-24	1 (0.5)	1 (0.5)	0.822 (0.051—13.338)	0.891	0.672 (0.041–10.994)	0.781
GGTA/TTCA-34	10 (5.3)	11 (5.0)	0.905 (0.367–2.229)	0.828	0.885 (0.357–2.190)	0.791
TTCA/TTCA-44	6 (3.2)	6 (2.7)	0.822 (0.256–2.640)	0.742	0.803 (0.247–2.60)	0.715
TTCA/TGTA-45	1 (0.5)	0 (0)	NA	1.000	NA	1.000
GCCG/TCCA-64	1 (0.5)	0 (0)	NA	1.000	NA	1.000
Haplotype 355G > T,4326C > G, 4379C > T,4390A > G						
GCCA-0000	254 (67.9)	306 (69.9)	1	–	–	–
GCTA-0010	2 (0.5)	2 (0.5)	0.830 (0.116–5.934)	0.853	0.782 (0.109–5.607)	0.807
GGCA-0100	1 (0.3)	3 (0.7)	2.490 (0.257–24.086)	0.431	2.046 (0.210–19.903)	0.537
GGTA-0110	24 (6.4)	39 (8.9)	1.349 (0.790–2.303)	0.273	1.343 (0.784–2.301)	0.282
TCCA-1000	76 (20.3)	78 (17.8)	0.852 (0.596–1.217)	0.379	0.837 (0.582–1.205)	0.339
TGTA-1110	1 (0.3)	0 (0)	NA	1.000	NA	1.000
GCCG-0001	16 (4.3)	10 (2.6)	0.519 (0.231–1.163)	0.111	0.499 (0.221–1.127)	0.095

Note. *p*-value (K^2) = 0.000 for diplotype; *p*-value (K^2) = 0.401 for haplotype.

Table 4 Frequencies of diplotype and haplotype with the risk of emdometriosis among Leu432Val, Asp^{449}(C > T) and Asn453Ser polymorphisms of the *CYP1B1* genotype

	Number (%)				Adj OR (95% CI)	
	Control	Endometriosis	OR (95% CI)	*p*-value	Age, BMI	*p*-value
Diplotype 4326C > G, 4379C > T, 4390A > G						
CCA/CCA	143 (76.1)	169 (76.4)	1	–	–	–
CCA/TCA	1 (0.5)	3 (1.4)	2.524 (0.260–24.526)	0.425	2.281 (0.230–22.589)	0.481
CCA/TTA	25 (13.3)	35 (15.8)	1.178 (0.673–2.060)	0.567	1.144 (0.650–2.013)	0.641
CCA/CCG	3 (1.6)	4 (1.8)	1.122 (0.247–5.091)	0.882	1.208 (0.259–5.641)	0.810
CCA/TCG	15 (8.0)	7 (3.2)	0.393 (0.156–0.989)	0.047	0.346 (0.135–0.884)	0.027
CTA/TTA	1 (0.5)	3 (1.4)	2.524 (0.260–24.526)	0.425	2.715 (0.276–26.681)	0.392
Haplotype 4326C > G, 4379C > T, 4390A > G						
CCA	330 (87.8)	387 (87.6)	1	–	–	–
CTA	1 (0.3)	3 (0.7)	2.558 (0.265–24.710)	0.417	2.761 (0.283–26.907)	0.382
TCA	1 (0.3)	3 (0.7)	2.558 (0.265–24.710)	0.417	2.323 (0.237–22.732)	0.469
TTA	26 (6.9)	38 (8.6)	1.246 (0.741–2.096)	0.407	1.228 (0.727–2.074)	0.442
CCG	3 (0.8)	4 (0.9)	1.137 (0.253–5.117)	0.867	1.225 (0.266–5.652)	0.794
TCG	15(4.0)	8 (1.8)	0.398 (0.160–0.988)	0.047	0.357 (0.143–0.892)	0.028

Note. *p*-value (K^2) = 0.394 for diplotype; *p*-value (K^2) = 0.256 for haplotype.

Table 5 Linkage disequilibrium (LD) between CYP1B1 polymorphisms in the Korean populations

CYP1B1	LOD	D′	r^2
355G > T–1719T > C	0.02	0.12	0.0
355G > T–4326C > G	0.29	0.415	0.0040
355G > T–4390A > G	0.38	1.0	0.0020
1719T > C–4326C > G	67.7	1.0	0.933
1719T > C–4390A > G	0.27	1.0	0.0010
4326C > G–4390A > G	0.28	1.0	0.0010

Results

We determined the frequency of the Ala119Ser, Leu432Val, Asp^{449}(C > T), Asn453Ser polymorphisms of the CYP1B1 gene among 188 healthy control subjects in the Korean women. The distribution of the three genotypes of Ala119Ser, Leu432Val, Asp^{449}(C > T) and Asn453Ser, polymorphisms of the CYP1B1 genes were consistent with a Hardy–Weinberg equilibrium. The allelic frequencies of Ala119Ser is 0.782 for Ala and 0.218 for Ser, allelic frequencies of Leu432Val is of 0.918 for Leu and 0.082 for Val, allelic frequencies of Asp^{449}(C > T) is 0.920 for T and 0.080 for C and allelic frequencies of Asn453Ser is 0.992 for Asn and 0.008 for Ser encoding alleles. None of the patients or controls had homozygous variant type genotype (Ser/Ser) of CYP1B1 Asn453Ser polymorphisms in this study.

Genotype frequencies did not show significant differences between the endometriosis group and the control group. Using the homozygous wild type genotype as a reference group for each polymorphisms, the odds ratio of homozygous variant genotype, heterozygous plus homozygous variant type genotype showed no significant differences, even after adjusting for age and BMI (Table 2).

Individual haplotype and diplotype of SNPs were determined using Haplotyper program. Using four SNPs of Ala119Ser (355G > T), Leu432Val (4326C > G), Asp^{449} (C > T), Asn453Ser (4390A > G) polymorphisms for the analysis, only 7 haplotypes were found among 16 possible haplotypes. The most common haplotype and diplotype were GCCA and GCCA/GCCA. The other haplotype and diplotype were not significantly associated with the risk of endometriosis in this study. The last three SNP loci are close, so we analyzed using those loci, the most common diplotype and haplotype are CCA/CCA and CCA, which frequencies of diplotype CCA/CCA was 0.761 for control and 0.759 for endometriosis and haplotype CCA was 0.878 for control and 0.876 for endometriosis. Using the most common haplotype and diplotype as a reference group, other haplotypes and diplotypes were not significantly associated with the risk of endometriosis in this study (Table 3 and 4). Linkage disequilibrium among the four and three CYP1B1 SNPs was shown at Table 5.

Discussion

To our knowledge this is the first case–control study to investigate the association between CYP1B1 polymorphisms and endometriosis in Asian women, in which we found no associations between the risk of endometriosis and various CYP1B1 genotypes and haplotypes. With respect to endometriosis, a considerable number of studies on SNPs of candidate genes focusing on estrogen-metabolizing pathway and dioxin detoxification pathway have been published, among them 17-β-hydroxysteroid dehydrogenase type 1, catecho-O-methyltransferase, glutathione-S-transferase (GST) M1, GSTT1, GSTP1, and CYP1A1. However the results have not been consistent [8, 23–28].

Wild-type and variant-type CYP1B1 show significant differences in estrogen hydroxylation activity, which may result in different concentrations of 2-OH-E2, 4-OH-E2, and 16α-hydroxyoestradiol (16α-OH) [29]. Variant enzymes exceeded wild-type CYP1B1, with especially the activities for the 4-hydroxylation of estradiol being higher for variant type 2 combinations (Arg^{48}, Ser^{119}, Val^{432} and Asn^{453}) [30]. That result is consistent with the recent report of Shimada et al. [7], who examined the functional effects of two CYP1B1 polymorphisms (Ala119Ser and Leu432Val) on estrogen metabolism. They found that the gene expression was lowest in subjects carrying two wild-type CYP1B1 alleles, while it was highest in subjects carrying the CYP1B1 Val432Val genotype [7]. Moreover, the risk of breast cancer may be higher in long-term hormone users with the latter genotype [31]. However, in our study the frequency of the CYP1B1 Val432Val genotype was 1.2% and was not associated with an increased risk of endometriosis. This discrepancy between the studies might be attributable to the genotype distribution differing with ethnicity.

A recent case-control study of 32 endometriosis patients and 790 healthy controls in Austria showed that the risk of endometriosis was not significantly increased in carriers of the Leu432Val and Asn453Ser alleles [19]. Our results are consistent with these, with our study also being the first to show that the Ala119Ser and Asp^{449}(C > T) polymorphisms are not associated with endometriosis. The results of association studies of CYP1B1 polymorphisms and estrogen-dependent cancers have varied with ethnicity [10, 12, 13, 15, 31–33].

CYP1B1 is also induced by Benzo[a]pyrene as well as dioxin. Aryl hydrocarbon receptor(AhR) protein exists on both eutopic and ectopic endometrium in human, as well as the mRNA of AhR and AhR translocator (ARNT). Chemical substances such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) or aryl amines interact with the liganded-AhR/ARNT heterodimer and activate the phase I detoxification enzymes, CYP1A1, CYP1A2 and CYP1B1 [34]. Both the oxidation rates of benzo[a]pyrene and the inducibility tend to be higher for the Leu432Leu form of CYP1B1 than for

the Val432Val form [35]. But the effect of the CYP1B1 Leu432Val polymorphism on CYP1B1 gene expression and its inducibility are not consistent with other reports [36, 37]. Our results suggest that the CYP1B1 Ala119Ser, Leu432Val, Asp^{449}(C > T) and Asn453Ser polymorphisms do not influence the risk of endometriosis. Differences in the activations of environmental carcinogens caused by CYP1B1 polymorphisms should be studied in the future, including the levels of TCDD in individual subjects.

We also found that the CYP1B1 haplotypes were not associated with endometriosis, and to our knowledge this is the first study to investigate this association. The complete haplotypes of enzyme variants were analyzed so as to evaluate the functional consequences *in vivo* and the relation between genetic polymorphisms and the risk of endometriosis. GTG (Gly^{48}, Ser^{119} and Val^{432}; *CYP1B1* ∗ 6) and GTGG (Gly^{48}, Ser^{119}, Val^{432} and Gly^{443}; *CYP1B1* ∗ 7) haplotypes showed significantly reduced Vmax and increased apparent Km for both the 2- and 4-hydroxlyation of estradiol. But other haplotypes showed no significant alterations in the kinetic properties [36]. However, in our study, only one case in the control group had the TGTA (Ser^{119}, Val^{432}, Asp^{449}(T), and Asn^{453}) haplotype, and we did not perform the kinetic anlaysis of the 4- and 2-hydroxylation of the estradiol among the study group. Thus, we cannot compare the effect of haplotype differences or the functional differences by each haplotypes. And to our knowledge there are no reports about this information. On the other hand, the Asn453Ser allele is associated with lower intracellular protein levels and is degraded more rapidly than the other CYP1B1 variants by post-translational regulation. The individuals with the CGCG (Arg^{48}, Ala^{119}, Leu^{432} and Ser^{453}; *CYP1B1* ∗ *4*) haplotype display reduced metabolic activation of certain endogenous and exogenous carcinogens [38]. In Koreans, the allele frequency of the Ser variant of the Asn453Ser polymorphism is only 1.7%, substantially lower than that reported for other populations, which shows the ethnic variations. Thus, we could not compare the effect of GCG (Ala^{119}, Leu^{432} and Ser^{453}) haplotype. In our study population, the most frequent haplotypes were GCCA (68.5%), TCCA (18.8%), GGTA (7.7%) and GCCG (3.2%), and we found that these did not influence the CYP1B1 enzymatic effects.

There is considerable interest in the interaction between polymorphic genetic variants and endometriosis risk associated with environmental risk factors. Since CYP1B1 is involved in estrogen metabolism, the BMI was chosen based on the biological plausibility of CYP1B1 polymorphisms potentially influencing estrogen levels. Our results suggest that BMI does not modify the relationship between CYP1B1 genotypes and endometriosis. This result is consistent with McGrath et al, who studied the association between CYP1B1 polymorphisms and endometrial cancer [15].

In conclusion, we found no association between endometriosis and the genetic polymorphisms of CYP1B1 Ala119Ser, Leu432Val, Asp^{449}(C > T), and Asn453Ser, including their possible haplotypes. Further studies should focus on associations between the various polymorphisms and haplotypes of CYP1B1 by measuring the levels of dioxin and enzymatic activity of each CYP1B1 genes in patients with and without endometriosis.

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