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Cytochrome P450 1A1 (CYP1A1) polymorphisms and breast cancer risk in Korean women

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Abbreviations: CYP1A1, cytochrome P450 1A1; BMI, body mass index; OR, odds ratio

Abstract

Cytochrome P450 1A1 (CYP1A1) is involved in the 2-hydroxylation of estrogen, the hormone that plays a critical role in the etiology of breast carcinoma. We evaluated the associations between two *CYP1A1* polymorphisms [*Msp*I (rs4646903); Ile462Val (rs1048943)] and breast cancer in a multicenter case-control study of 513 breast cancer cases and 447 controls in Korea. Women carrying the T allele of the *CYP1A1 Msp*I polymorphism were found to have a 1.72-fold (95% CI 1.11-2.68) greater risk of developing breast cancer. No association was found between any *CYP1A1* Ile462Val polymorphism and breast cancer. Haplotype analysis of the two loci showed that the CA haplotype was associated with the lowest risk of breast cancer, and CA/CA diplotypes were associated with a lower risk of breast cancer [OR = 0.28 (0.13-0.61)] than others/others diplotypes. Moreover, this reduced risk was more pronounced among women with a lower body mass index (BMI) [OR = 0.18 (0.06-0.58)] or with a shorter lifetime exposure to estrogen [OR = 0.23 (0.07-0.81)]. The results obtained suggest that the *CYP1A1 Msp*I polymorphisms could affect susceptibility to breast cancer.

Keywords: Asian continental ancestry group; breast neoplasms; cytochrome P450 CYP1A1; Korea; polymorphism, genetic

Introduction

Genes involved in estrogen metabolism are prime targets of breast cancer etiological studies, because estrogen and its metabolites play critical roles in breast cancer initiation and promotion (Service, 1998; Zhu and Conney, 1998). Cytochrome P450 1A1 (CYP1A1) is involved in the conversion of estrone (E_1) and estradiol (E_2) to 2-hydroxyestradiol (2-OHE_1) (Cavalieri *et al.*, 2001). CYP1A1 also has aryl hydrocarbon hydroxylase activity which is responsible for metabolizing polycyclic aromatic hydrocarbons (PAH) to aryl epoxides (Law, 1990).

The *CYP1A1 Ile462Val* polymorphism in exon 7 and the *T6235C* polymorphism on the 3' non-coding region (*Msp*I) have been intensively studied in relation to breast cancer risk (Ambrosone *et al.*, 1995; Taioli *et al.*, 1995; Bailey *et al.*, 1998; Ishibe *et al.*, 1998; Huang *et al.*, 1999; Basham *et al.*, 2001; Krajinovic *et al.*, 2001; da Fonte de Amorim *et al.*, 2002; Laden *et al.*, 2002; Miyoshi and Noguchi, 2003; Hefler *et al.*, 2004; Li *et al.*, 2004; Boyapati *et al.*, 2005; Masson *et al.*, 2005). The Val allele of the *Ile462Val* polymorphism was found to be related with increased *CYP1A1* gene inducibility (Cosma *et al.*, 1993b; Crofts *et al.*, 1994), and the *Msp*I polymorphic site was observed to have an effect on gene inducibility in subjects with variant alleles when combined with the *Ile462Val* polymorphism (Crofts *et al.*, 1994). In this study, we evaluated the potential association between two polymorphisms of the *CYP1A1* gene and breast cancer risk in Korean women.

Materials and Methods

Study subjects and data collection

Breast cancer patients and controls were selected from three teaching hospitals in Seoul (Seoul National University Hospital, Borame Hospital, and the Asan Medical Center) between 1995 and 2001. Histologically confirmed incident breast cancer patients who agreed to participate in this study were potential cases, and patients that admitted or who visited the Department of General Surgery in the same hospitals with no previous or present cancer history were recruited as controls. Participants with a history of amenorrhea or oophorectomy were excluded from both groups during data analysis. Informed consents were obtained at the time of blood sampling, and information on demographic characteristics, education, marital status, reproductive history, family history of illness including cancer, smoking, alcohol drinking, and food consumption frequencies was obtained using a structured questionnaire administered by trained interviewers. In the present, 513 breast cancer patients and 447 controls were included.

Genotyping

DNA was extracted using standard methods from blood drawn into 10ml heparinized tubes and stored at -20°C until use. Polymorphisms of *CYP1A1* were determined using single base extension assays (SnapShot assays). PCR products were obtained using 500 nM oligonucleotide primers (*Msp*I: (F) 5'-GCTTGCATGCTTGCATAAGA-3', (R) 5'-TAATCCCAGCACTTTGGGAG-3'; codon 462: (F) 5'-GGTCAACCCATCTGAGTTCCTAC-3', (R) 5'-TCATGTCCACCTTCACGC-3') in a total volume of 20 μl of reaction mixture. The PCR amplification profile consisted of an initial denaturation at 95°C for 5 min, followed by 35 cycles of 30 s at 94°C, 60 s at 54°C (58°C for *Msp*I) and 120 s at 72°C.

Primer extension reaction was performed by combining 1 μl of exonuclease I and alkaline phosphatase treated PCR product with a 5 μl SNaPshot kit (which included DNA polymerase, fluorescently labeled ddNTPs, and control primers), 0.15 pmol extension primer (*Msp*I: 5'-TTTCACTGTAACCTCCACCTCC-3'; codon 462: 5'-CAAGCGGAAGTGTATCGGTGAGACC-3') and 3 μl water. The reaction mixture was incubated for 2 min at 94°C and then subjected to 25 amplification cycles (5 s at 95°C, 5 s at 50°C, and 5 s at 60°C). Aliquots of 1 μl of single base extension product and 9 μl Hi-Di formamide were combined in a 96-well 3100 optical microamp plate, and then loaded into a 3100 DNA sequencer (Applied Biosystems, Foster City, CA). Electrophoresis data were processed using Genescan Analysis version 3.7 (Applied Biosystems). Finally, genotyping was successfully performed for 493 cases and 437 controls for the *Ile462Val* polymorphism and for 380 cases and 286 controls for the *Msp*I polymorphism.

Statistical analysis

Odds ratios and 95% confidential intervals were estimated using unconditional logistic regression models adjusted for age, a family history of breast cancer, and lifetime exposure to estrogen. Haplotypes for *CYP1A1* were estimated using the Bayesian method with PHASE version 2.1 (Stephens *et al.*, 2001). Subjects who had missing data with at least one polymorphism were excluded from the haplotype analysis. The Chi-square test was used for comparing the haplotype frequencies of cases and controls. Diplotypes were categorized as others/others, others/CA, and CA/CA, because the CA haplotype was associated with the lowest risk of breast cancer.

Table 1. Characteristics of breast cancer cases and controls.

Participants characteristics	Cases (%) (n = 513)	Controls (%) (n = 447)	OR (95% CI)[a]
Age (years)			
-39	102 (20.7)	146 (33.4)	1.0
40-49	223 (45.2)	121 (27.7)	2.20 (1.44-3.38)
50-59	100 (20.3)	93 (21.3)	1.14 (0.68-1.90)
60-	68 (13.8)	77 (17.6)	0.98 (0.58-1.65)
Family history of breast cancer in first and second degree relatives			
No	475 (92.7)	423 (96.8)	1.0
Yes	36 (7.3)	14 (3.2)	2.36 (1.26-4.41)
Total lifetime estrogen exposure (years)			
< 27 years	176 (34.5)	203 (46.3)	1.0
≥ 27 years	334 (65.5)	235 (53.7)	1.35 (0.91-1.99)
Body mass index (BMI, kg/m^2)			
< 22.77	245 (47.8)	233 (52.1)	1.0
≥ 22.77	268 (52.2)	214 (47.9)	1.10 (0.84-1.44)
Cigarette smoking			
< 400 cigarettes /lifetime	458 (92.9)	404 (92.5)	1.0
≥ 400 cigarettes /lifetime	35 (7.1)	33 (7.5)	0.94 (0.57-1.57)

[a]adjusted for age, total lifetime exposure to estrogen, and a family history of breast cancer.

Table 2. Association between the *CYP1A1 Ile462Val* and *Msp*I polymorphisms and breast cancer risk.

CYP1A1 genotypes	Cases (%)	Controls (%)	OR (95% CI)[a]	*P* for trend
All participants				
*Msp*I (rs4646903)	(*n* = 380)	(*n* = 286)		
CC	60 (15.8)	73 (25.5)	1.0	
CT	173 (45.5)	118 (41.3)	1.80 (1.17-2.75)	
TT	147 (38.7)	95 (33.2)	1.72 (1.11-2.68)	0.03
CT + TT	320 (61.1)	213 (67.4)	1.76 (1.19-2.61)	
Ile462Val (rs1048943)	(*n* = 493)	(*n* = 437)		
Ile/Ile	252 (51.1)	232 (53.1)	1.0	
Ile/Val	213 (43.2)	175 (40.0)	1.17 (0.89-1.54)	
Val/Val	28 (5.7)	30 (6.9)	0.98 (0.56-1.71)	0.51
Ile/Val + Val/Val	241 (48.9)	205 (46.9)	1.14 (0.88-1.49)	
Number of risk alleles[b]	(*n* = 360)	(*n* = 276)		
0	95 (26.4)	105 (38.0)	1.0	
1-3	265 (73.6)	171 (62.0)	1.39 (1.04-1.85)	

[a]adjusted age, total lifetime estrogen exposure, and family history of breast cancer. [b]Val allele for *Ile462Val* and T allele for *Msp*I.

Results

The demographic characteristics and the known risk factors of breast cancer of study subjects are presented in Table 1. Significant differences between cases and controls were found for age distribution and a family history of breast cancer among first and second degree relatives.

The distributions of the *CYP1A1* gene polymorphisms are presented in Table 2. The genotype frequencies of the *Ile462Val* polymorphisms in controls did not deviate from Hardy-Weinberg equilibrium, however, the genotype frequencies of the *Msp*I polymorphisms in controls did deviate from Hardy-Weinberg equilibrium (P = 0.02). Overall, no association was found between the *Ile462Val* polymorphism and breast cancer risk. However, women with the T allele of the *Msp*I polymorphism showed a 1.76-fold higher risk of breast cancer (95% CI = 1.19-2.61). When the risk alleles of the two polymorphisms combined (Val allele for Ile462Val and T allele for *Msp*I), women who had at least one risk allele showed a 1.4-fold increase in risk for breast cancer compared to women who did not have risk allele.

Table 3 presents the distributions of the *CYP1A1* gene haplotypes in breast cancer cases and controls. Two alleles were found to be in strong linkage disequilibrium with each other (D' =0.77, $P < 0.001$) with 1,344 base pairs apart. Four haplotypes were estimated from the genotype data, and haplotype frequencies differed for cases and controls ($P_{Global\ test} < 0.001$). The frequency of the CA haplotype was lower in breast cancer cases than in controls. Compared to others/others diplotypes, others/CA diplotypes showed a reduced risk of breast cancer [OR = 0.72 (0.50-1.04)] (Table 4), and the CA/CA diplotype showed a further reduction in risk [OR = 0.28 (0.13-0.61)], and this trend toward a reduced risk was significant ($P < 0.001$).

Table 3. Distributions of *CYP1A1* haplotypes in breast cancer cases and controls.

Haplotypes[a]	Cases (%)	Controls (%)	aOR (95% CI)[b]	*P* (χ^2 test)
TA	418 (58.1)	273 (49.5)	1.0	
CG	170 (23.6)	132 (23.9)	0.91 (0.69-1.21)	
CA	105 (14.6)	127 (23.0)	0.59 (0.43-0.80)	
TG	27 (3.7)	20 (3.6)	0.82 (0.44-1.52)	< 0.001

[a]Composed of two polymorphic sites: *Msp*I T > C and *Ile462Val* A > G. [b]Adjusted for age, total lifetime exposure to estrogen, and a family history of breast cancer.

Table 5 presents the results of analyses stratified by hormone-related factors. Body mass index, age at menarche, and total estrogen exposure were divided about median values. The reduced risk of the CA/CA diplotype was pronounced for those with a low BMI (< 22.77 kg/m^2) [OR = 0.18 (0.06-0.58)], a short total estrogen exposure (< 27 years) [OR = 0.23 (0.07-0.81)], and for premenopausal women

Table 4. *CYP1A1* diplotypes and breast cancer risk.

Diplotype[a]	Cases (%)	Controls (%)	OR (95% CI)[b]	*P* for trend
Others/others	265 (73.6)	171 (61.9)	1.0	
Others/CA	85 (23.6)	83 (30.1)	0.72 (0.50-1.04)	
CA/CA	10 (2.8)	22 (8.0)	0.28 (0.13-0.61)	< 0.001

[a]Composed of two polymorphic sites: *Msp*I T > C and *Ile462Val* A > G. [b]Adjusted for age, total lifetime exposure to estrogen, and a family history of breast cancer.

Table 5. Association of *CYP1A1* diplotypes with breast cancer risk by selected breast cancer risk factors.

	Others/others		Others/CA		CA/CA	
	Cases/ controls	OR[b]	Cases/ controls	OR (95% CI)[b]	Cases/ controls	OR (95% CI)[b]
Body mass index (BMI, kg/m^2)						
< 22.77	129/83	1.0	43/43	0.73 (0.43-1.24)	5/11	0.18 (0.06-0.58)
≥ 22.77	136/88	1.0	42/40	0.71 (0.42-1.19)	5/11	0.35 (0.12-1.05)
Menopausal status						
Premenopausal	171/98	1.0	54/46	0.76 (0.47-1.23)	6/15	0.23 (0.08-0.62)
Postmenopausal	94/72	1.0	31/36	0.66 (0.37-1.18)	4/7	0.43 (0.12-1.56)
Lifetime estrogen exposure (years)						
< 27	98/74	1.0	30/39	0.72 (0.40-1.30)	4/12	0.23 (0.07-0.81)
≥ 27	167/97	1.0	55/44	0.76 (0.47-1.22)	6/10	0.33 (0.12-0.96)

[a]Composed of two polymorphic sites: *Msp*I T > C and *Ile462Val* A > G. [b]adjusted for age, total lifetime exposure to estrogen, and a family history of breast cancer

[OR = 0.23 (0.08-0.62)].

Discussion

Our results suggest that *CYP1A1 Msp*I polymorphisms may affect breast cancer susceptibility in Korean women, and haplotype analysis may provide more information on the risk assessment.

Several studies have been conducted on the association between polymorphisms of the *CYP1A1* gene and breast cancer risk (Ambrosone *et al.*, 1995; Taioli *et al.*, 1995; Bailey *et al.*, 1998; Ishibe *et al.*, 1998; Huang *et al.*, 1999; Basham *et al.*, 2001; Krajinovic *et al.*, 2001; da Fonte de Amorim *et al.*, 2002; Laden *et al.*, 2002; Miyoshi and Noguchi, 2003; Hefler *et al.*, 2004; Li *et al.*, 2004; Boyapati *et al.*, 2005; Masson *et al.*, 2005). Overall, the *Ile462Val* polymorphism was found to show a null or weak association with breast cancer risk (Masson *et al.*, 2005), whereas 1.36-5.22 fold increases in breast cancer risk were reported in subgroups of smokers or in women with higher serum polychlorinated-biphenyls (PCB) levels (Miyoshi and Noguchi, 2003). In our study, the proportion of women who smoked was low (7-7.5%), which concurs with the prevalence of smoking among Korean women (http://www.nso.go.kr/newnso/s_data/search_kosis.html), therefore subgroup analysis for smokers did not have sufficient statistical power (data not shown). The C allele of the *Msp*I polymorphism was found to be associated with a significantly higher risk of breast cancer in African-American women (Taioli *et al.*, 1995), in smokers (Ishibe *et al.*, 1998), and in Taiwanese postmenopausal women (Huang *et al.*, 1999), whereas it was found to be associated with a reduced risk in Japanese (Miyoshi and Noguchi, 2003) and Chinese women (Boyapati *et al.*, 2005), which concurs with the findings of the present study, whereas another study performed in the Caucasian and African-American population did not produce a significant result (Bailey *et al.*, 1998). Interestingly, substantial racial differences in variant allele frequencies and risk estimates were observed. Asian and African-American showed higher frequencies of variant allele of *Msp*I polymorphisms

(22-40%) than Caucasian women (11-12%) (Cosma *et al.*, 1993a; Taioli *et al.*, 1995; Ishibe *et al.*, 1998; Huang *et al.*, 1999; Boyapati *et al.*, 2005). Moreover, an opposite association was observed between *Msp*I polymorphisms and breast cancer risk in Caucasian and African-American women, although the individual associations for either race were not statistically significant (Bailey *et al.*, 1998).

It is known that the *Msp*I and *Ile462Val* polymorphisms are in linkage disequilibrium (Whitlock, 1999). However, no study has used haplotypes to assess breast cancer risk to date. A study in Chinese women used combinations of two polymorphisms and observed a significantly reduced risk of breast cancer in women homozygous for both variant alleles (CC for *Msp*I and GG for *Ile462Val*) compared with those homozygous for both wild-type alleles (TT for *Msp*I and AA for *Ile462Val*) (Boyapati *et al.*, 2005), which was the same direction with our result regarding *Msp*I genotype. Interestingly, risk reduction was prominent among postmenopausal women with a lower waist-to-hip ratio or a lower BMI (Boyapati *et al.*, 2005). However, in the present study, the CA haplotype was associated with the lowest risk of breast cancer.

Our result should be interpreted with caution, since genotype frequency for *Msp*I among control population was deviated from Hardy-Weinberg equilibrium. There has been one report for genotype frequency of *Msp*I polymorphism in Korean population (Hong *et al.*, 1998). Hong *et al*. (1998) reported a minor allele frequency of 0.3 for *Msp*I polymorphism in a control group consisted with 63 subjects. Therefore, the genotype frequency of this polymorphism among Korean population needs to be confirmed in other studies.

In conclusion, our study suggests that the *Msp*I polymorphisms are associated with breast cancer risk in Korean women. Further large-scales studies on this association are required to confirm the differential effects of the haplotypes of the *CYP1A1* gene among races.

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