Genetic polymorphisms of cytochrome P450 19 and 1B1, alcohol use, and breast cancer risk in Korean women

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A case–control study was performed to assess the potential influence of CYP19 Arg264Cys and CYP1B1 Leu432Val polymorphisms on breast cancer risk in a series of Korean breast cancer patients and controls. The results suggest that the CYP19 Arg264Cys polymorphism modifies breast cancer risk (OR = 1.5, 95% CI = 1.1 – 2.2), especially in association with alcohol consumption (P for interaction = 0.04), whereas the CYP1B1 Leu432Val polymorphism appears to play no role here.

Keywords: CYP19; CYP1B1; alcohol; breast cancer

Breast cancer is the second most frequent cancer in Korean women and its incidence is increasing (Yoo et al., 1998). Lifetime cumulative exposure to oestrogens is known as the most important risk factor for breast cancer (Yager, 2000). A variety of different enzymes are involved in the synthesis of oestrogen from cholesterol and further metabolism of oestrogen (Thompsen and Ambrosone, 2000). Polymorphisms of genes encoding for these proteins are regarded as the candidates for elevated breast cancer risk.

The CYP19 gene encodes aromatase, which catalyses the formation of oestrogens from testosterone and androstenedione. To date, several polymorphisms have been found in the CYP19 gene (i.e. Polymeropoulos et al., 1991; Sourdaine et al., 1994; Siegelmann-Danieli and Buetow, 1999; Healey et al., 2000; Miyoshi et al., 2000). One of these, a C-to-T variation in exon 7 resulting in an Arg264Cys amino-acid exchange, has been shown to be very common in Asians (Watanabe et al., 1997; Miyoshi et al., 2000) and could thus be an important modifier of breast cancer risk in this ethnic group.

The CYP1B1 enzyme is known to be involved in the formation of 4-hydroxyoestradiol, which is a catechol metabolite of oestrogen (Hayes et al., 1996). A C-to-G variation in exon 3 of the CYP1B1 gene results in a Leu432Val amino-acid exchange. The Leu/Leu genotype has been associated with increased breast cancer risk in an Asian population (Zheng et al., 2000) but controversial results have been reported as well (Bailey et al., 1998; Watanabe et al., 2000).

In this study, we have evaluated the potential influence of CYP19 Arg264Cys and CYP1B1 Leu432Val polymorphisms on the breast cancer risk among Korean women as an extension of our previous work in this study population (Park et al., 2000; Yim et al., 2001).

MATERIALS AND METHODS

Study subjects

The criteria of subject selection and details of data collection on lifestyle have been described elsewhere (Park et al., 2000; Yim et al., 2001). Eligible study population consisted of 389 incident breast cancer cases and 346 controls with no other known cancer or systemic disease, admitted in 1995–2001 to three teaching hospitals located in Seoul, Korea (Seoul National University Hospital, Borame Hospital, and Asan Medical Center). Each patient was frequency-matched to one control in the following age groups: under 30, 30 – 34, 35 – 39, 40 – 44, 45 – 49, 50 – 54, 55 – 59, 60 – 69, and over 70 years; 288 cases and 288 controls were selected.

Genotyping

DNA was isolated using standard methods from blood drawn into 10 ml heparinised tubes and stored at −70°C until use. The CYP19 genotypes were determined by dynamic allele-specific hybridisa-
tion system (DASH, Hybaid). Briefly, a 63 bp long amplification product was obtained using about 10 ng DNA as template in a polymerase chain reaction (PCR) with 20 pmol of oligonucleotide primer P1 (5’-TGC CAT AGA AGT CCT GTT GAC-3’) and 4 pmol of primer P2 (biotin-labelled 5’-TCT TCC AGT TCC TCT GCT-3’) (Bioneer: Seoul, Korea) in a total volume of 20 μl. The amplification conditions were: initial denaturation at 95°C for 5 min followed by 45 cycles of 15 s at 94°C and 30 s at 58°C (Multiblock System, Hybaid). The PCR products were hybridised with the following probes: 5’-AAA AAA GAC GCA GGA TT-3’ and 5’-AAA AAA GAT GCA GGA TT-3’. The CYP19 was genotyped for 241 cases and 290 controls recruited from 1995 to 1999 by a real-time PCR-based method as described by Bruning et al (1999).

Statistical analyses

Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated by unconditional logistic regression analysis adjusting for age, education, body-mass index, age at first full-term pregnancy, family history of breast cancer, duration of breast feeding, and alcohol consumption. Information on alcohol consumption was collected by three questions: (1) How frequently do you drink alcohol (i.e., per week, month, year)? (2) How long have you been drinking? (3) Have you ever quitted drinking? Alcohol intake was categorised as: never-drinking (never and less than once per month) or ever drinking (at least once per month). Interactions between known risk factors (alcohol consumption and age at first full-term pregnancy) and each genotype were evaluated by likelihood ratio test. The difference of two –2log L values of logistic models with and without interaction terms was referred to tables of χ² on one degree of freedom.

RESULTS

The distribution of established breast cancer risk factors was compared in cases and controls (Table 1). These two groups differed statistically significantly in education (OR = 2.1, 95% CI = 1.5–3.0), age at first full-term pregnancy (OR = 1.8, 95% CI = 1.1–2.9), family history of breast cancer in first and second degree relatives (OR = 2.6, 95% CI = 1.2–5.6), and alcohol consumption (OR = 1.5, 95% CI = 1.0–2.2). The distributions of CYP19 and CYP1B1 genotypes are shown in Table 2. The genotype distributions in the control subjects agreed with those predicted by the Hardy–Weinberg equilibrium. The frequency of CYP19 Cys allele-containing genotypes (39%) was somewhat lower than that previously found in Japanese women (52–54%) (Watanabe et al, 1997; Miyoshi et al, 2000), whereas the frequency of CYP19 Cys allele-containing genotypes was very low, less than 10% for Caucasians (Siegelmann-Danielli and Buetow, 1999; Healey et al, 2000). The frequency of CYP1B1 Val allele-containing genotype (21%), on the other hand, was similar to that (29%) found in Japanese women (Watanabe et al, 2000). However, it was rather surprisingly lower than that found in Chinese women (85%) (Zheng et al, 2000), Caucasians (70–81%), and African Americans (95%) (Bailey et al, 1998; Ko et al, 2001).

The CYP19 Cys allele-containing genotypes showed a significantly increased risk of breast cancer (OR = 1.5, 95% CI = 1.1–2.2), whereas the CYP1B1 Val allele-containing genotypes had no effect in this context (OR = 1.0, 95% CI = 0.7–1.6) (Table 2).

When the genotype effects were evaluated in relation with the known risk factors for breast cancer (alcohol consumption, age at first full-term pregnancy), a significant interactive effect was observed between CYP19 genotype and alcohol consumption (P for interaction = 0.044). Ever-drinking women with CYP19 Cys allele-containing genotypes showed a 3.3-fold risk (95% CI = 1.7–6.5) for breast cancer compared with never-drinking women with the Arg/Arg genotype (Table 3).

In contrast to the CYP19 genotypes, no interaction was observed between the known risk factors for breast cancer and the CYP1B1 genotypes. Neither was there a gene–gene interaction between the CYP19 and CYP1B1 genotypes.

### Table 1

|                          | Cases N (%) | Controls N (%) | OR (95% CI) |
|--------------------------|-------------|----------------|-------------|
| **Age**                  |             |                |             |
| ≤ 39                     | 79 (27.4)   | 79 (27.4)      |             |
| 40–49                    | 90 (31.3)   | 90 (31.3)      |             |
| 50–59                    | 74 (25.7)   | 74 (25.7)      |             |
| ≥ 60                     | 45 (15.6)   | 45 (15.6)      |             |
| **Education**            |             |                |             |
| Under high school        | 154 (54.2)  | 204 (71.6)     | 1.0         |
| At and over high school  | 130 (45.8)  | 81 (28.4)      | 2.1 (1.5–3.0) |
| **Age at first full-term pregnancy (FFTP)** |             |                |             |
| FFTP < 30                | 242 (84.0)  | 259 (90.2)     | 1.0         |
| Nulliparous or FFTP ≥ 30 | 46 (16.0)   | 28 (9.8)       | 1.8 (1.1–2.9) |
| **Family history of breast cancer in first and second degree relatives** |             |                |             |
| No                       | 263 (91.3)  | 278 (96.5)     | 1.0         |
| Yes                      | 25 (8.7)    | 10 (3.5)       | 2.6 (1.2–5.6) |
| **Alcohol consumption**  |             |                |             |
| <1/month                 | 208 (72.2)  | 229 (79.5)     | 1.0         |
| ≥1/month                 | 80 (27.8)   | 59 (20.5)      | 1.5 (1.0–2.2) |
| **Cigarette smoking**    |             |                |             |
| <400 cigarettes/lifetime | 270 (93.8)  | 271 (94.1)     | 1.0         |
| ≥400 cigarettes/lifetime | 18 (6.2)    | 17 (5.9)       | 1.1 (0.5–2.1) |

*ORs were not adjusted for other covariates.*
cases and controls), and inadequate statistical power, marked differences in frequency of Cys allele (52–54% for Japanese women, 39% for Korean women, and less than 10% for Caucasian), and different genotyping methods (single-strand conformation polymorphism, sequencing, and dynamic allele-specific hybridisation).

The Arg284Cys polymorphism is located in or near recognition site of CYP19 aromatase and thus, it might enhance the oestrogen synthesis and exposure to endogenous oestrogen. However, it is also be possible that CYP19 Arg284Cys polymorphism is in linkage disequilibrium with other important polymorphic site such as TATA repeat polymorphism (Kristensen et al, 1998; Haiman et al, 2000; Baxter et al, 2001). Watanabe et al (1997) reported, however, that Arg284Cys genotype did not affect the aromatase activity in vitro test so further studies on the potential role of this polymorphism in breast cancer aetiology are required.

One mechanism by which alcohol consumption may increase breast cancer risk is through increased circulating oestrogen and androgen levels (Singletary and Gapstur, 2001). In a controlled feeding study, serum levels of estrone sulphate and dehydroepiandrosterone sulphate were significantly increased by the consumption of 30 g of alcohol per day in postmenopausal women (Dorgan et al, 2001). Since CYP19 is also involved in the biosynthesis of oestrogen, the CYP19 genotype and alcohol consumption may synergistically increase breast cancer risk by affecting both synthesis and metabolism of oestrogen.

In contrast to the CYP19 genotype, we found no association between CYP1B1 genotype and breast cancer. While our finding is consistent with some previous studies (Bailey et al, 1998; Watanabe et al, 2000), Zheng et al (2000) reported that Chinese women with Leu/Leu genotype had a 2.3-fold elevated risk of breast cancer among 186 cases and 200 controls. This inconsistency might at least partly be explained by the remarkable difference in the frequency of Val allele-containing genotypes (21 vs 85%).

In conclusion, the results of this study support the hypothesis that CYP19 genotype and alcohol consumption play important roles in breast cancer development and that these factors may synergistically increase the risk of this malignancy in Korean women. Epidemiological studies with large sample sizes are, however, required to confirm these preliminary findings and to evaluate the role of the other CYP19 genotypes of potential interest in breast cancer development in this ethnic group.

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DISCUSSION
Our results suggest that both CYP19 genotype and alcohol consumption play important roles in breast cancer development, and that these factors could synergistically increase the risk of breast cancer in Korean women. The contrasting findings with previous studies (Watanabe et al, 1997; Siegelmann-Danieli S and Buetow, 1999; Healey et al, 2000; Miyoshi et al, 2000) may be because of the differences in subject selection (noncomparable controls in the above studies), sample size (less than 200 in both cases and controls), and inadequate statistical power, marked differences in frequency of Cys allele (52–54% for Japanese women, 39% for Korean women, and less than 10% for Caucasian), and different genotyping methods (single-strand conformation polymorphism, sequencing, and dynamic allele-specific hybridisation).

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