Genetic Polymorphism of Glutathione S-transferase P1 and Breast Cancer Risk

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To evaluate the potential association between the GSTP1 genotype and the development of breast cancer, a hospital based case-control study was conducted on Korean women. The study population consisted of 171 histologically confirmed incident breast cancer cases and 171 age-matched controls with no present or previous history of cancer. PCR-RFLP was used for the GSTP1 genotyping and statistical evaluations were performed using an unconditional logistic regression model. Postmenopausal women with the GSTP1 Val allele were found to have a reduced risk of breast cancer (OR = 0.3, 95% CI = 0.10 – 0.74). A significant interaction was observed between the GSTP1 genotype and alcohol consumption (p for interaction = 0.01); compared with never-drinking women with Ile/Ile genotype, ever-drinking women with the GSTP1 Val allele had almost a three-fold risk of breast cancer (OR = 2.9, 95% CI = 1.05 – 7.85), whereas never-drinking women with Val allele had half this risk (OR = 0.5, 95% CI = 0.27 – 0.93). Our findings suggest that the GSTP1 polymorphism influences individual susceptibility to breast cancer in the Korean women and this effect may be modified by alcohol consumption.

Keywords: Alcohol consumption, Breast cancer, Genetic polymorphism, GSTP1

Introduction

Breast cancer is the second most frequent cancer in Korean women, and its incidence is increasing (Yoo et al., 1998). The major risk factors of breast cancer are primarily related to reproductive events that influence lifetime estrogen exposure levels (Thompsen and Ambrosone, 2000). However, the carcinogenesis of breast cancer cannot be wholly explained by these factors, and in most cases, it is presumed to be related to environmental exposure (Lee, 2001). Thus, the identification of genetic susceptibility factors associated with the metabolism of environmental agents might provide further insight into the etiology of breast cancer (Smith et al., 1995).

Glutathione S-transferases (GSTs) are a superfamily of phase II enzymes that are involved in conjugating the reactive intermediates of endogenous and exogenous toxicants with soluble glutathione. Moreover, alterations in the structure, function, or expression of GST genes could alter the ability of a cell to inactivate carcinogens or mutagens, and thus modify an individual’s risk. Our previous study suggested that the GSTM1 and GSTT1 genotypes are important modifiers of susceptibility to breast cancer among premenopausal Korean women (Park et al., 2000), and here we extended this study to evaluate the potential effects of GSTP1 Ile105Val polymorphism in this context.

Glutathione S-transferase P1 is a major GST, which is ubiquitously expressed in both normal and tumor breast tissue (Forrester et al., 1990; Shea et al., 1990; Albin et al., 1993), and the GSTP1 gene has been mapped to a small region of chromosome 11q. Recently polymorphisms in exon 5 (Ile105Val) and exon 6 (Ala114Val) of the GSTP1 gene were identified (Zimniak et al., 1994), and both affected codons lie in close proximity to the hydrophobic binding site of GSTP1. Moreover, the 114Val variant has been demonstrated to have
either lower or higher specific activity and affinity than that of $^{106}$Ile depending on the substrate (Ali-Osman et al., 1997; Sundberg et al., 1998), whereas the Ala$^{114}$Val polymorphism seems not to influence enzyme activity.

Previous epidemiological studies on the potential association between genetic polymorphisms of GSTP1 and breast cancer have produced inconsistent results (Hezlsouer et al., 1998; Millikan et al., 2000; Mitrunen et al., 2001; Gudmundsdottir et al., 2001). These inconsistencies might be partly due to differences in population, and their exposures to factors of breast cancer development. We evaluated this issue for the first time in an Asian population.

Materials and Methods

**Study subjects** The study subjects consisted of a consecutive series of breast cancer patients and non-cancer controls admitted to three teaching hospitals located in Seoul, Korea (Seoul National University Hospital, Borame Hospital, and the Asan Medical Center), from March 1994 to September 1998. The study design and selection of study subjects have been described in detail elsewhere (Park et al., 2000). Women who were diagnosed as incident breast cancer cases with histopathological confirmation, and from whom a blood sample was available were selected as cases ($n = 204$). The controls ($n = 332$), who had no history of breast cancer, were recruited simultaneously at the same hospitals. Women with amenorrhea, a previous history of hysterectomy, oophorectomy, hormone replacement therapy, or hormone-related disease, such as thyroid problems were excluded. Those with benign breast tumor, other breast diseases (e.g., mastitis or a benign calcification), or other systemic problems like chronic liver diseases were also excluded from the controls. Using these criteria 189 cases and 233 controls were enrolled in the study. Each patient was then frequency-matched to control according to the following age groups: under 30, 30-34, 35-39, 40-54, 55-69, and over 70 years. Consequently, the final study population consisted of 171 cases and 171 controls.

Informed consent was obtained at the time of blood withdrawal. Information on demographic characteristics, education, marital status, family history of breast cancer in first and second relatives, reproductive factors, menstruation, lifestyle including alcohol consumption, and diet were collected using a questionnaire administered by trained interviewers.

**Genotyping** DNA was isolated using standard methods from blood drawn into 10 ml heparinized tubes and stored at $-70^\circ$C until use. The GSTP1 Ile$^{106}$Val genotypes were determined by PCR-RFLP as described by Saarikoski et al. (1998). Briefly, a 173 bp fragment was amplified using the primers 5'-CAG TGA CTG TGT GTT GAT CA-3' and 5'-TGC TCA CAT AGT TGG TGT AGA TGA GGG A(T)A-3'. Subsequently, aliquots of the PCR products were digested using SnaB I restriction enzyme (New England Biolabs, Beverly, USA) and run on agarose gels. The presence of the SnaB I restriction site identified the presence of the GSTP1 Ile$^{106}$Val allele and the absence of that restriction site identified the $^{106}$Ile allele. To confirm the reliability of the assay, 50 randomly selected samples were re-tested and the identical results were obtained.

**Statistical analyses** Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by unconditional logistic regression model. ORs were adjusted for age, education (at and over high school vs. under high school), body mass index, smoking, alcohol consumption, parity and age at the first full-term pregnancy (FFTP) (nullipara or FFTP $\geq 30$ vs. $< 29$), duration of breast feeding, and a family history of breast cancer. Subjects who had smoked more than 400 cigarettes in their lifetime were defined as ever-smokers. The frequency of alcohol intake was categorized as never-drinkers, never or less than once per month, and ever drinkers, at least once per month.

The frequencies of the GSTP1 Val/Val genotype were too small to evaluate the gene-dosage effect. Instead, the genotype data were divided into two groups (GSTP1 Ile/Ile vs. Ile/Val or Val/Val) in the statistical analyses to increase statistical power. The possible gene-environment interactions between the GSTP1 genotypes and other risk factors (e.g., body mass index, alcohol consumption, meat consumption, age at first full term pregnancy, and number of live-birth babies) on breast cancer development were evaluated using a likelihood ratio test.

**Results**

A higher education (OR = 2.7, 95% CI = 1.51-4.77), an older age at first full-term pregnancy (OR = 3.2, CI = 1.60-6.52), two or more live-birth babies (OR = 0.4, 95% CI = 0.25-0.79), and alcohol consumption (OR = 1.9, 95% CI = 1.16-3.25) were found to be significantly associated with the risk of breast cancer.

The GSTP1 genotype distribution in control subjects was in agreement with those predicted by the Hardy-Weinberg equilibrium ($p = 1.00$) (Table 1), and the frequency of GSTP1 Val allele containing genotypes in Korean women (34%) was lower than that reported for Caucasians (45-60%) (Hezlsouer et al., 1998; Millikan et al., 2000; Mitrunen et al., 2001; Gudmundsdottir et al., 2001).

Although no association was found between GSTP1 Val allele containing genotypes and breast cancer risk for the total study population, a significantly reduced risk of breast cancer was observed in postmenopausal women (OR = 0.3, 95% CI = 0.10-0.74) with these genotypes compared with women with GSTP1 Ile/Ile genotypes.

The GSTP1 Val allele showed a significant multiplicative interaction with alcohol consumption ($p$ for interaction $= 0.01$) (Table 2). Never-drinking women with the Val allele containing genotypes were found to have half the risk of never-drinking women with the GSTP1 Ile/Ile genotype (OR = 0.5, 95% CI = 0.27-0.93), and ever-drinking women with these genotypes were found to have almost a three-fold risk of developing breast cancer (OR $= 2.9$, 95% CI $= 1.05-7.85$). Therefore, the effect of alcohol consumption was only observed among those with the Val allele containing genotypes.

No significant interaction was observed between the GSTP1 genotypes and body mass index, meat consumption, age at first full-term pregnancy, or the number of live-births.
breast feeding, and family history of breast cancer.

Discussion

No significant overall association has been reported between the GSTP1 Ile<sup>105</sup>Val polymorphism in breast cancer in previous studies (Harries et al., 1997; Hezlsouer et al., 1998; Curran et al., 2000; Millikan et al., 2000; Gudmundsdottir et al., 2001; Krajnovic et al., 2001; Mitrunen et al., 2001). Our finding that the GSTP1 Val allele has a slight protective effect against breast cancer agrees with the findings of Millikan et al. (2000), and Mitrunen et al. (2001), but disagrees with the findings of Helzlsouer et al. (1998) and Gudmundsdottir et al. (2001), which found that the GSTP1 Val allele had the opposite overall effect. Inconsistent results might be that the enzyme encoded by GSTP1 Val allele exhibit different activity, affinity, and thermostability according to substrates. For example, a recent fundamental study reported that the GSTP1 Val variant showed lower catalytic activity for 1-chloro-2,4-dinitrobenzene (CDNB), but higher activity for 4-vinylpyridine and (+)-anti benzo[a]pyrene-7,8-diol-9,10-oxide. Therefore, it is possible that more reactive intermediate metabolites are generated by the GSTP1 Val variant during alcohol metabolism. However this issue remains to be further explored.

To conclude, although our study has several limitations, i.e., the moderate sample size and the use of hospital controls, it is the first epidemiological evaluation of the relationship between GSTP1 polymorphism and breast cancer in Asian women. Our results also suggest for the first time that the GSTP1 Ile<sup>105</sup>Val polymorphism may influence the alcohol consumption associated individual susceptibility to breast cancer.

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References


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