Null Results in Brief

No Apparent Association of *GSTP1* $A^{313}G$ Polymorphism with Breast Cancer Risk among Postmenopausal Iowa Women[1]

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Introduction

GSTP1[3] catalyzes the conjugating reactions of PAHs and their electrophilic compounds to facilitate their excretion (1). Burned foods and cigarette smoke contain mammary carcinogens such as PAHs (2). Mice treated with PAHs had an elevated risk of skin tumors, particularly among those without the *GSTP1* gene (3). The expression of the *GSTP1* gene has been observed in many human tissues including breast epithelium (4).

A polymorphic adenine to guanine transition at nucleotide 313 ($A^{313}G$) in exon 5 results in an isoleucine to valine substitution in codon 105 ($I^{105}V$) (5). This codon is located in the substrate-binding site of GSTP1, and the corresponding allozymes exhibited differential catalytic activities toward diverse substrates (5). Epidemiological studies associating *GSTP1* polymorphism with breast cancer risk are few and inconsistent (6, 7). To evaluate the role of this polymorphism and its joint effect with PAH exposure in the risk of breast cancer, we analyzed data from a case-control study conducted among postmenopausal women in Iowa.

Materials and Methods

A nested case-control study of breast cancer was conducted within the Iowa Women's Health Study, a prospective cohort study of 41,836 postmenopausal Caucasian women who completed a self-administered baseline questionnaire in January 1986. A supplementary survey on meat-eating habit was completed during 1995 to 1996 in 273 breast cancer cases diagnosed during 1992 to 1994 and 657 women randomly selected from the cohort members without any cancer diagnosis in 1992. Of them, blood samples were obtained from 488 women (156 cases and 332 controls). Genomic DNA from peripheral white blood cells was used to determine the genotypes of the *GSTP1* gene using the PCR-RFLP method. The primers for the PCR reactions were *GSTP1* forward 5′-ccagtgactgtgtgttgatc-3′ and reverse 5′-caaccctggtgcagatgctc-3′. The PCR reactions were carried out in a 50-μl mixture containing sample DNA, 20 mM Tris-HCl (pH 8.4), 5.0 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM deoxynucleotide triphosphate 1 unit of Taq polymerase, and 0.4 μM of each oligonucleotide primer. Amplification, which resulted in a 189-bp fragment, was achieved by 35 cycles of 30 s at 94°C, 30 s at 62°C, and 30 s at 72°C. At the end, the reactions were extended for 7 min at 72°C. Each PCR product (5 μl) was subjected to *Bsm*AI digestion and analyzed by gel electrophoresis (3% 2:1 Nosier/SeaKem agarose). The presence of the polymorphic *Bsm*AI restriction site yields 148- and 41-bp fragments, indicating the presence of the *G* allele.

ORs and their 95% CIs were derived from unconditional logistic regression models and were adjusted for potential confounding variables. Trend tests for dose-response across levels of the exposure variables were performed by treating ordinal-score variables as continuous variables in logistic regression models. Reported *P*s are based on two-sided probability tests, with a significance level of $P < 0.05$.

Results

The frequency of the variant allele was 29%, consistent with that reported from a previous study (7). There is no statistical significant association between $A^{313}G$ polymorphism and breast cancer risk (Table 1). This polymorphism was not found to modify the association of well-done meat intake or cigarette smoking with breast cancer risk.

Discussion

It was reported that the catalytic efficiency of the valine-containing allozyme was elevated in conjugating several carcinogenic intermediates of PAHs but reduced for other substrates, such as 1-chloro-2,4-dinitro-benzene (5). A recent study reported no clear relationship between the genotypes of *GSTP1* $A^{313}A$ polymorphism and the activities of corresponding allozymes (8). Furthermore, the 5′ promoter region of *GSTP1* contains GC-rich regions that are prone to be hypermethylated and lose gene expression (9). Therefore, *GSTP1* 5′-end hypermethylation may overwrite or mask the functional variations of GSTP1 $I^{105}V$ allozymes. The functional significance of the $A^{313}G$ polymorphism of the *GSTP1* gene remains unclear.

One potential concern of the study may be its low response rate. However, it is unlikely that selection bias can explain the null association, because the *GSTP1* genotype was unlikely to be associated with study participation.

Our study has an 80% statistical power to detect an OR of 1.74 for the *GSTP1 AG* and *GG* genotypes compared with the *AA* genotype at the significance level of 0.05. The statistical power to examine the interaction was further limited. Nevertheless, this study showed that there was no apparent associa-

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[3] The abbreviations used are: GSTP1, glutathione *S*-transferase π; OR, odds ratio; CI, confidence interval; PAH, polycyclic aromatic hydrocarbon.

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Table 1 Evaluation of *GSTP1* polymorphism and its modifying effect on the risk of breast cancer among postmenopausal Iowa women

	AA genotype		*AG* genotype		*GG* genotype	
	Case/Control	OR (95% CI)[a]	Case/Control	OR (95% CI)[a]	Case/Control	OR (95% CI)[a]
All subjects	87/170	1.0 (reference)	58/133	0.9 (0.6–1.3)	10/29	0.7 (0.3–1.4)
Trend test						$P = 0.27$
Meat doneness levels						
Rare/Medium	20/64	1.0 (reference)		14/58	1.0 (reference)	
Mostly well-done	19/36	1.6 (0.8–3.4)		21/37	2.4 (1.1–5.5)	
Consistently well- or very well-done	39/51	2.3 (1.2–4.4)		26/52	2.1 (1.0–4.4)	
Trend test		$P = 0.02$			$P = 0.07$	
Cigarette smoking						
Never	57/118	1.0 (reference)		45/111	1.0 (reference)	
Ever	28/50	1.1 (0.6–2.0)		22/48	1.2 (0.6–2.2)	
Former	18/28	1.2 (0.6–2.4)		16/29	1.5 (0.7–3.0)	
Current	10/22	0.9 (1.4–2.2)		6/19	0.8 (0.3–2.2)	

[a]Adjusted for age, number of live births, and waist:hip ratio.

tion of *GSTP1* polymorphism with breast cancer risk, and the modifying effect of *GSTP1* polymorphism, if any, on the association of well-done meat intake and smoking with breast cancer risk was unlikely to be substantial.

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