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GSTP1 Polymorphism, Cigarette Smoking and Cervical Cancer Risk in Korean Women

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Previous studies have suggested that glutathione S-transferase (GST) genotypes may play a role in determining susceptibility to cervical cancer, though the data have often been conflicting. The objective of this study was to examine the effect of GSTP1 polymorphism on cervical carcinogenesis. The studied subjects, patients who were pathologically diagnosed with invasive cervical cancer yielding positive results for human papillomavirus (HPV) (n=342), were compared to healthy, normal, female controls (n=707). DNA from peripheral blood samples from studied subjects whose GSTP1 specific sequences had been determined by PCR with allele-specific primers were reviewed in comparison with the normal controls. The genetic susceptibility of GSTP1 (11q 13.1) in cervical carcinogenesis was determined by examining the effect of gene and environmental factors by the different histopathologic types of invasive cervical cancers. In assessing polymorphism GSTP1, the percentages of individuals homozygous for the A allele, homozygous for the G allele, and heterozygous for the two alleles were 66.8%, 3.9%, and 29.3%, respectively, in the control group, and 64.3%, 4.1%, and 31.6%, respectively, among in women with cervical cancer. Compared with GSTP1 G allele positive (GA or G/G), the odds ratio (OR) (95% confidence interval) for GSTP1 A/A was 1.0 (0.7-1.4) for invasive cervical cancer. However, the risk increased with GSTP1 A/A among ever smokers (3.9, 1.7-8.9, p-value=0.0012) compared with GSTP1 G allele positive among nonsmokers. In particular, this risk was higher among women with squamous cell carcinoma (4.7, 2.0-10.8, p=0.0003). Polymorphism of GSTP1 among smoking women was associated with a higher risk of developing cervical cancer.

Key Word: Polymorphism, GSTP1, cervical cancer, smoking

INTRODUCTION

A number of genetic and biochemical studies have shown that human papillomavirus (HPV) E6 and E7 proteins cooperatively exert cellular immortality and transformation by interfering with the function of the cellular tumor suppressor proteins.[1,2] A polymorphism in the GSTP1 gene in which valine replaces isoleucine has been reported to affect the activity of the enzyme for some but not all electrophilic substrates.[3,4] The GSTP1 gene appears to be particularly susceptible to carcinogen from cigarette smoking, and cigarette smoking is an epidemiologic risk factor for cervical cancer.[5,6] However, no study has investigated whether GSTP1 is a potential genetic link between cigarette smoking and cervical cancer.

This study examined the hypothesis that the genetic susceptibility of GSTP1 on cervical cancer may differ among various histopathologic types of invasive cervical cancer.

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MATERIALS AND METHODS

Participants

The studied population consisted of Korean women who underwent outpatient and/or inpatient evaluation at the Catholic University Medical Center from October 2000 to December 2001. During this period, 1,572 patients agreed to participate in a case-control study to assess the risk factors for cervical cancer. As of December 2001, data was collected on 308 patients with HPV-infection associated invasive cervical cancer (adenocarcinomas, 53, and squamous cell carcinomas, 287). All cases were classified histopathologically according to the 1991 Bethesda classification. The control group consisted of 707 healthy patients who underwent routine physical examination at the Catholic University Medical Center at the same time.

Data collection protocols

Age, gender, education, menarche, first sexual intercourse, number of children, menopausal status, family history, cigarette smoking history, spouse's smoking, and alcohol consumption were obtained from an interviewer-administered questionnaire. Each individual's height and weight were measured and body mass index (kg/m^2) was calculated.

Women were identified as smokers if they had a lifetime history of smoking more than one hundred cigarettes. Exposure to passive smoking was assessed by questioning spouse's smoking history.

Blood samples were drawn between 8 and 11 AM after overnight fasting while the subject was in a supine position after 5 minutes of supine rest. For the purpose of genetic testing, whole blood was collected in 1 volume of EDTA and centrifuged at 2,500 × g for 15 minutes within 2 hours of blood collection. Informed consent was previously obtained.

Genomic DNA preparation and genotyping of GSTP1 genotype

The genomic DNA was prepared from peripheral blood samples using a Puregene blood DNA kit (Gentra Inc. Minneapolis, MN55447, U.S.A.) following the manufacturer's protocol. The genotypes of the patients and control samples were assayed by single base primer extension assay using a SNaPShot assay kit according to the manufacturer's recommendation (ABI). Briefly, the GSTP1 region of interest was amplified with PCR reaction with 5′- CTCCGCTGCAAATAC (Forward) and 5′- TCTCCCTCATCTACA (Reverse) primer pairs. The PCR conditions were: 10 min at 94℃ for 1 cycle, 30 cycles of 94℃ for 30s, 63℃ for 1 min, 72℃ for 1 min followed by 1 cycle of 72℃ for 7 mins. After amplification, the PCR products were treated with 2.0 units of SAP (shrimp alkaline phosphatase) and 2.0 units of EXO I (Exonuclease I) at 37℃ for 60 minutes and 72℃ for 15 minutes. The SNP genotyping primer (5′-GTCTCCAGCCTTCCTGGGAAGAAC) was added to the reaction products with an appropriate amount of SNaPShot mix and cycled on the PCR machine for 25 cycles of 96℃ for 10s, 50℃ for 5s, 60℃ for 30s. Half a unit of SAP was added to the product and incubated for 60 min at 37℃ followed by 15 minutes at 72℃. The final products were analyzed on an ABI 3700 automated sequence analyzer. Genescan program was used to call genotypes for each sample.

Statistical analysis

Statistical analysis was conducted using the package SAS for windows, version 6.1. Throughout this study a p-value of <0.05 was deemed to be significant. The t-test was used to assess the differences between the controls and study groups for continuous covariates such as age, height, weight, body mass index, education, menarche, age at first intercourse, and number of children. Multiple logistic regression (MLR) analysis was performed with these covariates to assess the independent effect of each environmental factor or genotype, and their combined effect. Odds ratios (OR) with 95% confidence intervals (CI) were calculated to examine the strength and precision of the statistical associations between GSTP1 and the environmental risk factors of cervical neoplastic disease after adjusting for age and other covariates using an MLR model.

RESULTS

Study sample

The characteristics of the study population are shown in Table 1. The mean age was 46.3 years for controls and 50.3 years for cases, with a wide range (20 through 77 years). Compared with controls, the study group had lower levels of education, earlier age of first intercourse, and more children. All of these factors are independently significant ($p<0.0001$).

Association between GSTP1 polymorphism and cervical cancer risk

The calculation of the Hardy-Weinberg equilibrium was performed for both controls and cases. Comparison of the observed with the expected relative frequencies in the control and case groups produced a level of significance of $p>0.05$ by the X^2 test, a finding consistent with the Hardy-Weinberg equilibrium (Table 2).

The distribution of the GSTP1 genotypes is presented in Table 2. In assessing polymorphism GSTP1, the percentages of individuals homozygous for the A allele, homozygous for the G allele, and heterozygous for the two alleles were 66.8%, 3.9, and 29.3%, respectively, in the control group, and 64.3%, 4.1%, and 31.6%, respectively, among in women with cervical cancer. There was no significant variation in GSTP1 genotype frequencies between cases and controls. Compared with GSTP1 G allele positive (G/G, G/A), OR (95% CI) for GSTP1 A/A was 1.0 (0.7 - 1.4) for invasive cervical cancer.

The distribution of the GSTP1 genotypes according to histopathologic cell type is presented in

Table 1. General Characteristics of Study Subjects

Characteristics	Controls (n=707)		nvasive cancer (n=342)		t-value	*p*-value
	Mean	SD	Mean	SD		
Age, year	46.3	10.3	50.3	10.9	5.8	<0.0001
Height, cm	158.1	4.9	157.1	4.9	3.1	0.0021
Weight, kg	56.6	7.2	58	8.2	2.8	0.0048
Body mass index, wt/kg^2	22.7	2.7	23.5	3.2	4.5	<0.0001
Education, year	12.1	3.3	9.6	4.1	10.5	<0.0001
Menarche, year	15.5	1.7	15.8	1.7	2.5	0.0013
First intercourse, year	24.7	3.4	22.6	3.1	9.6	<0.0001
Number of children	1.8	1.3	2.7	1.5	9.6	<0.0001
	n	%	N	%	X^2-value	
Ever smoking history	22	3.9	26	9.8	22	0.0007
Husband's smoking history	329	61	182	72.2	26.3	0.0022
Family history	51	7.4	32	9.6	8.4	0.2338

Table 2. Odds Ratio of GSTP1 Genotypes on Invasive Cervical Cancer

Genotype	Controls N=707 N (%)	Invasive Cervical Cancer N=342 N (%)	OR (95% CI)	*p*-value
A/A	472 (66.8)	220 (64.3)	1.0 (0.7 - 1.4)	0.9108
G/A	207 (29.3)	108 (31.6)		
G/G	28 (3.9)	14 (4.1)	1.0	
HW[a]	NS	NS		

Adjusted for age, education level, age at first intercourse, number of children and smoking status using multivariate logistic model.
[a]Goodness of fit to the Hardy-Weinberg equilibrium for genotype distribution (NS, not significant).

Table 3. Compared to women with GSTP1 G allele positive, women with GSTP1 A/A had increased risk of contracting squamous cell carcinoma (1.6, 0.8 - 3.2) but not adenocarcinoma (0.9, 0.7 - 1.3).

The risk increased with GSTP1 A/A among ever smokers compared with GSTP1 G allele positive among nonsmokers. In detail, compared with nonsmokers with GSTP1 positive, ever smokers with GSTP1 A/A had 3.9 times higher risk of cervical cancer (Table 4). In particular, this risk was higher among women with squamous cell carcinoma (4.7, 2.0 - 10.8, p=0.0003).

DISCUSSION

A gene at chromosome region 11p13.1, glutathione S-transferase (GST), has been hypothesized to be involved in the detoxification of polycyclic hydrocarbon and is inactivated in epithelial tumors, particularly in tumors resulting from exposure to cigarette smoking. GSTs are multifunctional, phase II enzymes that detoxify activated forms of chemical carcinogens, such as polycyclic hydrocarbons. Lack of GST activities caused by an inherited deletion of the GST gene has also been reported to increase the risk of lung and other tobacco-related cancers.[7]

This study examined the effect of the GSTP1 gene on cervical cancer in Korean women. The study produced two major findings. The first is that GSTP1 may play a direct role in increasing the risk of squamous cell carcinoma. The second is that there were significant combined effects of ever smoking and GSTP1 genotypes.

The pathogenesis of adenocarcinomas is considered to be somewhat different from that of squamous cell carcinomas. Epidemiologically, the age at the time of first coitus and the number of sexual partners appear to be less important for the

Table 3. Odds Ratio of GSTP1 Genotypes on Cervical Cancer according to Cellular Type

	Controls N=707	Invasive Cervical Cancer N=342					
		Adenocarcinoma N=53			Squamous cell carcinoma N=287		
Genotype	N (%)	N (%)	OR (95% CI)	p-value	N (%)	OR (95% CI)	p-value
A/A	472 (66.8)	39 (73.6)	0.9 (0.7 - 1.3)	0.7311	180 (62.7)	1.6 (0.8 - 3.2)	0.2283
G/A	207 (29.3)	12 (22.6)			96 (33.5)		
G/G	28 (4.0)	2 (3.8)	1.0		11 (3.8)	1.0	

Adjusted for age, education level, age at first intercourse, number of children, and smoking status using multivariate logistic model.

Table 4. Adjusted Odds Ratio of GSTP1 Genotype and Cigarette Smoking Interaction on Cervical Cancer

Smoking status	Genotype	Model I			Model II		
		Total invasive cervical cancer N=342	95% C.I.	p-value	Squamous cell carcinoma N=287	95% C.I.	p-value
Ever smoker	A/A	123	3.9 (1.7 - 8.9)	0.0012	167	4.7 (2.0 - 10.8)	0.0003
Non smoker	A/A	26	0.9 (1.4 - 8.2)	0.7917	17	0.9 (0.6 - 1.2)	0.4418
Ever smoker	G/A or G/G	66	1.4 (0.5 - 4.6)	0.619	92	1.6 (0.5 - 5.4)	0.4692
Non smoker	G/A or G/G	6	1.0		7	1.0	

OR, odds ratio; C.I., confidence interval.
Adjusted for age, education level, age at first intercourse and number of children using multivariate logistic model.

development of adenocarcinomas.[8] Adenocarcinomas of the uterine cervix are more frequently associated with HPV-18.[9] However, Croce et al.[10] reported that there was considerable difference in the frequency of loss of heterozygosity (LOH) in squamous cell carcinoma (87%) versus adenocarcinoma (59%). The present study also found that cervical cancer patients with GSTP1 A/A had more risk of squamous cell carcinoma than adenocarcinoma.

The GSTP1 gene appears to be particularly susceptible to carcinogens from cigarette smoking, and cigarette smoking is an epidemiologic risk factor for cervical cancer. If the mode of action is through the activation and detoxification of tobacco carcinogen, one might expect the relationship between polymorphism and risk to be stronger among smokers. Interestingly, we did find a significant interaction between smoking and background at the risk of cervical cancer. Croce et al.[10] also found that LOH was higher in tumors of smokers and lower in tumors of nonsmokers. Interestingly, in the present study, we found a significant combined effect of smoking and GSTP1 gene on cervical cancer. Smokers with GSTP1 A/A had a risk of cervical cancer about three times higher than nonsmokers with GSTP1 G allele positive.

In our samples of all groups, the distributions of the GSTP1 alleles fit the Hardy-Weinberg equilibrium. Our study presents a large study sample size. However, selection bias may have been introduced when selecting blood donors used in the reference group. In our study, although selection of the study group was not population based, their blood samples were collected within the same hospital as that of the control group. At least to our knowledge, there was no evidence of detection bias or any significant differences between our study groups compared to the general Korean population. On the other hand, the DNA source and the techniques used for genotyping might have affected the results. Poor quality DNA, such as that derived from formalin-fixed tissue, can inhibit PCR amplification, with failure being correlated with the length of the fragment.[11] To assure adequate DNA quality, we used fresh whole blood to extract the DNA for our study.

Future study will be required to understand the physiological role of GSTP1 gene on cervical carcinogenesis. In conclusion, GSTP1 gene was associated with a higher risk of developing cervical cancer, in particular among ever smokers.

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