Dietary Selenium Intake and Genetic Polymorphisms of the *GSTP1* and *p53* Genes on the Risk of Esophageal Squamous Cell Carcinoma

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Abstract

Few studies have assessed potential effect modifications by polymorphisms of susceptibility genes on the association between selenium intake and esophageal squamous cell carcinoma (ESCC). We studied the joint effects of dietary selenium and the *GSTP1* and *p53* polymorphisms on ESCC risk in a population-based case-control study with 218 ESCC cases and 415 controls in Taixing City, China. Dietary selenium intake was estimated from a food frequency questionnaire with 97 food items. *GSTP1* and *p53* polymorphisms were detected by RFLP-PCR assays. Logistic regression analyses were done to estimate odds ratios (OR) and 95% confidence intervals (95% CI). Reduced ESCC risk was observed among individuals in the highest quartile of dietary selenium intake (adjusted OR, 0.31; 95% CI, 0.13-0.70) with a dose-dependent gradient (P_{trend} = 0.01). The *p53 Pro/Pro* genotype was associated with increased risk of ESCC compared with the *Arg/Arg* genotype (adjusted OR, 2.02; 95% CI, 1.19-3.42). When combined with selenium consumption, an obvious increased risk was observed among individuals with the *p53 Pro/Pro* or *GSTP1 Ile/Ile* genotype with adjusted ORs of 3.19 (95% CI, 1.74-5.84) and 1.90 (95% CI, 1.03-3.51), respectively. Among smokers and alcohol drinkers, elevation of ESCC risk was more prominent among *p53 Pro/Pro* individuals who consumed a low level of dietary selenium (adjusted OR, 3.59; 95% CI, 1.49-8.66 for smokers and 6.19; 95% CI, 1.83-20.9 for drinkers). Our study suggests that the effect of dietary selenium on the risk of ESCC may be modulated by tobacco smoking, alcohol drinking, and *p53 Pro/Pro* and *GSTP1 Ile/Ile* genotypes. (Cancer Epidemiol Biomarkers Prev 2006;15(2):294–300)

Introduction

The potential protective role of selenium is suggested by several epidemiologic, preclinical, and clinical studies in the United States and abroad (1-4). A randomized nutritional intervention trial in Linxian, China, a region with high incidence of esophageal cancer, observed that the baseline serum selenium concentrations in 1,103 subjects randomly selected from a cohort over 15 years of follow-up (1986-2001) were inversely associated with ESCC mortality (relative risk, 0.83; 95% CI, 0.71-0.98; ref. 5). A dietary survey conducted among households using a method of food inventory changes showed very low selenium intake among the residents in Linxian (6). Participants who received a combination of selenium, β-carotene, and vitamin E supplements had notably reduced total cancer mortality than those who did not receive the supplements (7-10).

Selenium is an essential trace element involved in several key metabolic activities via selenoproteins, enzymes essential in protecting against oxidative damage and in regulating immunity functions (11). Selenium is potentially useful in oncology because this element possesses anticarcinogenic and chemopreventive properties. Selenium-containing enzymes, such as glutathione peroxidase, play an important role in polycyclic aromatic hydrocarbon metabolism and detoxification (12). Selenium is seen as either a beneficial scavenger of DNA-damaging oxygen free radicals or as a potent inducer of apoptosis that eliminates damaged, potentially cancerous cells (13). It was recently reported that a high level of selenomethionine, the primary organic form of selenium, prompts cells in culture to initiate DNA repair, a key mechanism in preventing cancer (14). Selenomethionine can activate the *p53* tumor suppressor protein by a redox mechanism (15). Selenium may modify *p53* for DNA repair or apoptosis in conjunction with a given level of endogenous or exogenous DNA damage (14). Selenium compounds, which are the most extensively studied cancer chemopreventive agents, may induce apoptotic death of tumor cells (16).

Accumulating evidence indicates that susceptibility to cancer is mediated by genetically determined differences in the process of activation (phase I) or detoxification (phase II) of potential carcinogens. The glutathione *S*-transferase (*GST*) supergene family, the phase II enzyme, plays an important role in detoxification of certain carcinogens. *GSTs* are categorized into four main classes: *GSTA, GSTM, GSTT*, and *GSTP* (17). The *GSTP1* gene displays a polymorphism at codon 105, resulting in an *Ile*-to-*Val* substitution (rs947894), which alters the enzymatic activity of the protein (18). This has been suggested as a putative high-risk genotype in various cancers (19). The *GSTP1* gene, which encodes the *GST* π isoenzyme, is the most important form in the esophagus. Although the association between the *GSTP1* polymorphism and risk of ESCC has been examined by

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several epidemiologic studies (20), results have been conflicting (19, 21).

The *p53* gene plays an important role in DNA transcription, cell cycle regulation, tumor suppression, DNA damage repair, and apoptosis (22, 23). Its mutations are widely detected in all types of cancer, including esophageal cancer (24, 25). A single-base change from the arginine (CGC) or proline (CCC) was found at codon 72 (rs1042522; ref. 26). This polymorphism may be associated with tumor susceptibility to a variety of cancers (27-29). The polymorphism of the *p53* gene at codon 72 is considered as a risk factor of the human papillomavirus–associated cervical neoplasia and ESCC (30, 31). However, it remains controversial in several studies (32).

Although genetic factors may modulate the role of dietary factors on cancer risk, the data are limited about the interplay between nutrients and genes. To the best of our knowledge, no study has evaluated the role of dietary selenium intake and polymorphisms of the *p53* and *GSTP1* genes in esophageal squamous cell carcinoma (ESCC). We hypothesized that polymorphisms of the *GSTP1* and *p53* genes that influence enzyme activity, DNA repair, and apoptosis might modify selenium-ESCC association. The current analyses were therefore conducted to test these hypotheses.

Materials and Methods

Background. This population-based case-control study was conducted in Taixing City of Jiangsu Province, China. Taixing City has one of the highest risks for esophageal cancer in the world, with an incidence rate of 65.2/100,000 in 2000. The population-based tumor registry is within the Division of Chronic Disease Prevention, Taixing City Center for Disease Prevention and Control. Taixing City has 23 townships (rural areas) and one central town (urban area). Each township or city has 10 to 12 villages (or resident blocks in the urban areas). Each village (or resident block) has one county doctor who is responsible for reporting new cancer cases and deaths to the disease prevention and control division of the district (or township) hospital; after which, the information is reported by the district hospital to the Taixing City Center for Disease Prevention and Control population-based tumor registry twice a month. The central town has a similar reporting system with resident blocks and a town hospital.

Subjects. A detailed description of the study has been published previously elsewhere (33). The study was restricted to people who lived in Taixing for at least 10 years. Eligible cases were patients diagnosed with ESCC from June 1, 2000 to December 30, 2000, with pathologically or clinically confirmed diagnoses reported to the Taixing Tumor Registry. We intended to interview all incident cases with primary ESCC who consented to participate in the study with the following restrictions: patients must be newly diagnosed, of ages 20 years or older, in stable medical condition as determined by their physicians, and willing to participate. A total of 220 ESCC cases were recruited, which represents 66.7% of all new cases ($n = 330$) diagnosed within the 6 months of the study period in Taixing. Among these cases, 218 patients completed interviews and 204 cases had DNA sample available. Considering that esophageal cancer is an extremely fatal disease, we could not recruit all eligible cases into our study because some cases died before we could approach and interview them. Additional reasons for the relatively low response rate are patients were too ill to get interviewed or patients were not willing to participate in the study.

Eligible controls were healthy individuals randomly selected from the general population in Taixing. Because the original study included three upper gastrointestinal cancers (stomach, liver, and esophagus), we used a common control group for all three cancer sites. The control group was selected according to the frequency distribution of the sex and age of cases interviewed from each village where cancer cases originated. For each village, a list of residents was generated with the same gender and age group as cases, and random numbers were used to select the healthy controls according to the control-to-case ratio of 2:3. If the control did not fit the criteria or he/she refused to be interviewed, we recorded his/her basic demographic data and used the same selection process to choose another control. A total of 464 potential healthy controls were selected from the entire population of 1,280,000 residents in the Taixing area and 415 controls completed interviews (89.9%).

Epidemiologic Data Collection. Our trained interviewers questioned cases and controls using a standard questionnaire. An informed consent was obtained for an interview and a blood sample from each study participant. Interviews were frequently monitored by the professional staff in the Division of Chronic Disease Prevention of the Taixing Center for Disease Prevention and Control. For cases, the interviews took place either in the hospital or at the study subjects' homes. All healthy controls were interviewed at their homes or in the county doctors' offices. Using a standard questionnaire, we attempted to include all possible risk and protective factors that were considered important in the Chinese population. The questionnaire included (*a*) demographic factors, including age of subject, gender, residence, place of birth, level of education, annual income, blood type, and disease diagnostic information; (*b*) residence and water drinking history; (*c*) detailed dietary history; (*d*) detailed smoking history; (*e*) alcohol drinking habits; (*f*) tea drinking habits; (*g*) detailed information on disease history; (*h*) occupational history and related exposures; (*i*) family history of esophageal cancer and other cancers; and (*j*) physical activities. A quantitative food-frequency questionnaire was used to assess dietary intake in the year before the interview. A total of 97 specific foods according to local residents' customs and 33 Chinese dietary habits were selected for investigation. Each participant was asked to report how frequently per day, week, month, or year he/she ''usually'' ate each food and the usual serving size of each food item during the past year. The nutrient components were estimated from food items, serving sizes, and consumption frequency using the Chinese Standard Tables of Food Composition (34).

Genotyping Methods. Among those who completed the in-person interviews, 8-mL blood specimens were collected from 205 (93.2%) cases and 394 (95%) controls. Genomic DNA was isolated from blood clots by using a modified phenol-chloroform protocol (35). *GSTP1* and codon 72 *p53* gene polymorphisms were examined by the RFLP-PCR method (11, 36). Briefly, 100 ng of the DNA sample were amplified using 0.2 μmol/L of primers for *GSTP1* (5′-ACCCCAGGGCTC-TATGGGAA-3′ and 5′-TGAGGGCACAAGAAGCCCCT-3′) and primers for codon 72 *p53* (5′-TTGCCGTCCCAAGCAATG-GATGA-3′ and 5′-TCTGGGAAGGGACAGAAGATGAC-3′), 20 μmol/L deoxynucleotide triphosphates, 1 unit of Taq DNA polymerase (Promega), and 1.5 mmol/L $MgCl_2$ in a total volume of 20 μL. Thermal cycling was carried out under the following conditions: for *GSTP1*, initial denaturation at 95°C for 5 minutes, followed by 35 cycles at 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds. We used an annealing temperature of 60°C for codon 72 *p53*. A final polymerization step of 72°C for 5 minutes was carried out to complete the elongation processes. The PCR product, 17.5 μL, was then digested with 5 units of Alw261 (Promega) and 5 units of *Bst*UI (New England Biolabs, Ipswich, MA) for *GSTP1* and codon 72 *p53*, respectively, in a total volume of 20 μL, and the products were separated on a 4% NuSieve 3:1 plus agarose (BMA Biomedicals, Rheinstrasse, Switzerland).

Table 1. General characteristics of ESCC cases and controls

Variable	Case (N = 218)	Control (N = 415)	P
	N (%)	N (%)	
Gender			
Male	141 (64.7)	287 (69.2)	0.25*
Female	77 (35.3)	128 (30.8)	
Age (y)			
<50	31 (14.2)	100 (24.1)	0.03*
50-59	77 (35.3)	136 (32.8)	0.01†
60-69	68 (31.2)	116 (27.9)	
70+	42 (19.3)	63 (15.2)	
Mean ± SD	63.67 ± 9.64	60.92 ± 12.06	
BMI (kg/m^2)			
<18.50	37 (17.0)	50 (12.0)	0.27*
18.50-23.89	136 (62.4)	259 (62.4)	0.07†
23.90-27.89	35 (16.1)	82 (19.8)	
≥27.90	10 (4.6)	24 (5.8)	
Education			
Illiteracy	83 (38.6)	73 (17.6)	<0.001*
Primary school	101 (47.0)	142 (34.2)	<0.01†
Middle school	28 (13.0)	124 (29.9)	
≥High school	3 (1.4)	76 (18.3)	
Monthly income (RMB)‡			
<100	134 (65.7)	199 (51.7)	0.001*
100+	70 (34.3)	186 (48.3)	
Total energy intake (kcal/d)			
<1,609	71 (32.6)	104 (24.9)	0.06*
1,609-2,178	60 (27.5)	104 (25.1)	<0.01†
2,179-2,890	50 (22.9)	104 (25.1)	
≥2,891	37 (17.0)	103 (24.9)	
Tobacco smoking			
Never	94 (44.6)	217 (52.4)	0.06*
Ever	117 (55.4)	197 (47.6)	
Pack-years			
0	94 (44.6)	217 (52.4)	0.07*
≤20	45 (21.3)	91 (22.0)	0.02†
20+	72 (34.1)	106 (25.6)	
Alcohol drinking			
Never	116 (55.0)	207 (50.2)	0.01*
Occasionally	18 (8.5)	72 (17.5)	
Usually	37 (17.5)	75 (18.2)	
Everyday	40 (19.0)	58 (14.1)	
Total years of drinking			
Never	116 (55.0)	207 (50.2)	0.04*
≤30	43 (20.4)	122 (29.6)	0.97†
>30	52 (24.6)	83 (20.2)	

*P values were obtained by χ^2 test.
†P values were obtained by test for trend.
‡The average monthly income per person, 10 years before.

Statistical Methods. Pearson's χ^2 test was used to compare the distribution of general characteristics between ESCC case patients and control subjects. The body mass index (BMI), total energy intake, and dietary selenium intake were categorized into quartiles. The threshold for quartiles was defined by values for controls. The median and quartile of selenium consumption from food for cases and controls were calculated. The observed genotype frequencies were compared with those calculated from the Hardy-Weinberg disequilibrium theory ($p^2 + 2pq + q^2 = 1$, where p is the frequency of the variant allele and $q = 1 - p$). Unconditional logistic regression analyses were conducted to estimate crude and adjusted odds ratios (OR) and their 95% confidence intervals (95% CI) for the association between dietary selenium intake and *GSTP1* and *p53* genotypes with ESCC risk. We adjusted for potential confounding factors including age (continuous), sex (male or female), educational level (categorized by four strata), income (average monthly income, RMB/person), BMI (continuous), total energy intake (continuous, kcal/d), smoking (ever versus never), and drinking (never = 1, seldom = 2, often = 3, everyday = 4). Stratified analyses were conducted to observe effect modification by genetic polymorphisms on the association between dietary selenium intake and ESCC risk. A logistic regression model was used to evaluate potential multiplicative interaction effects. The departures from multiplicative effects were assessed by including main effect variables and their product terms in the logistic regression model when adjusting for potential confounding factors. Statistical analyses were done using SAS 8.2 software.

Results

The demographics and potential risk or protective factors of 218 patients with ESCC and 415 healthy controls are summarized in Table 1. The distributions of cases and controls were similar in terms of gender. However, controls were much younger than cases (P = 0.02). The mean age ± SD was 63.7 ± 9.6 years for cases and 60.9 ± 12.1 years for controls ($P < 0.05$). We observed a higher percentage of cases (17.9%) in the underweight group (BMI < 18.5) compared with that among controls (12.0%). There are explanations for this observation: (*a*) cases with esophageal cancer have problems swallowing food, leading to reduced body weights after diagnosis; (*b*) low BMI may be related to low economic status as well as malnutrition in the general population of this rural area of China. Compared with cases, controls received more years of education. Obvious differences were observed for average income and total energy intake between cases and controls. A higher proportion of ESCC was distributed on lower social economic classes. As expected, the case group had a higher proportion of tobacco smokers and alcohol drinkers than the control group.

The frequencies of the *GSTP1 Ile* allele and *GSTP1 Val* allele in healthy controls were 82% and 18%, respectively. The genotype distribution of the *GSTP1* was in agreement with the Hardy-Weinberg equilibrium ($\chi^2_{HW} = 0.0806$, $P > 0.05$). The *p53 Pro* allele frequency was 58% among cases, which was higher than the frequency among controls (47%). No departure from the Hardy-Weinberg equilibrium was detected for the *p53* allelic frequency distribution in controls ($\chi^2_{HW} = 2.6285$, $P > 0.05$).

Table 2 shows the associations of dietary selenium intake and *GSTP1* and *p53* polymorphisms with ESCC risk. A strong inverse selenium-ESCC association was present in the highest quartiles of consumption. The adjusted OR was 0.31 (95% CI, 0.13-0.70) in a dose-dependent fashion ($P_{trend} = 0.01$). The median distribution of selenium intake among controls (25.9 μg/d) was used as the cutoff points for further stratified analyses. Compared with individuals with the *GSTP1 Val/Val* genotype, those with the *GSTP1 Ile/Ile* genotype had an adjusted OR of 1.40 (95% CI, 0.35-5.52). The *p53 Pro/Pro* genotype conferred a significantly higher risk of ESCC compared with the *p53 Arg/Arg* genotype (adjusted OR, 2.02; 95% CI, 1.19-3.42).

When combined genotypes were examined, individuals who were homozygous with the risk allele of both genes (*GSTP1 Ile* and *p53 Pro*) had a significantly elevated risk of ESCC (adjusted combined OR, 1.94; 95% CI, 1.09-3.64) compared with those with low-risk genotypes (*GSTP1 Val/Val* + *GSTP1 Ile/Val* and *p53 Arg/Arg* + *p53 Pro/Arg*). We examined the combined effects of selenium intake and *GSTP1* or *p53* polymorphisms in relation to ESCC risk. Elevation of ESCC risk was obvious among individuals who had the *GSTP1 Ile/Ile* or *p53 Pro/Pro* genotype who consumed a low level of dietary selenium with adjusted combined ORs of 1.90 (95% CI, 1.03-3.51) and 3.19 (95% CI, 1.74-5.84), respectively, compared with low-risk individuals (high selenium intake with *GSTP1 Val/Val* + *GSTP1 Ile/Val* or *p53 Arg/Arg* + *p53 Pro/Arg* genotype). Individuals who carried more than one risk genotype had higher ORs (adjusted OR, 1.96; 95% CI, 1.01-3.81) when their dietary selenium consumption was in the low level category although no obvious interactions were observed at the multiplicative scale (Table 3).

Table 2. Associations between dietary selenium intake, GSTP1, and p53 polymorphisms and risk of ESCC

Variable	Cases, *N* (%)	Controls, *N* (%)	Crude OR (95%CI)	Adjusted OR (95% CI)
Selenium (μg/d)				
Q1 (<17.0)	91 (41.4)	104 (25.1)	1	1
Q2 (17.0-25.9)	58 (26.4)	103 (24.8)	0.64 (0.42-0.99)	0.62 (0.37-1.05)*
Q3 (25.9-39.3)	44 (20.0)	105 (25.3)	0.48 (0.31-0.75)	0.53 (0.28-1.00)*
Q4 (39.3+)	27 (12.3)	103 (24.8)	0.30 (0.18-0.50)	0.31 (0.13-0.70)*
			$P_{trend} < 0.01$	$P_{trend} = 0.01$
GSTP1 (*Ile105Val*)				
Val/Val	3 (1.5)	12 (3.1)	1	1
Ile/Val	58 (28.4)	116 (29.5)	2.00 (054-7.37)	1.20 (0.29-4.87)†
Ile/Ile	143 (70.1)	265 (67.4)	2.16 (0.60-7.74)	1.40 (0.35-5.52)†
			$P_{trend} = 0.35$	$P_{trend} = 0.41$
p53 (*Arg72Pro*)				
Arg/Arg	41 (20.1)	117 (30.1)	1	1
Arg/Pro	89 (43.6)	178 (45.8)	1.43 (0.92-2.21)	1.21 (0.74-1.98)†
Pro/Pro	74 (36.3)	94 (24.2)	2.25 (1.41-3.59)	2.02 (1.19-3.42)†
			$P_{trend} < 0.01$	$P_{trend} = 0.01$

*Adjusted for age, gender, BMI, education level, income, total energy intake, smoking, and drinking.
†Adjusted for age, gender, BMI, educational level, smoking, and drinking.

Table 4 summarizes ORs for dietary selenium intake and the polymorphisms of the *GSTP1* and *p53* genes according to tobacco smoking. The *p53 Pro/Pro* genotype was associated with a higher risk among smokers who had a low consumption of dietary selenium (adjusted OR, 3.59; 95% CI, 1.49-8.66) than among nonsmokers (adjusted OR, 2.71; 95% CI, 1.17-6.26). A similar nonsignificant trend was observed with the *GSTP1 Ile/Ile* genotype. The combination of low selenium intake and *GSTP1 Ile/Ile* and *p53 Pro/Pro* genotypes was associated with a statistically significant increase in risk for ESCC only among smokers (adjusted OR, 2.47; 95% CI, 1.01-6.03).

The combined effects and interactions between selenium intake and polymorphisms of the *GSTP1* or *p53* genes were also explored when stratified by alcohol drinking. With respect to the *p53* polymorphism and selenium intakes, compared with low-risk individuals (*p53 Arg/Arg* or *p53 Pro/Arg* genotype with a higher selenium intake), increased ESCC risk was most pronounced among alcohol drinkers with the *p53 Pro/Pro* genotype who consumed a relatively lower level of dietary selenium (adjusted OR, 6.19; 95% CI, 1.83-20.87; data not shown).

Discussion

Several epidemiologic studies suggest that selenium may protect against the development of some cancers and may have an important role in chemoprevention of cancer (3, 4, 37, 38). Low selenium levels have been implicated as one of the risk factors associated with cancers (39-41). Findings from our study give support to the notion that selenium may have anticarcinogenic properties against cancers in low selenium geographic areas (42, 43). Food is considered as the major source of selenium intake. The organic form of selenium is found predominantly in grains, fish, meat, poultry, eggs, and dairy products and enters the food chain via plant consumption (37). Our study population investigated a low selenium

Table 3. Combined effects of GSTP1 and p53 polymorphisms and selenium intake on ESCC risk

Variable		Cases, *N*	Controls, *N*	Crude OR (95% CI)	Adjusted OR (95% CI)*
GSTP1	p53				
Val/Val + Ile/Val	*Arg/Arg + Arg/Pro*	41	98	1	1
Ile/Ile	*Arg/Arg + Arg/Pro*	89	197	1.08 (0.69-1.68)	1.05 (0.64-1.72)
Val/Val + Ile/Val	*Pro/Pro*	20	27	1.77 (0.89-3.51)	1.59 (0.74-3.44)
Ile/Ile	*Pro/Pro*	54	67	1.93 (1.16-3.21)	1.94 (1.09-3.46)
Interaction				1.01 (0.45-2.27)	1.17 (0.46-2.93)
Selenium intake†	*GSTP1*				
≥Median	*Val/Val + Ile/Val*	22	64	1	1
<Median	*Val/Val + Ile/Val*	39	64	1.77 (0.95-3.32)	1.54 (0.77-3.08)
≥Median	*Ile/Ile*	45	132	0.99 (0.55-1.79)	1.08 (0.57-2.07)
<Median	*Ile/Ile*	98	133	2.14 (1.24-3.72)	1.90 (1.03-3.51)
Interaction				1.22 (0.57-2.60)	1.14 (0.49-2.65)
Selenium intake	*p53*				
≥Median	*Arg/Arg + Arg/Pro*	42	146	1	1
<Median	*Arg/Arg + Arg/Pro*	88	149	2.05 (1.33-3.17)	1.73 (1.06-2.81)
≥Median	*Pro/Pro*	25	49	1.77 (0.98-3.20)	1.77 (0.92-3.41)
<Median	*Pro/Pro*	49	45	3.79 (2.23-6.44)	3.19 (1.74-5.84)
Interaction				1.04 (0.48-2.23)	1.05 (0.44-2.48)
Selenium intake	No. genotypes at risk‡				
≥Median	0	17	50	1	1
<Median	0	24	48	1.47 (0.70-3.07)	1.32 (0.59-2.98)
≥Median	≥1	50	146	1.01 (0.53-1.91)	1.06 (0.53-2.12)
<Median	≥1	113	146	2.28 (1.25-4.16)	1.96 (1.01-3.81)
Interaction				1.54 (0.66-3.56)	1.40 (0.55-3.55)

*Adjusted for age, sex, BMI, educational level, smoking, and alcohol drinking.
†Median of dietary selenium intake among controls: 25.9μg/d.
‡*p53 Pro/Pro* and *GSTP1 Ile/Ile* were defined as risk genotypes in this study.

Table 4. Stratified analysis of selenium intake and GSTP1 and p53 polymorphisms on ESCC risk by tobacco smoking

Variable			Never smokers		Ever smokers	
			Crude OR (95% CI)	Adjusted OR (95% CI)*	Crude OR (95% CI)	Adjusted OR (95% CI)*
Selenium intake†	*GSTP1*					
≥Median	*Val/Val + Ile/Val*		1	1	1	1
<Median	*Val/Val + Ile/Val*		1.31 (0.46-3.77)	1.16 (0.36-3.70)	2.30 (1.03-5.11)	2.00 (0.83-4.83)
≥Median	*Ile/Ile*		1.04 (0.39-2.76)	1.32 (0.45-3.88)	0.94 (0.46-2.09)	0.97 (0.43-2.21)
<Median	*Ile/Ile*		2.00 (0.80-5.02)	1.79 (0.65-4.93)	2.19 (1.08-4.46)	2.11 (0.96-4.64)
Interaction			1.47 (0.43-4.97)	1.17 (0.30-4.50)	0.98 (0.36-2.69)	1.09 (0.36-3.28)
Selenium intake	*p53*					
≥Median	*Arg/Arg + Arg/Pro*		1	1	1	1
<Median	*Arg/Arg + Arg/Pro*		1.17 (0.62-2.19)	0.91 (0.45-1.86)	3.35 (1.80-6.26)	3.09 (1.56-6.14)
≥Median	*Pro/Pro*		0.73 (0.27-2.01)	0.92 (0.30-2.82)	3.29 (1.50-7.20)	2.88 (1.30-6.71)
<Median	*Pro/Pro*		3.31 (1.57-6.96)	2.71 (1.17-6.26)	3.97 (1.82-8.66)	3.59 (1.49-8.66)
Interaction			3.88 (1.14-13.23)	3.245 (0.82-12.78)	0.36 (0.13-1.03)	0.40 (0.13-1.30)
Selenium intake	No. of genotypes at risk‡					
≥Median	0		1	1	1	1
<Median	0		0.79 (0.24-2.65)	0.86 (0.22-3.35)	2.29 (0.89-5.92)	1.95 (0.68-5.61)
≥Median	≥1		0.77 (0.29-2.08)	1.14 (0.38-3.42)	1.21 (0.52-2.78)	1.10 (0.44-2.74)
<Median	≥1		1.63 (0.64-4.14)	1.63 (0.58-4.53)	2.74 (1.23-6.12)	2.47 (1.01-6.03)
Interaction			2.67 (0.69-10.27)	1.66 (0.36-7.59)	0.99 (0.33-3.01)	1.14 (0.34-3.89)

*Adjusted for age, sex, BMI, educational level, and alcohol drinking.
†Median of dietary selenium intake among controls: 25.9μg/d.
‡*p53 Pro/Pro* and *GSTP1 Ile/Ile* were defined as risk genotypes in this study.

region in China where very few people took selenium supplements, which provides a unique opportunity in estimating selenium intake from food sources. In this population, the foods that had significant median differences between ESCC cases and controls were red meat, fish, eggs, milk, and fruits. The medians of selenium intake were markedly lower in the case group than in the control group. Compared with the U.S. Recommended Dietary Allowance of 55 μg/d for healthy adults (44), the median intake of dietary selenium in this population was considerably lower (25.9 μg/d). The protective effects of selenium might be stronger in low selenium areas and among individuals in the low selenium intake group. Our observation of the potential protective effects of dietary selenium intake is consistent with the results from a joint U.S.-China nutritional intervention study, which found highly significant inverse associations of serum selenium levels with the incidence of esophageal cancer (5, 7). The relative risk for comparison of the highest to lowest quartile of serum selenium was 0.56 (95% CI, 0.44-0.71) for esophageal cancer. However, very few studies have assessed the effect between dietary selenium intake and ESCC risk.

Glutathione and glutathione-related antioxidant enzymes are involved in the metabolism and detoxification of carcinogenic compounds. It has been reported that the genetic polymorphism of *GSTP1* exon 5 [rs947894, *Ile105Val* (A>G)] has functional relevance to the *GST* gene product resulting in reduced *GST* enzyme activity (45). The frequencies of the variant genotype of *GSTP1* are entirely dependent on the ethnic group being considered. In this study, the frequencies of the *GSTP1 Ile/Ile, Ile/Val*, and *Val/Val* genotypes were 64.7%, 29.5%, and 3.1%, respectively, among controls, which is similar to the frequencies in Japanese populations (68.9% of *Ile/Ile*, 29.3% of *Ile/Val*, 1.8% of *Val/Val*; ref. 21). Epidemiologic studies have been done to explore the associations between the various *GSTP1* polymorphisms and esophageal cancer (46-48). Although results have been inconsistent, an important role for the *GSTP1* polymorphism in cancer susceptibility was suggested. Our results suggest that the *GSTP1 Ile/Ile* genotype may contribute to ESCC risk in Chinese populations that have a low level of dietary selenium intake (adjusted OR, 1.90; 95% CI, 1.03-3.51).

p53 is one of the most established tumor suppressor genes and is involved in the pathogenesis of various human cancers. A polymorphism on *p53* codon 72, exon 4, which encodes either arginine (*Arg*) or proline (*Pro*) and involves a G→C transversion, has been proposed as a genetic susceptibility factor for cancer development (49). A recent study (50, 51) reported that a single nucleotide polymorphism at codon 72 (*p53 Arg72Pro*) affects its function. It is shown that *p53 Pro/Pro* exhibits a lower ability to induce apoptosis *in vitro* than *p53 Arg/Arg*. Our results showed that *p53 Pro/Pro* was significantly associated with ESCC risk as compared with *p53 Arg/Arg* homozygotes (adjusted OR, 2.02; 95% CI, 1.19-3.42). Our study is in agreement with Lee et al.'s study (25), which found that the codon 72 *p53 Pro* allele was more frequently found in ESCC patients in Taiwan with an OR of 1.86 (95% CI, 1.04-3.35) for the *Arg/Pro* genotype and an OR of 2.56 (95% CI 1.29-5.08) for the *Pro/Pro* genotype.

We found that elevation of ESCC risk was most pronounced among individuals who consumed low levels of dietary selenium and were exposed to tobacco carcinogens (adjusted OR, 3.59; 95% CI, 1.49-8.66) or alcohol drinking (adjusted OR, 6.19; 95% CI, 1.83-20.87). Furthermore, we observed an increased risk of ESCC in individuals with both the *GSTP1 Ile/Ile* and *p53 Pro/Pro* genotypes. Elevated risk was even stronger if the dietary selenium consumption of these carriers was low. Although no obvious multiplicative interaction was observed because of the relatively small sample size, these results give some support for the hypothesis that the polymorphisms of *GSTP1* and the *p53* gene may modify the relationship between dietary selenium intake and ESCC risk. In addition, ESCC risk may be further modified by tobacco smoking and alcohol drinking. It is evident that genes that have an effect on selenium might be involved in the development of ESCC (52). Selenium can alter phase II enzymes in a manner leading to inhibition of carcinogen-DNA adduct formation in the target organ (53). Such an effect can account for selenium protection during the initiation phase of carcinogenesis. Selenium arrests cells in the G_1 phase of the cell cycle and induces the expressions of *p53* (52). During the postinitiation phase of carcinogenesis, inhibition of cell proliferation and induction of apoptosis have been suggested as critical cellular events in cancer chemoprevention by selenium (54). The third National Health and Nutrition Examination Survey in the United States reported that serum selenium concentrations can be significantly influenced by cotinine concentrations and alcohol consumption ($P < 0.05$; refs. 55, 56). Further studies on the potential biological mechanism of selenium and these SNPs,

as well as smoking and drinking in the etiology of ESCC, are warranted.

The case-control study design is, however, subject to several limitations of retrospective nature. This study population takes selenium from natural food items rather than selenium supplementation. The intake is basically covered by a food-frequency questionnaire that consisted of 97 specific foods that were selected according to the customs of local residents. Compared with other dietary data collection methods, the food frequency questionnaire might be a better measurement method, which can be used to collect long-term patterns of food consumption with the assumption that people do not have obvious changes in their dietary habits. Despite this benefit, dietary selenium intake measured by a retrospective food frequency questionnaire in case-control studies could be considered as a potential limitation because of possible recall and misclassification bias of dietary history. Because dietary selenium intakes were not directly collected from interviews, the possible recall and misclassification bias might be nondifferential. It has been suggested that the direction of those biases on the observed relationship might be towards the null (57).

Another possible limitation is that the confounding effect of age might distort some associations in this study. The proportion of younger individuals (<50 years old) was higher among controls (24.1%) than among cases (13.8%). Because we used a common control group, the age and sex distributions of controls were correspondent to three cancer sites (stomach, liver, and esophagus) and were not identical to the distribution of ESCC cases. The higher proportion of younger cases for liver cancer resulted in a higher proportion of younger controls. The potential residual confounding effects by age might still persist although we adjusted for age as a continuous variable in multivariate analyses. We have done sensitivity analyses by using age and gender frequency-matching case-control subsets (218 cases and 218 controls). The adjusted point estimates for selenium intake and *p53*, as well as the interaction and combined ORs between selenium intake and *GSTP1* or *p53*, were similar to that of the overall analyses. The 95% CIs of the ORs for *GSTP1* main effect included null in both analyses although the point estimates were in different directions. Thus, based on the results of the sensitivity analyses, we believe that the potential residual confounding effects of age and gender are minimal.

Strengths of our study include a population-based study design, extensive relevant questionnaire data, and biological specimens for genetic polymorphisms. ESCC is a high-mortality cancer with complex etiology and the development of the disease may involve both genetic and environmental factors. Our findings support the hypothesis that polymorphisms of *p53* and *GSTP1* may modify the association between dietary selenium intake and ESCC risk.

In summary, low dietary selenium intake is a high-risk factor for ESCC, especially among smokers and heavy drinkers with *p53 Pro/Pro* and *GSTP1 Ile/Ile* genotypes. The findings from our study suggest that the high-risk populations to be targeted for selenium chemoprevention of ESCC are those who have low dietary selenium intake with *p53 Pro/Pro* and/or *GSTP1 Ile/Ile* genotypes and who are either smokers or heavy drinkers.

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