

## Green tea drinking and multigenetic index on the risk of stomach cancer in a Chinese population

Li-Na Mu<sup>1</sup>, Qing-Yi Lu<sup>2</sup>, Shun-Zhang Yu<sup>1</sup>, Qing-Wu Jiang<sup>1</sup>, Wei Cao<sup>3</sup>, Nai-Chieh You<sup>3</sup>, Veronica Wendy Setiawan<sup>3</sup>, Xue-Fu Zhou<sup>4</sup>, Bao-Guo Ding<sup>4</sup>, Ru-Hong Wang<sup>4</sup>, Jinkou Zhao<sup>5</sup>, Lin Cai<sup>6</sup>, Jian-Yu Rao<sup>7</sup>, David Heber<sup>2</sup> and Zuo-Feng Zhang<sup>3\*</sup>

<sup>1</sup>Department of Epidemiology, Fudan University School of Public Health, Shanghai, China

<sup>2</sup>UCLA Center for Human Nutrition, School of Medicine, Los Angeles, CA, USA

<sup>3</sup>Department of Epidemiology, UCLA School of Public Health, Los Angeles, CA, USA

<sup>4</sup>Taixing City Center for Disease Prevention and Control (CDC), Taixing, Jiangsu, China

<sup>5</sup>Department of Chronic Disease Prevention, Jiangsu CDC, Nanjing, Jiangsu, China

<sup>6</sup>Department of Epidemiology, Fujian Medical University, Fuzhou, Fujian, China

<sup>7</sup>Department of Pathology and Laboratory Medicine, UCLA School of Medicine, Los Angeles, CA, USA

The purpose of our study was to examine the roles of green tea drinking, other risk and protective factors, and polymorphism of susceptibility genes such as *GSTM1*, *GSTT1*, *GSTP1*, and *p53* codon 72 and their possible joint effects on the risk of stomach cancer. A population-based case-control study was conducted in Taixing, China, including 206 newly diagnosed cases with stomach cancer and 415 healthy control subjects. Epidemiological data were collected by in-person interviews using a standard questionnaire. Polymorphisms of susceptibility genes were assayed by PCR-RFLP techniques. A multigenetic index was created by summing up the number of risk genotypes. The data were analyzed using the logistic regression model. A reverse association between green tea drinking and risk of stomach cancer was observed with an adjusted odds ratio (OR) of 0.59 (95% confidence interval [CI] = 0.34–1.01). Dose-response relationship was shown ( $p$ -trend < 0.05). A higher score on the multigenetic index was associated with increased risk of stomach cancer with an adjusted OR of 2.21 (95% CI = 1.02–4.79) for those with at least 3 risk genotypes compared to those with <2 risk genotypes. Green tea drinking was suggested to have more than multiplicative interactions with alcohol consumption with an adjusted OR for interaction of 4.57 (95% CI = 1.62–12.89), and with higher multigenetic index with adjusted OR for interaction of 2.31 (95% CI = 0.88–6.03). The protective effect of green tea drinking was observed on the risk of stomach cancer and the possible effect modification by susceptibility genes was suggested.

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**Key words:** stomach cancer; green tea; alcohol; genetic polymorphism; multigenetic index

Stomach cancer is the fourth most frequent cancer worldwide, with 876,000 new cases (8.7% of the total) and 647,000 deaths (10.4% of cancer deaths) in 2000. Approximately two-thirds of the cases occurred in less developed countries.<sup>1</sup> Continual declines in incidence rates have been observed worldwide in the last few decades, but exact causes of the decline are not well understood.<sup>2</sup> China is a high-risk country for stomach cancer and 38% of stomach cancer cases in the world are diagnosed there. The estimated age-standardized incidence rates in 2000 were 36.1 per 100,000 for men and 17.5 per 100,000 for women; the age-standardized mortality rates were 32.7 per 100,000 for men and 15.0 per 100,000 for women.<sup>3</sup> Incidence and mortality in China were much higher than that in the rest of the world during the same period, with estimated age-standardized incidence rates of 21.5/100,000 for males and 10.4/100,000 for females; as well as mortality rates of 15.6/100,000 for males and 7.8/100,000 for females. Although slight declines in mortality rates for both men and women in China have been observed in the last decade, the absolute numbers of deaths from stomach cancer increased with population growth and aging by 19,037 and 7,152 for men and women, respectively.<sup>3</sup>

Tea is grown in about 30 countries and is the most widely consumed beverage in the world.<sup>4</sup> All tea is derived from one plant (*Camellia sinensis*) and 78% of that manufactured in the world is black, 20% is green and 2% is oolong. Green tea is consumed pri-

marily in Asian countries, such as Japan and China, and in some parts of North Africa and the Middle East.<sup>5</sup> Green tea contains polyphenols, commonly known as catechins. Some major green tea catechins are (–)-epigallocatechin-3-gallate (EGCG), (–)-epigallocatechin (EGC), (–)-epicatechin-3-gallate (ECG), (–)-epicatechin (EC), (+)-gallocatechin and (+)-catechin. From *in vitro* and animal studies,<sup>6,7</sup> extracts from green tea containing tea catechins have been shown to enhance the activities of Phase II detoxifying enzymes, known as glutathione-S-transferases. Animal and *in vitro* studies have provided evidence that polyphenol compounds, abundantly present in green tea, may play an important role in anti-carcinogenic progress.<sup>6,8–10</sup> Studies have suggested that green tea extract and its major component, epigallocatechin gallate (EGCG), may lead to growth inhibition and apoptosis of human stomach cancer line KATOIII, and may inhibit release of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) from the cells.<sup>11,12</sup> Green tea has also been shown to block heterocyclic aromatic amines formed during the cooking of meat, which plays a role in the development of gastric and colorectal cancers, and to induce Phase I and Phase II metabolic enzymes that increase the formation and excretion of detoxified metabolites of carcinogens.<sup>13,14</sup> In animal models, green tea has been shown to inhibit tumorigenesis in the mouse forestomach induced by NDEA or precursors of NMBzA or *N*-nitrososarcosine. The inhibitory effect of EGCG has been reported on *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG)-induced glandular stomach carcinogenesis in rats and on *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine (ENNG)-induced duodenal carcinogenesis in mice.<sup>15–17</sup> However, epidemiological studies on tea consumption and stomach cancer have yielded inconclusive results (Table I).<sup>18–35</sup>

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. *GSTT1*, *GSTM1*, and *GSTP1*, within the subfamily of GST, have been shown to be polymorphic. Individuals who lack enzyme activity may have higher levels of the mutagenic metabolites to bind to DNA, leading to DNA damage and an increased risk of cancer when exposed to potential carcinogenic compounds.<sup>36–40</sup> Results from one study suggested that green tea

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\*Correspondence to: Department of Epidemiology, UCLA School of Public Health, 71-225 CHS, Box 951772, 650 Charles Young Drive, Los Angeles, CA 90095-1772. Fax: +310-206-6039.

E-mail: zfzhang@ucla.edu

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TABLE I – EPIDEMIOLOGIC STUDIES OF GREEN TEA AND STOMACH CANCER

Author, year, reference	Country, study	Consumption	OR (95% CI)/RR (95% CI)	Confounder adjustment
<b>Case-control studies</b>				
Tajima, 1985 <sup>18</sup>	Japan 93 cases, 186 hospital controls	≥4 times/day	0.64 (NA)	Age and gender
Kono, 1988 <sup>19</sup>	Japan 139 cases, 2,574 hospital controls, 278 population controls	Cases vs. hosp controls Low Medium High High* (≥10 cups/day)	1.0 1.1 0.6 0.5 (0.3–1.1)	Age, gender, smoking, oranges and other fruits
Kato, 1990 <sup>20</sup>	Japan 427 cases, 3014 controls	Cases vs. pop. controls High* (≥10 cups/day) Male, hot green tea Non-drinkers 1–4 cups/day ≥5 cups/day Female, hot green tea Non-drinkers 1–4 cups/day ≥5 cups/day	0.3 (0.1–0.7) 1.00 1.14 (0.82–1.60) 1.01 (0.70–1.47) 1.00 0.71 (0.45–1.14) 0.81 (0.51–1.27)	Age and residence
	1414 chronic atrophic gastritis cases, 3014 controls	Male, hot green tea Non-drinkers 1–4 cups/day ≥5 cups/day Female, hot green tea Non-drinkers 1–4 cups/day ≥5 cups/day	1.00 1.04 (0.83–1.30) 1.00 (0.78–1.29) 1.00 1.46 (1.16–1.83) 1.19 (0.93–1.51)	
Lee, 1990 <sup>21</sup>	Taiwan 210 cases, 810 hospital controls	No Yes	1.0 (NA) 2.0 (NA)	Smoking, alcohol drinking, salted meat, fried food, fermented bean, milk intake
Yu, 1991 <sup>22</sup>	China 84 cases, 2,676 controls	Strong tea (type not specified)	0.3 (0.1–0.7)	Age, gender, income, family history of stomach cancer/ other cancer, history of TB, blood type, smoking, alcohol, fruit and milk intake
Hoshiyama, 1992 <sup>23</sup>	Japan 294 cases, 294 population controls, 202 hospital controls	Use vs. pop controls Low (≤4 cups/day) Medium (5–7 cups/day) High (≥8 cups/day) Use vs. hosp controls Low (≤4 cups/day) Medium (5–7 cups/day) High (≥8 cups/day)	1.0 1.0 (0.7–1.4) 0.8 (0.5–1.3) 1.0 1.3 (0.8–2.0) 1.3 (0.8–2.1)	Age, gender, administrative division and smoking status
Inoue, 1994 <sup>24</sup>	Japan 668 cases, 668 hospital controls	Not every day Every day	1.00 1.09 (0.83–1.43)	Gender
Yu, 1995 <sup>25</sup>	China 711 cases, 711 controls	Users Pylori Temperature Boiling hot Hot Warm/cold	0.71 (0.54–0.93) 0.29 (0.13–0.68) 1.18 (0.75–1.86) 0.63 (0.46–0.87) 0.51 (0.29–0.91)	Age, gender, residence, education, birthplace, alcohol drinking and smoking
Ji, 1996 <sup>26</sup>	China 1124 cases, 1451 controls	Men Non-drinkers Drinkers Tea leaf (g/year) ≤1,200 >1,200–≤2,000 >2,000–≤3,000 >3,000 Women Non-drinkers Drinkers Tea leaf (g/year) ≤1,200 >1,200	1.0 0.96 (0.77–1.21) 1.06 (0.76–1.49) 1.15 (0.82–1.61) 0.88 (0.64–1.24) 0.76 (0.55–1.27) 1.0 0.77 (0.52–1.13) 0.74 (0.45–1.21) 0.81 (0.46–1.43)	Age, income, education (women), alcohol drinking and smoking (men)
Inoue, 1998 <sup>27</sup>	Japan 896 cases, 21, 128 non-cancer outpatients	Rarely Occasional Daily 1–3 cups/day 4–6 cups/day 7+ cups/day	1.00 1.00 (0.77–1.44) 0.96 (0.70–1.32) 1.01 (0.74–1.39) 0.69 (0.48–1.00)	Age, gender, years and season of hospital visit, smoking, alcohol drinking, coffee and black tea drinking, physical exercise, fruit, rice and beef intakes

TABLE 1 – EPIDEMIOLOGIC STUDIES OF GREEN TEA AND STOMACH CANCER (CONTINUED)

Author, year, reference	Country, study	Consumption	OR (95% CI)/RR (95% CI)	Confounder adjustment
Setiawan, 2001 <sup>28</sup>	Yangzhong, China 133 cases, 433 healthy controls	Non-drinkers	1.00	Age, gender, education, body mass index, pack-years of smoking, alcohol drinking
		1–21 cups/week	0.47 (0.27–0.80)	
Gao, 2002 <sup>29</sup>	Huaian, China	0 g/month	1.00	Age and gender
		> 1 g/month	0.38 (0.24–0.62)	
Cohort studies Galanis, 1998 <sup>30</sup>	US (Hawaii) 5233 men, 6297 women	Men and women	1.0	Age, gender, education and birthplace
		None	1.3 (0.7–2.1)	
Tsubono, 2001 <sup>31</sup>	Japan 11902 men, 14409 women	2+	1.5 (0.9–2.3)	Age, gender, health insurance, peptic ulcer, smoking, alcohol drinking, rice, black tea, coffee drinking, meat intake, vegetables, fruits and bean-paste soup
		<1 cup/day	1.0	
		1–2 cups/day	1.1 (0.8–1.6)	
		3–4 cups/day	1.0 (0.7–1.4)	
Sun, 2002 <sup>32</sup>	China, prospective study, nested case-control 190 cases, 772 controls Urine EGC	Urinary EGC	1.00	Matched on age, date of sample collection, residence, and adjusted for helicobacter pylori seropositivity, smoking, alcohol drinking, and level of serum carotenes
		Negative Positive (4+ years)	0.52 (0.28–0.97)	
Hoshiyama, 2002 <sup>33</sup>	Japan, prospective cohort study (JACC) Men: 2,849,605 PY 240 cases Women: 4,024,456 PY 119 cases	Men	1.0	Age, smoking, gender, history of peptic ulcer, family history of stomach cancer, consumption of rice, miso soup, green-yellow vegetables, white vegetables, fruits, and preference for salty foods
		<1	1.0	
		1–2	1.6 (0.9–2.9)	
		3–4	1.1 (0.6–1.9)	
		5–9	1.1 (0.6–1.9)	
		≥10	1.0 (0.5–2.0)	
		Women	1.0	
		<1	1.1 (0.5–2.5)	
		102	1.0 (0.5–2.1)	
		3–4	0.8 (0.4–1.6)	
5–9	0.7 (0.3–2.0)			
≥10	0.7 (0.3–2.0)			
Koizumi, 2003 <sup>34</sup>	Japan, two cohort studies Cohort 1: 31,345 199,748 PY 419 cases Cohort 2: 47,605 290,599 PY 314 cases	<1	1.01	Age, gender, type of health insurance, parental history of gastric cancer, history of peptic ulcer, cigarette smoking, alcohol consumption, pickled vegetables, bean-paste soup.
		1–2	1.0 (0.80–1.27)	
		3–4	0.89 (0.70–1.13)	
		≥5	1.06 (0.86–1.30)	
			1.06 (0.86–1.30)	
			<i>p</i> for trend: 0.61	
Hoshiyama, 2004 <sup>35</sup>	Japan, Nested Case-Control Study from cohort (JACC study) 151 cases, 265 controls	<1 cup/day	1.0	Age, smoking, gender, <i>H. pylori</i> , history of peptic ulcer, family history of stomach cancer, education, consumption of rice, miso soup, green-yellow vegetables, white vegetables, fruits, and preference for salty foods
		1–2	1.3 (0.6–2.8)	
		3–4	1.0 (0.5–1.9)	
		5–9	0.8 (0.4–1.6)	
		≥10	1.2 (0.6–2.5)	
			<i>p</i> for trend: 0.899	

intervention may be effective in the subgroup of smokers who are *GSTM1*- or *GSTT1*-positive.<sup>41</sup> The *p53* gene, in its normal capacity, acts as a gatekeeper by invoking cell-cycle arrest or cell death in response to genetic mutations, facilitating DNA repair activities in response to DNA damage. The gene exhibits a single nucleotide change of G to C at codon 72 and leads to amino acid change from arginine to proline (*Arg*<sup>72</sup> *Pro*).<sup>42,43</sup> *p53* with *Pro*-72 is structurally different from *p53* with *Arg*-72, and the difference in function of this *p53* variant remains unclear. A number of studies have been conducted to estimate the relationship between the *p53* variant and cancer susceptibility; however, the results are controversial.<sup>42–46</sup>

Although the potential effects of selected single nucleotide polymorphisms (SNP) of susceptibility genes and their potential interactions with environmental exposures on stomach cancer

risk have been evaluated in several studies at single gene or limited gene levels, the role of multigenetic effects on the risk of stomach cancer are not known. The genetic susceptibility to stomach cancer may be attributed to the accumulation and combination of SNP of major genetic pathways including metabolic, DNA repair, and cell-cycle control pathways. We hypothesize that the accumulation and combination of SNP of major pathways may play an important role in the development of stomach cancer. The risk of stomach cancer will increase with the elevated numbers of risk genotypes. There may be interactions between multigenetic factors and green tea drinking on the risk of stomach cancer.

The purposes of this population-based case-control study were to evaluate the possible effect of green tea drinking on the risk of stomach cancer, and to explore potential interactions between

green tea, environmental risk factors and genetic susceptibility on the risk of the disease.

## Material and methods

### Background

Taixing City (formerly Taixing County before 1995) is located on the east bank of the Yangtze River in Jiangsu Province, in southeast China. The population-based tumor registry is within the Division of Chronic Disease Prevention, Taixing City Center for Disease Prevention and Control (CDC). Taixing City has 23 townships (rural areas) and one central town (urban area). Each township or city has 10–12 villages (or resident blocks in the urban area). 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 CDC population-based tumor registry twice a month. The central town has a similar reporting system (resident blocks and town hospital). Taixing is one of the highest-risk areas of alimentary cancer in the world. The incidence for the combined top 3 cancers (esophagus, liver and stomach) is 176 in 100,000. The incidence rate was 55 in 100,000 for stomach cancer in 2000. Among people diagnosed with cancer, stomach cancer is the third highest killer after esophageal cancer and liver cancer.

### Study population

A population-based case-control study was conducted in Taixing City, Jiangsu Province, China. Data collected included questionnaire data and blood samples for assaying molecular markers. Although the original study included 3 cancer sites (esophagus, stomach and liver) and one common population-control group, the case group for this analysis only included patients with newly diagnosed stomach cancer and population controls. The population healthy control group was a random sample from the local population from which the cases derived.

### Cases

Eligible cases were patients diagnosed with stomach cancer from June 1, 2000 to December 30, 2000 with pathologically or clinically confirmed diagnoses, reported to Taixing Tumor Registry at the Taixing CDC. During the study period, we intended to interview all incident cases with primary stomach cancer who consented to participate in our study with the following criteria: patients must be newly diagnosed, 20 years of age or older, in stable medical condition as determined by their physician and willing to participate. Our study was restricted to people living in Taixing for 10 years or more. In our study period, we recruited a total of 206 patients with primary stomach cancer. This represents 65% of all new cases ( $n = 316$ ) diagnosed within 6 months of the study period in Taixing. Among these cases, all 206 patients completed the questionnaires and 196 DNA samples were isolated. Five percent of cases ( $n = 10$ ) had inadequate blood samples for DNA extraction.

### Controls

Healthy individuals selected randomly from the general population in Taixing were eligible controls. Because our original study included 3 upper-GI cancers (stomach, liver and esophagus), we used a common control group for all 3 cancer sites. The control group was selected according to the frequency distribution of gender and age of all 3 cancer cases interviewed from each village (or resident block in the city) where cancer cases originated. For each village (or resident block), a list was generated of residents with the same gender and within the same age group and random numbers were used to select the healthy controls according to the control-to-case ratio of 2:3. When the control did not fit the criteria, or refused to be interviewed, we recorded their basic demographic data and used the same selection process to choose another con-

trol. On average, 18–20 healthy controls were selected for each township (center town). A total of 464 controls were finally selected from the whole population of 1,280,000 residents in the Taixing area. Due to the method of control selection, the age and gender distribution of controls were correspondent to all 3 cancer sites and might not have totally matched the distribution of stomach cancer cases. The higher proportion of younger cases for liver cancer resulted in a high proportion of younger controls.

We interviewed eligible controls during the study period with the following criteria: 20 years of age or older, in stable medical condition, and willing to participate. The study was restricted to people living in Taixing for 10 years or more. Following the selected list, the interviewer located the controls, explained the study, interviewed them at their homes and collected approximately 8 ml of blood. A total of 464 potential healthy controls were approached and 415 controls completed interviews (89.4%). Among the controls who were interviewed, 397 DNA samples were isolated from blood samples. Four percent of interviewed controls did not have DNA samples for analysis due to no or insufficient collected blood samples.

### Epidemiologic data collection

We interviewed cases and controls using a standard questionnaire. Our interviewers received rigorous training. Interviews were monitored frequently by professional staff in the Division of Chronic Disease Prevention of the Taixing CDC. 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 doctor's office.

We attempted to include all possible risk or protective factors that were considered important in the Chinese population using a standard questionnaire. The questionnaire included: (i) demographic factors, including the subject's age, gender, residence, place of birth, education, annual income, blood type and disease diagnostic information; (ii) residence and drinking water history; (iii) detailed dietary history; (iv) detailed smoking history; (v) alcohol drinking habits; (vi) tea drinking habits; (vii) detailed information on disease history; (viii) occupation history and related exposures; (ix) family history of stomach cancer and other cancers; and (x) physical activities.

### Laboratory assays

**DNA isolation.** Genomic DNA was isolated from blood clots using a modified phenol-chloroform protocol.

**PCR analysis of gene polymorphisms.** Genotyping was performed at the Molecular Epidemiology Laboratory at UCLA. All reagents were obtained from Promega Company (Madison, WI). *GSTM1*, *GSTT1* and *GSTP1* were analyzed using procedures published previously.<sup>47,48</sup> The fragments of *GSTM1*,  $\beta$ -globin and *GSTT1* were 215, 350 and 480 bp in size, respectively. Polymorphic bands of *GSTP1* were 176 bp (Ile/Ile), 91 bp and 85 bp (Val/Val), 176 bp, 91 bp and 85 bp (Ile/Val). Codon 72 polymorphisms of the *p53* gene were examined by restriction fragment length polymorphisms using the polymerase chain reaction (RFLP-PCR).<sup>49</sup>

**PCR-RFLP analysis for *p53* codon 72.** The reaction was carried out in a total volume of 20  $\mu$ l containing 50 mM KCl, 1.5 mM  $MgCl_2$ , 20  $\mu$ M dNTPs and 0.2  $\mu$ M each *p53* codon 72 primers, 1 U Taq DNA polymerase and 100 ng DNA. The primers were 5'-TTGCCGTCCCAAGCAATGGATGA-3' and 5'-TCTGGGAAGGGACAGAAGATGAC-3'. The PCR conditions were 95°C for 5 min for primary denaturation, followed by 35 temperature cycles of 95°C for 30 sec, 60°C for 30 sec and 72°C for 30 sec. The last extension step was 72°C for 5 min. PCR products then were digested overnight with 5 U of restriction enzyme BstU1 (Promega), which distinguishes between the Arg allele and the Pro allele. Polymorphic bands of *p53* codon 72 were 169 bp and 127 bp (Arg/Arg), 296 bp (Pro/Pro), 169 bp, 127 bp and 296 bp (Arg/Pro).<sup>50</sup>

**H. pylori measurement.** *H. pylori* was measured by assaying antibodies for (CagA-HP) IgG. The presence of anti-(CagA-HP) IgG in serum was measured by indirect enzyme immunoassay (EIA) using kits from the Reagent Company of the Shanghai Biotechnology Industry Park (Shanghai, China), according to the manufacturer's instructions. The serum samples were diluted (1:21) and pipetted into pre-coated micro-well plates; plates were then incubated at 37°C for 30 min and washed 5 times. After the conjugate was added, the plate was incubated at 37°C for 30 min again. The plate was then washed 5 times followed by dispensing the substrate solution and incubating at 37°C for 15 min. Stop solution was added and the plate was read under 450 nm wavelengths. Results were efficient when  $OD_{PC} > OD_{NC} + 0.5$ ; otherwise the measurements were repeated.  $OD_{PC}$  represents average OD value of positive controls;  $OD_{NC}$  represents average OD value of negative controls. Samples are considered positive when  $OD > OD_{NC} + 0.30$ .

#### Multigenetic index

A multigenetic index is the number of risk genotypes for each participant in our study. We assume that there are additive interactions among risk genotypes of susceptibility genes from different pathways and that the contribution to the carcinogenesis of each SNP is similar to each other, although weighted approaches of individual SNP of different pathways may be developed in the future studies. The multigenetic index was generated based on the sum of risk genotypes of all susceptibility genes with prior knowledge on susceptibility genes for each individual. We defined *GSTM1* null, *GSTT1* null, *GSTP1* Val/Val and *p53* codon72 Pro/Pro genotype as risk genotypes, and summed the numbers of risk genotypes for each participant in our study.

#### Statistical analysis

All analysis was performed using SAS 8.0 software. We evaluated relationships between stomach cancer and putative risk factors by crude and adjusted odds ratios (OR) and their 95% confidence intervals (CI) derived from an unconditional logistic regression model. Crude OR and adjusted OR were estimated for each independent variable. We adjusted for potential confounding factors including age (continuous), gender (male = 1, female = 0), education (continuous), income (continuous variable), body mass index (continuous variable), pack-years of smoking (continuous), alcohol drinking, very hot food eating habit (yes or no), *H. pylori* infection (CagA + or -), stomach disease history (any one of the following stomach related diseases: chronic atrophic gastritis, other chronic gastritis, acute gastritis, gastric ulcer, gastric polypus, pernicious anemia) and family history of stomach cancer. In data analysis, dummy variables were used in a logistic regression model to estimate the OR for each exposure category. We explored the relationship between green tea and risk of stomach cancer and interactions with other risk factors. A logistic regression model was used to evaluate the multiplicative interaction effects. When the interactions were assessed, OR were further adjusted by the same confounding variables described above. A more than multiplicative interaction was indicated when:  $OR_{11} > OR_{10} * OR_{01}$ .  $OR_{11}$  represents the OR for combined effect when both factors were present,  $OR_{10}$  represents the OR when only Factor 1 was present and  $OR_{01}$  represents the OR when only Factor 2 was present. 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.

#### Results

The distributions of potential confounders in cases and controls are shown in Table II. The proportion of males in cases (66.99%) was similar to that in controls (69.16%). More cases were distributed in the age groups >60 years than controls ( $p < 0.05$ ). Compared to cases, more controls received more years of education.

Obvious differences were observed for average income and body mass index between cases and controls. A higher proportion of stomach cancer was distributed on lower social economic class. Rates of tobacco smoking, alcohol drinking and *H. pylori* infection (CagA) were not found to be significantly different between cases and controls. The case group, however, was observed to have high proportions of very hot food eating habit, stomach disease history and family history of stomach cancer.

The relationships between green tea drinking and risk of stomach cancer are presented in Table III. A total of 6.3% of cases (13 cases) and 4.3 % of controls (18 subjects) with only black tea drinking habits were excluded from analysis. About 32.1% of cases and 45.6% of controls had ever drunk green tea, which was defined as drinking at least one cup of green tea per day for more than half a year. Decreased risk was observed between green tea drinking and the risk of stomach cancer, with a crude OR of 0.57 (95% CI = 0.39~0.81), and an adjusted OR of 0.59 (95% CI = 0.34~1.01). No obvious monotonic trend was observed between years of green tea drinking and risk of stomach cancer; however, the risk was decreased for increased years of green tea drinking. Tea concentration was categorized into 3 levels: low (tea leaves were <25% of the volume of the cup), moderate (tea leaves were between 25~50% of the volume of the cup) and high (volume of tea leaves was >50% of cup volume). A strong dose-response relationship was observed between an increased green tea concentration and a decreased risk of stomach cancer ( $p$ -value for the trend was 0.01). In addition, higher consumption of green tea was strongly related to a decreased risk of stomach cancer and a monotonic trend was shown ( $p$ -value for trend was 0.01). For subjects who drank >250 g of green tea per month, which is equivalent to 2 or more cups per day, the adjusted OR was 0.39 (95%CI = 0.17~0.91), in comparison with non-green tea drinkers.

Table III shows the associations between genetic susceptibility markers and stomach cancer. The prevalence of *GSTM1*, *GSTT1* null genotype was 64.8%, 47.5% in cases and 59.8%, 48.9% in controls, respectively. No obvious associations of *GSTM1* and *GSTT1* with the risk of stomach cancer were observed. A total of 31.6% of cases and 29.5% of controls were heterozygous for Ile/Val genotype, 4.6% of cases and 3.1% of controls were homozygous for Val allele of *GSTP1*. Compared to subjects with Ile/Ile or Ile/Val genotype, adjusted OR for subjects with Val/Val genotype was 1.53 (95% CI = 0.50~4.56). Those who were heterozygous for Arg/Pro genotype of *p53* codon 72 were similar among cases and controls (45% for both) whereas more cases were homozygous for Pro allele (33.0%) than controls (24.1%). For those who possessed both Pro alleles, the adjusted OR was 1.56 (95% CI = 0.96~2.52) in comparison with other genotypes. When individuals with a multigenetic index of 0~1 served as a reference group, the adjusted OR were 1.22 (95% CI = 0.76~1.96) for those with a multigenetic index of 2, and 2.21 (95% CI = 1.02~4.79) for those with 3 or more high-risk genotypes. There was a borderline dose-response relationship between increased numbers of risk genotypes and the risk of stomach cancer ( $p$ -value for trend = 0.0613). When treating the multigenetic index as a continuous variable in logistic regression model, the crude and adjusted OR were 1.23 (95% CI = 0.99~1.52,  $p = 0.06$ ) and 1.25 (95% CI = 0.96~1.63,  $p = 0.09$ ).

Possible interactions were explored between green tea drinking and susceptible genotypes, as well as alcohol drinking, tobacco smoking and *H. pylori* infection (CagA), when adjusting for potential confounders. A more than multiplicative interaction was indicated between green tea drinking and alcohol drinking. Crude and adjusted OR for interaction were 3.37 (95% CI = 1.51~7.55) and 4.57 (95% CI = 1.62~12.89), respectively (Table IV). When using the green tea drinkers with a multigenetic index of 0 or 1 as a reference group, the adjusted OR for joint effect was 2.03 (95%CI = 0.99~4.15) for the non-green tea drinkers with a multigenetic index of 2~4. The adjusted OR for interaction between green tea drinking and multigenetic index was 2.31 (95%CI =

TABLE II – DISTRIBUTION OF POTENTIAL CONFOUNDERS AMONG CASES AND CONTROLS

Variables	Cases n (%)	Controls n (%)	Total n (%)	p-value <sup>1</sup>
Total <sup>3</sup>	206	415		
Gender				
Male	138 (66.99)	287 (69.16)	425 (68.44)	0.5844
Female	68 (33.01)	128 (30.84)	196 (31.56)	
Age (years)				
<40	5 (2.43)	31 (7.47)	36 (5.80)	0.0019
40–49	19 (9.22)	69 (16.63)	88 (14.17)	
50–59	65 (31.55)	136 (32.77)	201 (32.37)	
60–69	73 (35.44)	116 (27.95)	189 (30.43)	
>69	44 (21.36)	63 (15.18)	107 (17.23)	
Education				
Illiteracy	66 (32.04)	73 (17.59)	139 (22.38)	<0.0001 <sup>2</sup>
Primary	107 (51.94)	142 (34.22)	249 (40.10)	
Middle	30 (14.56)	124 (29.88)	154 (24.80)	
High	2 (0.97)	66 (15.90)	68 (10.95)	
College	1 (0.49)	10 (2.41)	11 (1.77)	
Income per capita				
<60	59 (28.64)	88 (21.21)	147 (23.67)	0.0472
60–100	41 (19.90)	74 (17.83)	115 (18.52)	
100–160	66 (32.04)	135 (32.53)	201 (32.37)	
>160	40 (19.42)	118 (28.43)	158 (25.44)	
Body mass index				
≤22	122 (59.22)	180 (43.37)	302 (48.63)	0.0002
>22	84 (40.78)	235 (56.63)	319 (51.37)	
Tobacco smoking				
Never	92 (45.77)	217 (52.42)	309 (50.24)	0.1222
Ever	109 (54.23)	197 (47.58)	306 (49.76)	
Alcohol drinking				
Never	111 (55.22)	207 (50.24)	318 (51.88)	0.6987
Few	31 (15.42)	72 (17.48)	103 (16.80)	
Often	32 (15.92)	75 (18.20)	107 (17.46)	
Everyday	27 (13.43)	58 (14.08)	85 (13.87)	
Very hot food eating habit				
No	169 (84.92)	379 (92.67)	548 (90.13)	0.0027
Yes	30 (15.08)	30 (7.33)	60 (9.87)	
<i>H. pylori</i> infection				
No	130 (64.68)	251 (68.77)	381 (67.31)	0.3208
Yes	71 (35.32)	114 (31.23)	185 (32.69)	
Family history of stomach cancer				
No	168 (81.95)	392 (94.69)	560 (90.47)	<0.0001
Yes	37 (18.05)	22 (5.31)	59 (9.53)	
Stomach disease history				
No	103 (51.76)	361 (87.20)	464 (75.69)	<0.0001
Yes	96 (48.24)	53 (12.80)	149 (24.31)	

<sup>1</sup>Based on Chi-square testing.–<sup>2</sup>Fisher's exact testing.–<sup>3</sup>There are less than 5% missing values for some variables excepting *H. pylori* infection with about 8.9% missing value due to less blood serum samples.

0.88–6.03), indicating a potential more than multiplicative interaction between both factors (Table V).

## Discussion

Selection bias and information bias may exist with case-control studies. Because this is a population-based case-control study, it is less susceptible to selection bias because healthy controls were selected randomly from the same base population as the cases. There is a possibility for information bias, in that exposures under study might have been misclassified during the interviews due to recall bias or reporting bias. Cases might think more about the possible causes of their illness, and therefore recall their exposure experience differently from healthy controls. Recall bias would lead to an overestimation of the observed association. For example, cases may report gastric related disease more often than controls. The misclassification bias for molecular and serological assays may be minimized because we have used standard protocols, and the case-control status was blinded for the laboratory testing. A possibility of potential protopathic bias on the relationship between green tea drinking and risk of stomach cancer may exist in a case-control study. The symptoms of early-stage stomach cancer such as stomach ache and upset stom-

ach may lead to reduced green tea drinking, which would result in an overestimated effect of green tea drinking on the risk of stomach cancer. In our previous study of green tea,<sup>28</sup> we found that green tea drinking not only protected against the development of stomach cancer, but also reduced the risk of chronic atrophic gastritis, a premalignant lesion of the stomach. Because chronic atrophic gastritis may last for several years before development of stomach cancer, the protective effect of green tea drinking on the premalignant lesion suggests that potential protopathic bias on the association between green tea drinking and risk of stomach cancer might be reduced.

Our study has a relatively large sample size, which enables us to detect moderate associations for selected risk or protective factors. Because the majority of our cases (>90%) had adenocarcinoma of the distal stomach, we were not able to stratify our analysis for other tumor sites such as gastric cardia and cell histology. Our results may only indicate the associations for adenocarcinoma of the distal stomach. When evaluating potential interactions, the precision of measurements would be compromised by our limited sample size. The potential confounding factors may also distort the association under investigation in observation studies. Although we could not entirely control for all potential confounding factors, we have tried to control for potential known confound-

TABLE III – GREEN TEA DRINKING, GENE POLYMORPHISMS AND THE RISK OF STOMACH CANCER

Variables	Cases n (%)	Controls n (%)	Crude OR (95% CI)	Adjusted OR (95% CI) <sup>1</sup>
Green tea drinking status				
Never	131 (67.9)	216 (54.4)	1	1
Ever	62 (32.1)	181 (45.6)	0.57 (0.39~0.81)	0.59 (0.34~1.01)
Years of green tea drinking				
Never	131 (67.88)	216 (54.41)	1	1
0~15	14 (7.25)	38 (9.57)	0.61 (0.32~1.16)	0.60 (0.24~1.51)
15~25	14 (7.25)	50 (12.59)	0.46 (0.25~0.87)	0.47 (0.20~1.12)
≥25	34 (17.62)	93 (23.43)	0.60 (0.39~0.94)	0.64 (0.34~1.19)
<i>p</i> for trend			0.0057	0.1034
Green tea concentration				
Never	131 (67.88)	216 (54.41)	1	1
Low	17 (8.81)	20 (5.04)	1.40 (0.71~2.77)	1.39 (0.57~3.42)
Moderate	37 (19.16)	125 (31.49)	0.49 (0.32~0.75)	0.48 (0.26~0.90)
High	8 (4.15)	36 (9.07)	0.37 (0.17~0.81)	0.40 (0.14~1.09)
<i>p</i> for trend			0.0002	0.0100
Green tea consumption (g/month)				
Never	131 (68.59)	216 (57.14)	1	1
<125	33 (17.28)	42 (11.11)	1.30 (0.78~2.15)	1.09 (0.53~2.23)
125~250	15 (7.85)	50 (13.23)	0.50 (0.27~0.92)	0.44 (0.19~1.01)
≥250	12 (6.28)	70 (18.52)	0.28 (0.15~0.54)	0.39 (0.17~0.91)
<i>p</i> for trend			<0.0001	0.0118
<i>GSTM1</i>				
Normal	69 (35.20)	158 (40.20)	1	1
Null	127 (64.80)	235 (59.80)	1.24 (0.87~1.77)	1.11 (0.70~1.76)
<i>GSTT1</i>				
Normal	103 (52.55)	201 (51.15)	1	1
Null	93 (47.45)	192 (48.85)	0.95 (0.67~1.33)	1.12 (0.72~1.74)
<i>GSTP1</i>				
Ile/Ile	125 (63.78)	265 (67.43)	1	1
Ile/Val	62 (31.63)	116 (29.52)	1.13 (0.78~1.65)	1.02 (0.63~1.66)
Val/Val	9 (4.59)	12 (3.05)	1.59 (0.65~3.87)	1.53 (0.50~4.65)
<i>p</i> for trend			0.2831	0.6106
Ile/Ile	125 (63.78)	265 (67.43)	1	1
Ile/val or Val/val	71 (36.22)	128 (32.57)	1.18 (0.92~1.69)	1.07 (0.67~1.71)
Ile/Ile or Ile/Val	187 (95.41)	381 (96.95)	1	1
Val/Val	9 (4.59)	12 (3.05)	1.53 (0.63~3.69)	1.51 (0.50~4.56)
<i>p53</i> codon 72				
Arg/arg	42 (21.65)	118 (30.26)	1	1
Arg/pro	88 (45.36)	178 (45.64)	1.39 (0.90~2.15)	1.00 (0.57~1.76)
Pro/pro	64 (32.99)	94 (24.10)	1.91 (1.19~3.07)	1.56 (0.86~2.82)
<i>p</i> for trend			0.0072	0.1284
Arg/arg	42 (21.65)	118 (30.26)	1	1
Arg/pro or Pro/pro	152 (78.35)	272 (69.74)	1.57 (1.05~2.35)	1.21 (0.72~2.02)
Arg/arg or Arg/pro	130 (67.01)	296 (75.90)	1	1
Pro/pro	64 (32.99)	94 (24.10)	1.55 (1.06~2.26)	1.56 (0.96~2.52)
Multi-genetic index <sup>2</sup>				
0-1	96 (49.48)	222 (57.07)	1	1
2	75 (38.66)	145 (37.28)	1.20 (0.83~1.73)	1.22 (0.76~1.96)
≥3	23 (11.86)	22 (5.65)	2.42 (1.29~4.55)	2.21 (1.02~4.79)
<i>p</i> for trend			0.0140	0.0613

<sup>1</sup>Adjusted on age (continuous variable), gender (male or female), education (continuous), income (continuous variable), body mass index (continuous variable), pack-year of smoking (continuous), alcohol drinking (1, never; 2, seldom; 3, often; 4, every day), very hot food eating habit, *H. pylori* infection (CagA + or -), stomach disease history and family history of stomach cancer.<sup>2</sup>Multigenetic index is the sum of the risk genotypes defined as *GSTM1* null, *GSTT1* null, *GSTP1* Val/Val, *p53* Pro/Pro.

ers including age, gender, education, income, body mass index, pack-years of smoking, alcohol drinking, very hot food eating habit, *H. pylori* infection (CagA), stomach disease history and family history of stomach cancer. In comparison with crude OR, the adjusted OR for relationship between green tea drinking and risk of stomach cancer were very similar, indicating that the impact of potential confounding factors was limited. Because we have used the common controls for all 3 cancer sites, potential residual confounding effects of age and gender might still exist even though these 2 factors were adjusted in the multivariate analysis. We have carried out sensitivity analyses by using age and gender frequency matching case-control subset (206 cases and 206 controls). The adjusted point estimates for green tea drinking, multigenetic index, the interaction OR between green tea drinking and alcohol drinking as well as multigenetic index were similar to

that of the overall analyses. We believe that the potential residual confounding effects by age and gender are minimal.

Although numerous epidemiological studies including our previous study (Table I) have explored the possible association between green tea drinking and the risk of stomach cancer, very few studies have explored the possible effect modification on the green tea by genetic susceptibility genes.<sup>6,7</sup> Compared to these prior studies, the major advantage of the present study is that we have evaluated the possible effect modification on green tea by looking at multiple pathway and multigenetic factors, in addition to evaluating the main effect of green tea on the risk of stomach cancer. Our results provide clues for future mechanistic research of green tea on cancer prevention and epidemiological studies and offer the potential strategy of chemoprevention aimed at individuals with high genetic susceptibility.

TABLE IV – INTERACTIONS BETWEEN GREEN TEA DRINKING AND TOBACCO SMOKING, ALCOHOL DRINKING, OR *H. PYLORI* INFECTION

Variables	Green tea drinking <sup>2</sup>	Cases <i>n</i>	Controls <i>n</i>	Crude OR (95% CI)	Adjusted OR (95% CI) <sup>1</sup>
Alcohol drinking <sup>3</sup>					
No	Yes	37	84	1	1
Yes	Yes	25	96	0.59 (0.33–1.06)	0.45 (0.21–0.98)
No	No	99	185	1.22 (0.77–1.92)	0.92 (0.47–1.82)
Yes	No	32	30	2.42 (1.29–4.55)	1.91 (0.86–4.22)
OR for interaction					
3.37 (1.51–7.55) 4.57 (1.62–12.89)					
Tobacco smoking <sup>4</sup>					
No	Yes	11	60	1	1
Yes	Yes	51	121	2.30 (1.12–4.73)	1.30 (0.44–3.79)
No	No	78	153	2.78 (1.38–5.59)	1.77 (0.69–4.55)
Yes	No	53	63	4.59 (2.19–9.61)	2.17 (0.77–6.11)
OR for interaction					
0.72 (0.31–1.69) 1.05 (0.35–3.18)					
Pack-year					
<20	Yes	28	104		
≥20	Yes	34	77	1.64 (0.92–2.93)	0.89 (0.41–1.91)
<20	No	101	190	1.97 (1.22–3.20)	1.22 (0.62–2.41)
≥20	No	30	26	4.29 (2.19–8.38)	2.40 (0.98–5.88)
OR for interaction					
1.32 (0.58–3.00) 2.22 (0.76–6.45)					
<i>H. pylori</i> infection					
No	Yes	36	107	1	1
Yes	Yes	25	55	1.35 (0.74–2.47)	1.50 (0.70–3.19)
No	No	82	133	1.83 (1.15–2.92)	1.76 (0.92–3.36)
Yes	No	45	55	2.43 (1.41–4.20)	2.41 (1.17–4.95)
OR for interaction					
0.98 (0.45–2.13) 0.91 (0.35–2.37)					

<sup>1</sup>Adjusted on age (continuous variable), gender (male or female), education (continuous), income (continuous variable), body mass index (continuous variable), pack-year of smoking (continuous), alcohol drinking (1, never; 2, seldom; 3, often; 4, every day), very hot food eating habit, *H. pylori* infection (CagA + or –), stomach disease history and family history of stomach cancer. –<sup>2</sup>Green tea consumption status: current drinker vs. non-current drinker. –<sup>3</sup>Ever alcohol consumer vs. never alcohol consumer. –<sup>4</sup>Ever-smoker vs. never-smoker.

In our present study, an inverse association between green tea drinking and risk of stomach cancer was suggested, with crude and adjusted OR of 0.57 (0.39–0.81) and 0.59 (0.34–1.01), respectively. Dose-response relationships were shown between increased concentration, as well as increased consumption, of green tea, and decreased risk of stomach cancer. For individuals who drank green tea more than 250 g/month (around 8 g/day, or 2 or more cups per day with at least moderate concentration), risk of developing stomach cancer might be decreased as much as 60%. Our findings are in general agreement with most previous studies<sup>19,22,25,27–29,32</sup> and the suggested effective consumption quantity from our study was relatively lower than that suggested by some previous epidemiological studies, even though the Japanese tea cup is much smaller than Chinese tea cup.<sup>19,27</sup> These data indicate that recommendations of drinking high concentrated green tea and having 2 or more cups per day may be appropriate to protect the general population from stomach cancer.

There is great interest in green tea as a potential chemo-preventive agent for stomach cancers. Six cohort and 12 case-control studies have been conducted to elucidate the relationship between green tea and stomach cancer. The results of these epidemiologic studies are summarized in Table I. Among all 18 studies, 5 (4 case-control and 1 cohort studies) of 7 studies (71%) in China<sup>22,25,28,29,32</sup> and 2 (case-control studies) of 11 studies (18%) in Japan<sup>19,27</sup> suggested a strong relationship between green tea drinking and decreased risk of stomach cancer. Three studies observed an inverse association, but the CI included null value.<sup>18,20,26</sup> The rest of the studies observed no obvious association between green tea drinking and stomach cancer risk.<sup>21,23,24,30,31,33–35</sup> A higher percentage (71%) of studies in China observed a protective effect of green tea drinking on risk of stomach cancer and most of these studies were conducted in the major green tea production areas (Jiangsu and Zhejiang provinces and the Shanghai area), where the proportion of non-green tea drinkers in the population is approximately 40–60%. A relatively lower percentage (18%) of studies conducted in Japan, however, showed no association between green tea drinking and stomach cancer. One explanation for this may lie in the selection of the non-

green tea drinkers as a reference comparison group. Studies in Japan had insufficient non-green tea drinkers as a reference comparison group, and investigators had to include both non-green tea drinkers and those who drank <1 cup per day as the reference group, which led to relatively lower power to detect the protective effect of green tea drinking. For example, the results of a cohort study reported by Tsubono *et al.*<sup>31</sup> did not suggest a protective effect of green tea on stomach cancer. The investigators recruited very few people who never drank green tea, and combined them with people who drank <1 cup of green tea per day as a reference group in their analysis. The comparison made in this study was among green tea drinkers, not between drinkers and non-drinkers, which may partially explain why they did not observe the protective effect of green tea. Another possible explanation is that the production of Japanese green tea involves a steaming process at a high temperature to keep the green color of the tea. This process may lead to changes in chemical composition and in the concentration of bioactive constituents in green tea such as vitamins C and E, which may also contribute to the preventive properties of green tea.

In our previous case-control study conducted in Yangzhong, Jiangsu Province, China,<sup>28</sup> we found that green tea drinkers had a 48% reduced risk of stomach cancer compared to nondrinkers after adjusting for potential confounders, which is consistent with the current study. In addition, we observed a decreased risk of stomach cancer with increasing frequency and duration of tea drinking among green tea drinkers. A case-control study conducted in the same province in China reported that green tea drinkers had a 62% reduced risk of stomach cancer compared to nondrinkers after adjusting for age and gender.<sup>29</sup> In a prospective nested case-control study from a prospective cohort in Shanghai, China,<sup>32</sup> a total of 190 incident cases and 722 controls were included and urinary tea polyphenols, including epigallocatechin (EGC) and epicatechin (EC), and their respective metabolites 5-(3',4',5'-trihydroxyphenyl)- $\gamma$ -valerolactone and 5-(3',4'-dihydroxyphenyl)- $\gamma$ -valerolactone, were measured by HPLC in all study subjects. After exclusion of cases diagnosed within 4 years of follow-up, urinary EGC levels showed a statistically significant



**TABLE V – GENE-GREEN TEA INTERACTIONS BETWEEN *GSTM1*, *GSTT1*, *GSTP1*, P53 CODON 72 AND GREEN TEA DRINKING ON THE RISK OF STOMACH CANCER**

Genes	Green tea drinking	Cases <i>n</i>	Controls <i>n</i>	Crude OR (95% CI)	Adjusted OR (95% CI) <sup>1</sup>
<i>GSTM1</i>					
Normal	Yes	20	67	1	1
Normal	Yes	40	103	1.30 (0.70–2.42)	0.88 (0.40–1.93)
Null	No	43	82	1.76 (0.94–3.27)	1.31 (0.56–3.08)
Null	No	81	124	2.19 (1.23–3.88)	1.64 (0.76–3.56)
OR for interaction				0.96 (0.44–2.07)	1.43 (0.53–3.83)
<i>GSTT1</i>					
Normal	Yes	35	82	1	1
Normal	Yes	25	88	0.67 (0.37–1.21)	0.84 (0.40–1.77)
Null	No	65	109	1.40 (0.85–2.31)	1.36 (0.66–2.79)
Null	No	59	97	1.43 (0.86–2.38)	1.71 (0.84–3.47)
OR for interaction				1.53 (0.73–3.22)	1.49 (0.58–3.83)
<i>GSTP1</i>					
Ile/ile or Ile/Val	Yes	57	163	1	1
Val/Val	Yes	3	6	1.43 (0.35~5.91)	0.79 (0.34~1.79)
Ile/ile or Ile/Val	No	118	202	1.67 (1.15~2.44)	1.43 (0.76~2.69)
Val/Val	No	6	5	3.43 (1.01~11.67)	1.69 (0.84~3.38)
OR for interaction				1.44 (0.22~9.26)	1.25 (0.13~12.17)
<i>p53</i> codon 72					
Arg/Arg or Arg/Pro	Yes	39	126	1	1
Pro/Pro	Yes	20	41	1.58 (0.83~3.00)	1.07 (0.48~2.37)
Arg/Arg or Arg/Pro	No	82	158	1.68 (1.07~2.62)	1.37 (0.73~2.60)
Pro/Pro	No	41	48	2.76 (1.59~4.78)	2.75 (1.28~5.93)
OR for interaction				1.04 (0.46~2.35)	1.88 (0.68~5.21)
Multi-genetic Index					
0~1	Yes	33	93	1	1
2~4	Yes	26	74	0.99 (0.55~1.80)	0.82 (0.38~1.74)
0~1	No	57	119	1.35 (0.81~2.24)	1.08 (0.52~2.22)
2~4	No	66	86	2.16 (1.30~3.60)	2.03 (0.99~4.15)
OR for interaction				1.62 (0.77~3.42)	2.31 (0.88~6.03)

<sup>1</sup>Adjusted on age (continuous variable), gender (male or female), education (continuous), income (continuous variable), body mass index (continuous variable), pack-year of smoking (continuous), alcohol drinking (1, never; 2, seldom; 3, often; 4, every day), very hot food eating habit, *H. pylori* infection (CagA + or -), stomach disease history and family history of stomach cancer.

inverse association with stomach cancer (OR = 0.52, 95% CI = 0.28–0.97) after adjustment for *H. pylori* seropositivity, smoking, alcohol drinking and level of serum carotenes. In addition, similar urinary EGC level cancer risk associations were observed when the stomach cancer and esophageal cancer (42 cases) sites were combined (OR = 0.58, 95% CI = 0.34–0.98). Our study is the only prospective study in a Chinese population that provides direct evidence that tea polyphenols may act as chemopreventive agents against gastric and esophageal cancer development.<sup>32</sup>

Tea polyphenols as antitumor agents have been reported to target multiple organs including the digestive tract, liver, lung, pancreas, mammary gland and skin.<sup>13,51,52</sup> Most animal models and *in vitro* studies have shown that they have anti-mutagenic and anti-oxidative properties that can inhibit various human cancer cell lines and also have no serious adverse effects.<sup>53–56</sup> *N*-nitroso compounds formed in the human stomach have been implicated as etiological factors of stomach cancer. Animal experiments have suggested that green tea extract can reduce carcinogenic activity of *N*-nitroso-compounds by combining with them.<sup>53,57,58</sup> These laboratory results are supported by an epidemiological study in China, which reported that intake of green tea extracts could effectively inhibit *N*-Nitrosoproline (NPRO) formation.<sup>59</sup> Polyphenols have been suggested by several animal studies to inhibit not only serum insulin-like growth factor-1 (IGF-1) levels but also the IGF-1 signal transduction pathway.<sup>60–62</sup> IGF-1 has been reported to be linked to initiation, progression and metastasis of cancers.<sup>63,64</sup> The decreased risk of stomach cancer among green tea drinkers might be related to the IGF-1 pathway. Green tea and tea polyphenols are bacteriostatic and bactericidal, thus they might lower the titers of *H. pylori* and the associated stomach cancer. Green tea drinkers were also observed to have a 51% lower risk of chronic gastritis than nondrinkers by one of our previous studies conducted in a Chinese population.<sup>28</sup> Chronic gastritis has been

suggested to have a strong relationship with stomach cancer. Green tea might decrease the risk of stomach cancer by protecting the stomach from chronic gastritis. In addition, green tea not only has an inhibitory effect on the progression of stomach cancer, but also has a protective effect after cancer treatment.<sup>10,52</sup>

Among the Chinese population, green tea drinking is often accompanied by alcohol drinking and cigarette smoking.<sup>32</sup> Alcohol and tobacco consumption have long been suspected as important risk factors for stomach cancer. We further explored the possible interactions between green tea drinking and alcohol drinking and tobacco smoking. We observed a strong, more than multiplicative interaction between green tea and alcohol drinking (adjusted OR = 4.57, 95% CI = 1.62–12.89). We found that the highest risk of stomach cancer was among individuals who were alcohol drinkers without drinking green tea. Epidemiological studies have been conducted to evaluate potential interaction between alcohol and green tea drinking. One epidemiological study has shown that green tea might protect gastric mucosa from damage caused by alcohol.<sup>65</sup> The mucosal and villous atrophy induced by fasting was reverted to normal by the ingestion of green tea. More research on this mechanism is suggested by the study.<sup>66</sup> Alcohol drinking may lead to increased oxidative stress and reduced antioxidants. Green tea may protect cellular components such as lipids and proteins against alcohol-related oxidative modification, and can effectively protect blood serum against oxidative stress produced by ethanol.<sup>67</sup>

A reduced risk was found for heavy smokers (pack-years  $\geq 20$ ) with green tea drinking habits. Among heavy smokers, green tea drinkers had an approximately 60% lower risk for stomach cancer than that of non-green tea drinkers (OR = 0.37). The OR for interaction was 2.22 (95%CI = 0.76–6.45), suggesting a more than multiplicative interaction between green tea drinking and pack-years of tobacco smoking, although the 95% CI include a null

value. Results from research experiments have demonstrated that green tea could lower the risk of cancers associated with tobacco use.<sup>10,54,68,69</sup> These findings were supported by epidemiological studies.<sup>70,71</sup> One animal experiment found that polyphenolic fraction isolated from green tea (GTP) offered a significant protection against both diethylnitrosamine (DEN)- and benzo(a)pyrene(BP)-induced forestomach and lung tumorigenesis in A/J mice.<sup>72</sup> Green tea may protect cells from oxidative stress produced by tobacco smoking and may inhibit tobacco-carcinogen-induced DNA damage and cell proliferation.

The members of the GST supergene family have diverse structures and catalyze GSH conjugation to identical as well as very different substrates. As reported from *in vitro* and animal studies, extracts from green tea might enhance the activity of GST enzymes and induce more effective detoxification for their corresponding substrates.<sup>6,7</sup> As an important tumor suppressor gene, *p53* codon 72 arg/arg genotype was reported in an *in vitro* study that it might induce apoptosis with faster kinetics and suppress transformation more efficiently than the pro/pro variant.<sup>73</sup> We have considered genes from both metabolic and cell-cycle regulation pathways in our study. Although no obvious relationship was observed for each individual susceptibility gene, including Phase II genes such as *GSTM1*, *GSTT1*, *GSTP1*, and a cell-cycle regulation gene (*p53* codon72 genotype), on the risk of stomach cancer, higher multigenetic index was associated with higher risk of stomach cancer in our study. With elevated numbers of risk genotypes, the risk of stomach cancer increases. The combination of multiple genes from different pathways yielding significant effects on stomach cancer risk might suggest that the interaction of genes in different pathways may contribute to the development of stomach cancer.

With the development of high-throughput technology for detecting SNP of susceptibility genes, the field of molecular epidemiology is facing tremendous challenges in the methodology of data analysis. Some of these challenges include: (i) there are an extremely large number of SNP from each single pathway, e.g., more than 129 genes in the inflammation pathway, and approximately 5,000 SNP identified in these genes; (ii) multiple pathways: metabolic (Phases I and II), DNA repair (BER, NER, etc.),

inflammation, cell-cycle regulation, etc; and (iii) low-risk susceptibility genes, which usually require a very large sample size to detect the association. At this point, a simple straightforward method needs to be developed as a first step to evaluate the joint effects on the risk of the disease, although there are several methods available such as haplotype block and htSNP and hierarchical modeling. The development of multigenetic index may serve as a very first step. Future development of multigenetic index should employ a weighted approach using the available results from functional analysis, correlations between SNP with and without functional analysis and results of epidemiological meta-analysis. The application of multigenetic indices will help us to study comprehensively the co-effects of several SNP, to discover potential mechanisms of SNP in the process and give us better ability to identify the important group of SNP that contribute to the development of stomach cancer.

In summary, green tea drinking was suggested as a protective factor of stomach cancer in a Chinese population. Higher multigenetic index was associated with increased risk of stomach cancer. This is the first time that a strong, more than multiplicative interaction was found between green tea drinking and alcohol drinking, and potential interactions were observed between multigenetic index and green tea drinking on the risk of stomach cancer.

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