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Association between polymorphisms in the *CYP1A1, CYP2E1 and GSTM1* genes, and smoking, alcohol and upper digestive tract carcinomas in a high-incidence area of northern China

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Abstract. Metabolic gene variants, smoking, and alcohol consumption are important upper digestive tract cancer (UDTC) risk factors. However, the gene-gene and gene-environment interactions remain unclear. A case-control study in a high incidence area for upper digestive tract cancer was conducted in China. DNA was extracted from buffy coat samples for PCR or PCR-restriction fragment length polymorphism. Smoking and alcohol drinking status was determined by questionnaires. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to assess the associations. After adjusting for confounding factors, smoking increased esophageal cancer (EC), gastric cardia cancer (GCC) and gastric antral carcinoma (GAC) risk by 3.594, 4.658, and 3.999-fold, respectively. Alcohol consumption increased EC, GCC and GAC risk by 1.953, 2.442 and 1.765-fold, respectively. The cytochrome P4501A1 (*CYP1A1)* rs4646903 T>C polymorphism increased GCC risk, the cytochrome P4502E1 (*CYP2E1)* rs2031920 C>T polymorphism increased EC risk, while the *GSTM1* null genotype decreased EC risk. An association existed between the following: *CYP1A1* rs4646903 and smoking in EC, GCC and GAC; *CYP1A1* rs4646903 and alcohol consumption in EC and GCC; *CYP2E1* rs2031920 and smoking in EC, GCC and GAC and *CYP2E1* rs2031920 and alcohol consumption in EC and GCC. No association was observed between *CYP1A1* and *CYP2E1*. The glutathione S-transferase mu 1 (*GSTM1*) null genotype decreased EC risk (OR=0.510). Smoking/drinking are upper digestive tract cancer risk factors. The *CYP1A1* rs4646903 and *CYP2E1* rs2031920 polymorphisms were risk factors of GCC or EC, and the *GSTM1* null genotype may serve a protective role against EC. The results of the present study indicated that gene-environment interactions increase the risk of UDTC.

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Introduction

Upper digestive tract cancers (UDTC) mainly include esophageal cancer (EC) and gastric cancer (GC). GC can be defined according to the tumor location as proximal or distal gastric adenocarcinoma (1). EC is the eleventh most common cancer and the sixth deadliest cancer worldwide, and GC is ranked fifth for cancer incidence and third for cancer-associated mortalities worldwide (2). Gastric cardia cancer (GCC), or esophagogastric junction cancer, has also become a public health concern (3). To date, several major risk factors have been reported to be associated with UDTC, including heavy smoking and alcohol consumption (4,5). It is widely accepted that the development of UDTC is a result of complex interactions between environmental triggers and genetic factors (6-8). However, these interactions and the exact mechanism of carcinogenesis are still not fully understood.

Metabolites of tobacco and alcohol are first metabolically activated by Phase I enzymes, including cytochrome P4501A1 (*CYP1A1)* and cytochrome P4502E1 (*CYP2E1)*, into their final forms and then combine with DNA, forming aromatic-DNA adducts that are considered to be an early stage in carcinogenesis (9). These activated forms are subsequently detoxified by Phase II enzymes, particularly *GSTM1*, a member of the glutathione S-transferases (GSTs) family (10). The *CYP1A1* rs4646903 T>C polymorphism (MspI), also known as the m1 allele, is a substitution of T to C in the non-coding 3'-flanking region which appears to be associated with increased

enzymatic activity (11). The *CYP2E1* rs2031920 C>T polymorphism (RsaI) also known as the c2 allele, involves a C to T transition in the 5'-flanking region of the *CYP2E1* gene, which appears to be associated with decreased enzymatic activity (12). Individuals who presents the null *GSTM1* alleles lack the respective enzyme function (13).

A number of studies have been performed to assess the association between gene polymorphisms and cancer susceptibility (14-18). One meta-analysis showed no association between *CYP1A1* rs4646903 polymorphism and digestive tract cancers risk (14), while another meta-analysis confirmed association existed between *CYP1A1* rs4646903 and gastric cancer (15). Zhang *et al* (16) indicated that *CYP2E1* rs2031920 polymorphisms revealed no association with the risk of GC, however when GSTM1 was null, the association became significant. GSTM1/T1 null genotype was reported to increase GC risk, and combination of the *CYP1A1* rs4646422 variant allele and GSTM1/T1 null genotypes was also associated with a statistically significant increased risk (17). A recent meta-analysis suggested the association between GSTM1 and digestive cancers, and two potential gene-smoking interactions were also found (18). The results from these studies have not always been consistent. In addition, to the best of our knowledge, the evaluation of gene-gene and gene-environment interactions regarding upper digestive cancer risk is insufficient at present. To clarify the combined effects of *CYP1A1* rs4646903, *CYP2E1* rs2031920, *GSTM1* null polymorphisms and smoking or alcohol consumption on upper digestive tract cancer risk, a population-based case-control study was performed in Anyang, a typical high-incidence area of upper digestive cancer in Northern China (19,20).

Materials and methods

Patient and control selection. This case-control study included 194 patients with EC, 212 patients with GCC, 135 patients with gastric antral carcinoma (GAC), and 212 controls. The mean ages ± standard deviation of these four groups were 63±7.179, 64±9.070, 63±6.852 and 63±4.646 years. The sex ratio (male vs. female) of these four groups were 65.5 vs. 34.5%, 67.9 vs. 32.1%, 67.4 vs. 32.6% and 66.5 vs. 33.5%. All subjects were recruited from Anyang Cancer Hospital (Henan, China) between July 2015 and July 2017, with the study conducted during the same period. Inclusion criteria were as follows: Age between 30-79 years old with Han ethnicity; pathological diagnosis confirming ECC, GCC or GAC and no simultaneous malignancies. Patients who had undergone chemotherapy or radiotherapy prior to surgery were excluded from the present study. The cancer diagnoses were confirmed histologically. Subjects with no sign of a tumor based on gastroscopy were recruited from a cancer screening program for early detection of upper digestive tract cancers in the same area. All subjects underwent a personal interview and provided information on sociodemographic characteristics, recent and prior tobacco or alcohol use, and family history of cancer. Smoking status was stratified into three levels: Never smoked, smoking for <30 years and smoking for ≥30 years; alcohol consumption status was stratified into three levels: Never to occasional; ≥1 day/week and <150 g/week; ≥1 day/week and >150 g/week. The Anyang Tumor Hospital Institutional Review Board approved the present study. All patients and controls signed a study-specific written informed consent form.

PCR analysis of gene polymorphisms. DNA was extracted from the buffy coat of blood samples from the patients and controls using a FlexiGene DNA kit (cat. no. 51206; Qiagen China Co., Ltd.) for PCR or PCR-restriction fragment length polymorphism (RFLP) experiments. The polymorphisms of *CYP2E1* rs2031920 C>T and *GSTM1* (21) were detected by PCR using the Thermal Cycler K640 (Hangzhou Jingle Scientific Instrument Co., Ltd.). Nested PCR (22) was used to amplify the *CYP1A1* rs4646903 T>C. The PCR thermocycling conditions included initial denaturation at 95˚C for 15 min followed by 35 cycles of denaturation at 95˚C for 1 min, annealing for 1 min (annealing temperatures are presented in Table I), and extension at 75˚C for 1 min; and a final extension at 72˚C for 10 min. The amplified products were digested and examined using 1.5% agarose gel electrophoresis, and were visualized using a UV transilluminator (Beijing Liuyi Biotechnology Co., Ltd.). Table I presents the primer sequences, annealing temperatures, and digestion enzymes used. A total of 15% of the PCR products were selected for direct sequencing to confirm the RFLP results. The primers used for *CYP1A1* and *CYP2E1* sequencing were the same as the primers used in PCR. For GSTM1, the primers used for sequencing were cited from Khabaz *et al* (23). No deviation was found between the RFLP results and the sequencing data.

Statistical analysis. SPSS 19.0 software (IBM Corp.) was used for statistical analysis, and all tests were repeated three times. Pearson's χ^2 test or Fisher's exact test were used to examine differences between groups and unpaired t-tests to compare means. All tests were two-sided. Hardy-Weinberg equilibrium test was used to confirm the CYP1A1 and CYP2E1 genotype distributions. The Bonferroni correction was used to evaluate the associations found and a P-value of <0.05/m was considered statistically significant (m=the total comparison times). Cancer risk associated with genotype or environmental exposure factors was estimated by calculating odds ratios (OR) and 95% confidence intervals (CI) using unconditional logistic regression. After adjusting for potential confounding factors, multivariate logistic regression was used to assess the association between smoking, alcohol, and the metabolic gene polymorphisms.

Results

Patient and control characteristics. Table II presents the demographic profiles of the 541 patients and 212 controls. There were no significant differences between the cases and controls in sex, mean age, marital status, education level, labor type and economic income. Upper digestive tract cancer and family history of cancer were significantly associated for EC (P=0.017), GCC (P=0.002) and GAC (P=0.001).

Detection of CYP1A1, CYP2E1 and GSTM1 variants in upper digestive tract cancers. A total of 194 EC, 212 GCC and 135 GAC cases, and 212 controls were examined to detect *CYP1A1* rs4646903, *CYP2E1* rs2031920 and *GSTM1* polymorphisms. Fig. 1 shows examples of gene polymorphisms in PCR-amplified fragments or digestion fragments. Fig. 2 shows the sequencing

Table I. PCR primers and restriction conditions used in the present study.

Gene	Primer	Annealing temperature	Restriction enzyme	Fragment length
CYP1A1	Forward 5'-TCACTCGTCTAAATACTCACCCTG-3' (C1F)	60˚C	*Msp*I	298 bp (wild-type)
rs4646903 T>C	Reverse 5'-TAGGAGTCTTGTCTCATGCCT-3' (C1R)			298, 135 and 160 bp (heterozygous)
	Forward 5'-CAGTGAAGAGGTGTAGCCGCT-3' (C2F)			135 and 160 bp (homozygous)
	Reverse 5'-GAGGCAGGTGGATCACTTGAGCTC3' (C2R)			
CYP2E1	Forward 5'-AACGCCCCTTCTTGGTTCAG-3'	60˚C	*Rsa*I	265 and 150 bp (wild-type);
rs2031920 C>T	Reverse 5'-CATACAGACCCTCTTCCACCTT-3'			416, 265 and 150 bp (heterozygous)
				416 bp (homozygous)
GSTM1	Forward 5'-GAACTCCCTGAAAAGCTAAAGC-3'	60˚C	-	215 bp (present)
	Reverse 5'-GTTGGGCTCAAATATACGGTGG-3'			No fragment (null)

CYP1A1, cytochrome P4501A1; *CYP2E1*, cytochrome P4502E1; *GSTM1*, glutathione S-transferase mu 1.

Figure 1. PCR analysis of polymorphisms of *CYP1A1* rs4646903, *CYP2E1* rs2031920 and *GSTM1*. (A) *CYP1A1* rs4646903 polymorphism: Lane 3, 6 and 7: Wild genotype (298 bp); lane 5 and 8: Homozygous variant (160 and 135 bp), and lane 1, 2 and 4: Heterozygous variant (298 and 160 bp and 135 bp). (B) *CYP2E1* rs2031920 polymorphism: Lane 4, 6 and 8: Wild genotype (416 and 265 and 150 bp); lane 2 and 7: Homozygous variant (416 bp), and lane 1, 3 and 5: Heterozygous variant (265 and 150 bp). (C) *GSTM1* genotypes: Lane 3, 5 and 6: Null genotype (no band) and lane 1, 2, 4 and 7: Wild genotype (215 bp band). *CYP1A1*, Cytochrome P4501A1; *CYP2E1*, Cytochrome P4502E1; *GSTM1*, Glutathione S-transferase mu 1.

chromatogram of *CYP1A1* rs4646903 and *CYP2E1* rs2031920. Among the controls, both the *CYP1A1* and *CYP2E1* genotype distributions were in Hardy-Weinberg equilibrium.

Association between smoking, alcohol consumption, CYP1A1, CYP2E1, GSTM1 and upper digestive tract cancers. Smoking and alcohol consumption were confirmed to be main risk factors for upper digestive tract cancers (Table III). After adjusting for matching variables and potential confounders, smoking increased EC, GCC and GAC risk compared with non-smoking status: EC [OR (95% CI)=3.594 (2.077-6.221); P<0.001]; GCC [OR (95% CI)=4.658 (2.654-8.174); P<0.001] and GAC [OR (95% CI)=3.999 (2.131-7.505); P<0.001], as did alcohol consumption: EC [OR (95% CI)=1.953 (1.210-3.151); P=0.006]; GCC [OR (95% CI)=2.442 (1.523-3.914); P<0.001] and GAC [OR (95% CI)=1.765 (1.030-3.025); P=0.039]. Dose-dependent trends were observed with these two risk factors, with ORs increasing as the total smoking years or alcohol consumption amount increased (Table III). It was indicated that the *GSTM1* null genotype had protective effects against EC, decreasing EC risk [OR (95% CI)=0.510 (0.340-0.765); P=0.001].

CYP1A1 rs4646903 polymorphism was significantly associated with GCC risk [CC vs. TT: OR (95% CI)=1.936 (1.035-3.620), P=0.039; CC vs. CT+TT: OR (95% CI)=2.263 (1.272-4.026), P=0.005]; *CYP2E1* rs2031920 was significantly associated with EC risk [c1/c2 vs. c1/c1: OR (95% CI)=1.673

Table II. Demographic characteristics of patients in the current study.

		EC			GCC			GAC		
Characteristics	Controls n=212	n=194	χ^2	P-value	n=212	χ^2	P-value	n=135	χ^2	P-value
Sex										
Male	141	127	0.049	0.824	144	0.096	0.756	91	0.030	0.862
Female	71	67			68			44		
Mean age ± SD[a], years	63±4.646	63±7.179	-	0.874	64±9.070	-	0.396	63±6.852	-	0.456
Marital status[b]										
Yes	209	190	-	0.836	208	-	0.685	134	-	0.147
No	3	4			4			1		
Education[b]										
≤Primary school	136	130	-	0.320	134	-	0.974	73	-	0.127
Junior or senior	73	64			75			58		
≥College	3	0			3			4		
Occupation										
Labor	22	18	3.793	0.285	25	2.567	0.463	19	1.475	0.688
Farmers	175	170			178			105		
Civil jobs	7	2			6			6		
Other jobs	8	4			3			5		
Income[c], yuan										
≤1,999	130	122	5.705	0.058	125	0.627	0.731	76	0.939	0.625
2,000-3,999	71	70			78			52		
≥4,000	11	2			9			7		
Family history										
Yes	37	141	5.716	0.017	64	9.475	0.002	45	11.526	0.001
No	175	53			148			90		

[a]t-test were used to compare means of age. χ^2 test was conducted if the total sample size was >40, and the minimum theoretical frequency was >5, otherwise, [b]Fisher's exact probability test was performed. [c]RMB per capita/month. *CYP1A1*, Cytochrome P4501A1; *CYP2E1*, cytochrome P4502E1; *GSTM1*, glutathione S-transferase mu 1; EC, esophageal carcinoma; GAC, gastric antral carcinoma; GCC, gastric cardia carcinoma.

(1.111-2.520), P=0.014; c1/c2+c2/c2 vs. c1/c1: OR (95% CI)=1.595 (1.071-2.375), P=0.022] (Tables IV and V).

Gene-gene and gene-environment association between smoking, alcohol consumption, and CYP1A1 or CYP2E1. Gene-gene and gene-environment association between cigarette smoking, alcohol consumption, and *CYP1A1* rs4646903 or *CYP2E1* rs2031920 polymorphisms are presented in Table VI. An association existed between *CYP1A1* and smoking in EC, GCC and GAC; *CYP1A1* and alcohol drinking in EC and GCC; *CYP2E1* and smoking in EC, GCC and GAC; and *CYP2E1* and alcohol drinking in EC and GCC. No association was observed between *CYP1A1* and *CYP2E1*. Compared with non-smokers with wild-type *CYP1A1* (TT), smokers with a *CYP1A1* heterozygous variant genotype had a 2.597, 4.359 and 3.503-fold increased risk of EC, GCC and GAC, respectively. Smokers with a *CYP1A1* homozygous variant genotype had a 5.125, 8.618 and 6.070-fold increased risk of EC, GCC and GAC, respectively. Compared with non-drinkers with wild-type *CYP1A1* (TT), alcohol drinkers with a *CYP1A1* homozygous variant genotype had a 4.124, 6.820 and 4.489-fold increased risk of EC, GCC and GAC, respectively. Compared with non-smokers with wild-type *CYP2E1* (c1/c1), smokers with a *CYP2E1* heterozygous variant genotype had a 6.345, 5.318 and 3.300-fold increased risk of EC, GCC and GAC, respectively. In addition, smokers with a *CYP2E1* homozygous variant genotype had 6.661 and 7.621-fold increased risk for GCC and GAC. Compared with non-drinkers with wild-type *CYP2E1* (c1/c1), alcohol drinkers with a *CYP2E1* heterozygous variant genotype had a 3.820 and 3.070-fold increased risk of EC and GCC, respectively. These results indicated the association between smoking or alcohol consumption and *CYP1A1* rs4646903 or *CYP2E1* rs2031920 in UDTC. No associations were observed between *CYP1A1* rs4646903 and *CYP2E1* rs2031920.

Discussion

In the present study, it was confirmed that smoking and alcohol consumption were the main risk factors of upper digestive tract cancers. In addition, it was indicated that *CYP1A1* rs4646903 polymorphisms increased GCC risk, *CYP2E1* rs2031920 increased EC risk, while the *GSTM1* null genotype decreased EC risk. Regarding the gene-gene

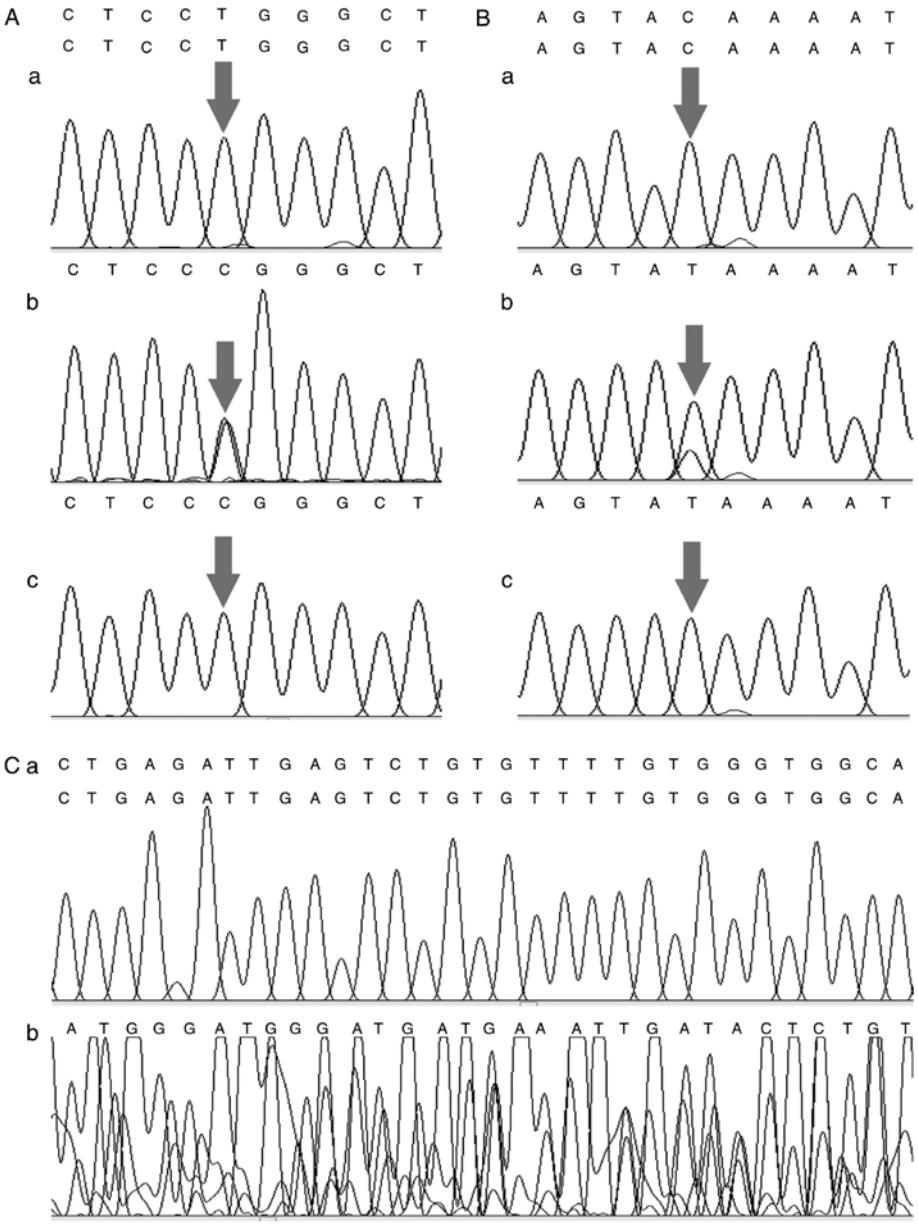

Figure 2. Sequencing chromatogram of *CYP1A1* rs4646903, *CYP2E1* rs2031920 and *GSTM1*. (A) Sequencing chromatogram of *CYP1A1* rs4646903. The arrow points at the *CYP1A1* rs4646903 SNP site. (Aa) Base at the SNP as a T (wild-type homozygous). (Ab) The base to be either a T or a C (heterozygous T/C). (Ac) Base to be a C (homozygous variant). (B) Sequencing chromatogram of *CYP2E1* rs2031920. The arrow points at the CYP2E1 rs2031920 SNP site. (Ba) Base at the SNP as a C (wild-type homozygous). (Bb) Base to be either a T or a C (heterozygous T/C). (Bc) Base to be a T (homozygous variant). (C) Sequencing chromatogram of *GSTM1*. (Ca) GSTM1 present genotype. (Cb) GSTM1 null genotype. SNP, single nucleotide polymorphism; *CYP1A1*, Cytochrome P4501A1; *CYP2E1*, Cytochrome P4502E1; *GSTM1*, Glutathione S-transferase mu 1.

or gene-environment associations in this study, associations between *CYP1A1* rs4646903, *CYP2E1* rs2031920 and smoking or alcohol were detected in UDTC.

To date, an increasing number of studies have investigated the associations between *CYP1A1* rs4646903 polymorphisms and digestive cancer risk (15,18,24,25). In a recent meta-analysis, seven articles reported on *CYP1A1* rs4646903 polymorphisms in four digestive cancers, and no associations were found in stratified analysis and subgroup analyses (18). In addition, in another meta-analysis, *CYP1A1* rs4646903 polymorphisms were confirmed to be associated with an increased susceptibility to colorectal cancer, however not to esophageal cancer or gastric cancer (24). In the present study, no association between the *CYP1A1* rs4646903 CC genotype and EC or GAC were detected, which was consistent with the aforementioned studies. However, in another meta-analysis, 11 studies about *CYP1A1* rs4646903 polymorphisms and GC were included, and significant results were found among a large sample-size subgroup (15). Furthermore, evidence was also found to support an association between *CYP1A1* rs4646903 polymorphisms and digestive tract cancer in the subgroups of Caucasian and mixed individuals (24). This suggested that the associations may vary across different sample sizes and ethnicities. This study found associations between *CYP1A1* rs4646903 polymorphisms and GCC. To the best of our knowledge, a limited number of studies have been performed in GCC. One report in Linzhou found an association between the *CYP1A1* rs4646903 variant allele, and a reduced risk of

Table III. Odds ratios and 95% Confidence Intervals of smoking, alcohol and *GSTM1* genotypes in upper digestive tract cancer.

		EC			GCC			GAC		
Factors	Controls n=212	n=194	OR[c] (95% CI)	P-value	n=212	OR[c] (95% CI)	P-value	n=135	OR[c] (95% CI)	P-value
Smoking										
Non-smokers	136	92	1.00 (reference)		89	1.00 (reference)		59	1.00 (reference)	
Smokers	76	102	3.594[b] (2.077-6.221)	<0.001	123	4.658[b] (2.654-8.174)	<0.001	76	3.999[b] (2.131-7.505)	<0.001
Smoking years										
<30	22	28	3.225[b] (1.570-6.626)	0.001	28	3.500[b] (1.672-7.327)	0.001	21	3.700[b] (1.657-8.264)	0.001
≥30	54	74	3.773[b] (2.096-6.790)	<0.001	95	5.185[b] (2.866-9.382)	<0.001	55	4.153[b] (2.115-8.156)	<0.001
Alcohol										
Never to occasional	135	103	1.00 (reference)		99	1.00 (reference)		70	1.00 (reference)	
Frequent drinkers	77	91	1.953[b] (1.210-3.151)	0.006	113	2.442[b] (1.523-3.914)	<0.001	65	1.765[a] (1.030-3.025)	0.039
Alcohol consumption										
≥1 day and <150 g/week	40	43	1.872[a] (1.044-3.355)	0.035	40	1.687[a] (0.933-3.051)	0.084	21	1.080 (0.535-2.182)	0.830
≥1 day and ≥150 g/week	37	48	2.024[a] (1.158-3.538)	0.013	73	3.139[b] (1.832-5.378)	<0.001	44	2.398[b] (1.310-4.389)	0.005
GSTM1										
Present	74	100	1.00 (reference)		84	1.00 (reference)		55	1.00 (reference)	
Null	138	94	0.510[b] (0.340-0.765)	0.001	128	0.862 (0.575-1.290)	0.470	80	0.823 (0.518-1.306)	0.408

χ^2 test was conducted to compare the differences between groups. [a]P<0.05; [b]P<0.01; [c]Adjusted for sex, age, marital status, education level, race, occupation, family per capita income/month, cigarette smoking, alcohol consumption and family history. *GSTM1*, glutathione S-transferase mu 1; EC, esophageal carcinoma; GAC, gastric antral carcinoma; GCC, gastric cardia carcinoma; OR, Odds ratio; CI, confidence interval.

Table IV. Adjusted odds ratios and 95% confidence intervals of the *CYP1A1* rs4646903 genotype in upper digestive tract cancer.

	Number (%)				Adjusted ORs[c] of different modes of inheritance (95% CIs)							
Factors	TT	CT	CC	P-value	③ vs. ①	P-value	② vs. ①	P-value	②+③ vs. ①	P-value	③ vs. ①+②	P-value
Controls (n=212)	74 (34.9)	116 (54.7)	22 (10.4)		1.00 (reference)		1.00 (reference)		1.00 (reference)		1.00 (reference)	
EC (n=194)	28 (14.4)	90 (46.4)	76 (39.2)	0.198	1.175 (0.607-2.274)	0.633	0.693 (0.449-1.069)	0.097	0.768 (0.507-1.162)	0.212	1.453 (0.790-2.674)	0.230
GCC (n=212)	76 (35.8)	96 (45.3)	40 (18.9)	0.028	1.936[a] (1.035-3.620)	0.039	0.760 (0.494-1.169)	0.212	0.940 (0.626-1.410)	0.764	2.263[b] (1.272-4.026)	0.005
GAC (n=135)	54 (40.0)	63 (46.7)	18 (13.3)	0.326	1.295 (0.617-2.721)	0.495	0.735 (0.451-1.199)	0.217	0.820 (0.515-1.307)	0.405	1.543 (0.774-3.076)	0.218

OR and P-values were calculated by multivariate unconditional logistic regression. [a]P<0.05; [b]P<0.01; [c]Adjusted for sex, age, marital status, education level, race, occupation, family per capita income/month, cigarette smoking, alcohol consumption, cancer in first degree relatives. TT, wild genotype; CC, homozygous variant genotype; CT, heterozygous variant genotype; ①, homogeneity wild genotype; ②, heterogeneity variant genotype; ③, homogeneity variant genotype; *CYP1A1*, cytochrome P4501A1; EC, esophageal carcinoma; GAC, gastric antral carcinoma; GCC, gastric cardia carcinoma; OR, odds ratios; CIs, confidence intervals.

Table V. Adjusted odds ratios and 95% confidence intervals of *CYP2E1* rs2031920 genotypes in upper digestive tract cancer.

	Number (%)				Adjusted ORs[b] of different modes of inheritance (95% CIs)							
Factors	c1/c1	c1/c2	c2/c2	P-value	③ vs. ①	P-value	② vs. ①	P-value	②+③ vs. ①	P-value	③ vs. ①+②	P-value
Controls (n=212)	118 (55.7)	84 (39.6)	10 (4.7)		1.00 (reference)		1.00 (reference)		1.00 (reference)		1.00 (reference)	
EC (n=194)	86 (44.3)	100 (51.5)	8 (4.1)	0.054	0.993 (0.367-2.686)	0.990	1.673[a] (1.111-2.520)	0.014	1.595[a] (1.071-2.375)	0.022	0.789 (0.297-2.094)	0.634
GCC (n=212)	115 (54.2)	87 (41.0)	10 (4.7)	0.955	0.974 (0.383-2.475)	0.956	1.051 (0.702-1.575)	0.808	1.043 (0.705-1.541)	0.834	0.955 (0.381-2.392)	0.921
GAC (n=135)	82 (60.7)	48 (35.6)	5 (3.7)	0.630	0.752 (0.238-2.380)	0.628	0.827 (0.517-1.321)	0.426	0.819 (0.520-1.288)	0.387	0.806 (0.258-2.519)	0.710

OR and P-values were calculated by multivariate unconditional logistic regression. [a]P<0.05. [b]Adjusted for sex, age, marital status, education level, race, occupation, family per capita income/month, cigarette smoking, alcohol consumption, cancer in first degree relatives. c1/c1, wild genotype; c2/c2, homozygous variant genotype; c1/c2, heterozygous variant genotype; ①, homogeneity wild genotype; ②, heterogeneity variant genotype; ③, homogeneity variant genotype; *CYP2E1*, cytochrome P4502E1; EC, esophageal carcinoma; GAC, gastric antral carcinoma; GCC, gastric cardia carcinoma; OR, odds ratios; CIs, confidence intervals.

Table VI. Association of smoking, alcohol, and *CYP1A1* rs4646903, *CYP2E1* rs2031920 variants in upper digest tract cancers.

			EC			GCC			GAC		
Factors[e]	Variant	Controls n=212	n=194	OR[c] (95% CI)	P-value	n=212	OR[c] (95% CI)	P-value	n=135	OR[c] (95% CI)	P-value
Smoking	rs4646903				0.011[d]			0.001[d]			0.049[d]
No	TT	41	32	1.00 (reference)		29	1.00 (reference)		19	1.00 (reference)	
	CT	79	44	0.700 (0.378-1.296)	0.257	37	0.704 (0.368-1.346)	0.288	29	0.907 (0.440-1.871)	0.791
	CC	16	16	1.175 (0.487-2.834)	0.719	23	2.494[a] (1.072-5.800)	0.033	11	1.722 (0.639-4.637)	0.282
Yes	TT	33	44	3.188[b] (1.482-6.857)	0.003	47	4.193[b] (1.863-9.438)	0.001	35	4.439[b] (1.846-10.674)	0.001
	CT	37	46	2.597[a] (1.225-5.505)	0.013	59	4.359[b] (1.979-9.601)	<0.001	34	3.503[a] (1.447-8.478)	0.005
	CC	6	12	5.125[b] (1.551-16.943)	0.007	17	8.618[b] (2.710-27.403)	<0.001	7	6.070[b] (1.580-23.325)	0.009
Smoking	rs2031920				0.002[d]			0.001[d]			0.017[d]
No	c1/c1	73	44	1.00 (reference)		49	1.00 (reference)		40	1.00 (reference)	
	c1/c2	55	43	1.336 (0.756-2.361)	0.319	36	1.046 (0.584-1.874)	0.880	19	0.646 (0.291-1.101)	0.204
	c2/c2	8	5	0.998 (0.289-3.439)	0.997	4	0.809 (0.224-2.922)	0.746	NA	NA	NA
Yes	c1/c1	45	42	2.834[b] (1.430-5.613)	0.003	66	4.236[b] (2.147-8.359)	<0.001	42	2.818[b] (1.345-5.904)	0.006
	c1/c2	29	57	6.345[b] (3.113-12.930)	<0.001	51	5.318[b] (2.546-11.106)	<0.001	29	3.300[b] (1.465-7.434)	0.004
	c2/c2	2	3	3.185 (0.467-21.740)	0.237	6	6.661[a] (1.202-36.901)	0.030	5	7.621[b] (1.277-45.480)	0.026
Alcohol	rs4646903				0.037[d]			0.002[d]			0.136[d]
No	TT	41	38	1.00 (reference)		34	1.00 (reference)		29	1.00 (reference)	
	CT	76	48	0.633 (0.353-1.135)	0.137	41	0.625 (0.340-1.149)	0.139	32	0.639 (0.332-1.230)	0.188
	CC	18	17	0.920 (0.406-2.088)	0.842	24	1.641 (0.749-3.593)	0.217	9	0.762 (0.292-1.991)	0.578
Yes	TT	33	38	1.579 (0. 786-3.172)	0.204	42	1.822 (0.892-3.722)	0.102	25	1.220 (0.550-2.705)	0.631
	CT	40	42	1.280 (0.649-2.522)	0.486	55	1.877 (0.948-3.714)	0.072	31	1.116 (0.518-2.402)	0.785
	CC	4	11	4.124[a] (1.122-15.155)	0.033	16	6.820[b] (1.974-23.561)	0.002	9	4.489[a] (1.185-17.002)	0.028
Alcohol	rs2031920				0.020[d]			0.016[d]			0.178[d]
No	c1/c1	71	46	1.00 (reference)		51	1.00 (reference)		45	1.00 (reference)	
	c1/c2	58	54	1.545 (0.901-2.651)	0.114	43	1.109 (0.638-1.928)	0.713	22	0.624 (0.330-1.181)	0.147
	c2/c2	6	3	0.782 (0.180-3.398)	0.743	5	1.271 (0.361-4.479)	0.709	3	0.972 (0.222-4.263)	0.970
Yes	c1/c1	47	40	1.789 (0.944-3.390)	0.075	64	2.467[b] (1.343-4.532)	0.004	37	1.380 (0.702-2.714)	0.351
	c1/c2	26	46	3.820[b] (1.913-7.629)	<0.001	44	3.070[b] (1.537-6.134)	0.001	26	1.801 (0.834-3.890)	0.134
	c2/c2	4	5	1.796 (0.444-7.272)	0.412	5	1.679 (0.415-6.797)	0.468	2	0.710 (0.118-4.273)	0.708
rs2031920	rs4646903				0.060[d]			0.976[d]			0.998[d]
c1/c1	TT	46	37	1.00 (reference)		49	1.00 (reference)		29	1.00 (reference)	
c1/c1	CT	25	42	2.256 (1.150-4.424)	0.018	24	0.857 (0.422-1.740)	0.669	23	1.604 (0.747-3.442)	0.225
c1/c1	CC	3	2	0.664 (0.100-4.389)	0.670	3	0.715 (0.128-4.004)	0.703	2	1.195 (0.181-7.905)	0.853
c1/c2	TT	66	39	0.678(0.372-1.237)	0.205	48	0.620 (0.353-1.090)	0.097	40	1.007 (0.530-1.911)	0.984

Table VI. Continued.

			EC			GCC			GAC		
Factors[e]	Variant	Controls n=212	n=194	OR[c] (95% CI)	P-value	n=212	OR[c] (95% CI)	P-value	n=135	OR[c] (95% CI)	P-value
c1/c2	CT	46	41	1.055 (0.567-1.961)	0.866	44	0.833 (0.459-1.510)	0.547	20	0.687 (0.329-1.432)	0.316
c1/c2	CC	4	5	1.328 (0.321-5.497)	0.696	4	0.861 (0.197-3.766)	0.842	3	1.228 (0.239-6.318)	0.806
c2/c2	TT	6	10	2.193 (0.713-6.746)	0.171	18	3.222 (1.149-9.039)	0.026	13	4.359 (1.433-13.260)	0.010
c2/c2	CT	13	17	1.477 (0.620-3.517)	0.378	19	1.398 (0.609-3.209)	0.430	5	0.682 (0.210-2.212)	0.524
c2/c2	CC	3	1	0.409 (0.039-4.330)	0.458	3	0.991 (0.183-5.364)	0.991	NA	NA	NA

OR and P-values were calculated by multivariate unconditional logistic regression. [a]P<0.05; [b]P<0.001; [c]Adjusted for sex, age, marital status, education level, race, occupation, cigarette smoking, alcohol consumption and cancer in first degree relatives; [d]P-value of gene-environmental interaction. [e]Association between *GSTM1* and other risk factors were not conducted, because *GSTM1* tended to be a protective factor. TT, wild genotype; CC, homozygous variant genotype; CT, heterozygous variant genotype; c1/c1, wild genotype; c2/c2, homozygous variant genotype; c1/c2, heterozygous variant genotype; *CYP1A1*, cytochrome P4501A1; *CYP2E1*, cytochrome P4502E1; *GSTM1*, glutathione S-transferase mu 1; EC, esophageal carcinoma; GAC, gastric antral carcinoma; GCC, gastric cardia carcinoma; OR, odds ratios; CIs, confidence intervals; NA, not available.

GCC in people with Dysplasia, who were at high risk for the development of GCC (25). However, the study only included 90 cases of GCC, decreasing the reliability of the results.

One meta-analysis in China suggested that the *CYP2E1* rs2031920 polymorphism was a risk factor for EC, and the c2 allele was demonstrated to be a factor that decreases the risk of EC in the mainland Chinese population (26). However, in this research, *CYP2E1* rs2031920 genotypes tended to increase EC risk. One report in Guangzhou Chinese population and another report in a Northern Jiangsu Chinese population also showed that the *CYP2E1* rs2031920 polymorphisms could be risk factors for the development of gastric cancer (27,28). Molecular biological evidence has shown that the *CYP2E1* rs2031920 variant in the *CYP2E1* promoter enhances gene transcriptional activity by altering its binding to its transcription factor, particularly, hepatocyte nuclear factor-1 (29), and influencing its susceptibility to N-nitrosamine-linked carcinogenesis (30), indicating that the *CYP2E1* rs2031920 variant may be associated with an increased cancer risk. The present study's results supported the aforementioned findings.

It was indicated that the *GSTM1* null genotype had protective effects against EC, decreasing EC risk. However, increased upper digestive tract cancer risk was associated with *GSTM1* non-null genotypes. To the best of our knowledge, this is not consistent with most other studies (17,18). A most recent meta-analysis on four digestive cancers showed that the *GSTM1* polymorphism was associated with the risk of the four digestive cancers among the Asian population, as subgroup analyses by cancer site showed that the *GSTM1* null genotype increased the total gastric cancer risk in the population (18). Another meta-analysis in a Japanese population showed that *GSTM1* null, *GSTT1* null and *GSTM1*/T1 both or either null genotypes were associated with increased risk, though this was not statistically significantly (15). However, there are a number of reports showing that cancer risk is associated with *GSTM1* non-null genotypes (30-33). There are several possible reasons for this observation. One is that the loss of one GST enzyme may be negligible compared with the large extended GST family (23). Even if the *GSTM1* detoxification function is lost, other GST family members can still act to decrease cancer risk. Furthermore, some carcinogens, including N-hydroxy-Trp-P-2, have enhanced genotoxicity and carcinogenicity after binding to glutathione (34). Furthermore, it appears that *GSTM1* null individuals have higher DNA adduct levels than *GSTM1*-expressing individuals (35).

Regarding the gene-gene or gene-environment associations in this study, an association between *CYP1A1* rs4646903, *CYP2E1* rs2031920 and smoking or alcohol was detected. Two meta-analyses showed that *CYP2E1* rs2031920 may modify the susceptibility to gastric cancer among individuals who have a smoking history, or when *GSTM1* or *GSTT1* are null, or *CYP2E1* rs2031920 is homozygous wild-type (16,36). An increased risk was seen in *CYP1A1* rs4646422 variant subjects whose smoking was categorized as ≤30 pack-years, or whose *GSTM1/T1* were both null genotypes, or who were null for either *GSTM1/T1* individually (17). These studies suggested that tumor incidence is often due to a combination of exposure to external environmental factors and internal gene aberrance. These interactions have a greater impact on cancer susceptibility compared with single genes.

Associations between metabolic gene polymorphisms and human cancers have been debated. The differences stem from several factors, including ethnic or geographic differences, as Asian populations have been reported to be more prone compared with Caucasian populations to show significant associations between metabolic gene polymorphisms and carcinogenesis (18,37,38). Even in populations containing the same ethnic group, the associations vary by region (14). It is believed that these inconsistent results across ethnicity and geographic areas derive mainly from the unequal frequency of genetic polymorphisms (30,39). Another factor is the different host habits and environmental factor exposure levels, including tobacco use and alcohol consumption (4,5), family history of cancer and *Helicobacter pylori* infection (40), which have been identified as risk factors for upper digestive tract cancers. Other environmental factors include low socioeconomic status (41), poor oral hygiene (42), nutritional deficiencies, diet (43) and high salt intake (44). It has been hypothesized that various living environments lead to different degrees of cancer susceptibility (45). Specific associations are easily found in subgroups with exposure to negative factors, including smoking, *H. pylori* infection, or low consumption of fruit. A lack of statistical power has also been identified as a contributing factor, as the number of subjects who carry the 'unfavorable' gene polymorphism combinations becomes visible and can be assessed only if sufficient subjects are available with the specific genetic profile required (46). Furthermore, the 'Berkson bias' is typically present in hospital-based studies, as the controls may only represent a sample of an ill-defined reference population and may not be representative of the general population (47). In addition, in terms of gene-gene and gene-environment interaction, tumor incidence is often a combination of multiple factors (48). A negative association between a gene and cancer susceptibility does not mean that the gene has no impact on cancer risk. In terms of methodological differences, the most popular method in previous studies has been PCR-RFLP (21,30). Although PCR-RFLP is a simple, specific and efficient method of SNP detection, it has obvious limitations with respect to accuracy, particularly for subjects who carry a heterozygous mutation (49). With the development of molecular detection technology, a number of researchers have begun to use TaqMan assays (25,50), which may be faster and more accurate compared with PCR-RFLP. A superior new method is genome sequencing (23,51), particularly genome-wide associated studies, which can assay huge amounts of SNPs in a large number of samples and facilitates rapid detection.

In conclusion, it was indicated that smoking/alcohol consumption are upper digestive tract cancer risk factors. The *CYP1A1* rs4646903 and *CYP2E1* rs2031920 genotypes may contribute to higher GCC and EC susceptibility, respectively. The *GSTM1* null genotype may serve a protective effect against EC. The gene-environment associations present increase the cancer risk. In the future, the present study may be improved by increasing the sample size and applying more advanced SNP detection methods, including a TaqMan assay or genome sequencing.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

FYZ, FZ and SML designed the experiment. FZ, JFS, SML, YJH, LJD, ZWG, JL, XJD, FFS, YWZ and NCW collected the data and performed the experiments. JFS analyzed and interpreted the data. FZ and JFS were major contributors in writing the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The Anyang Tumor Hospital Institutional Review Board approved the present study (no. AZLL022015005150701). All patients and controls signed a study-specific written informed consent form.

Patient consent for publication

All patients and controls have provided consent for publication.

Competing interests

The authors declare that they have no competing interests.

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