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.

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 present 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 an 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 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 of upper digestive tract cancers risk, a population-based case-control study was performed in Anyang, a typical high-incidence area of upper digestive cancer. 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 Khubaz 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 χ² 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...
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 chromogram of CYP1A1 rs4646903 and CYP2E1 rs2031920.

Among the controls, both the CYP1A1 and CYP2E1 genotype distributions were in Hardy-Weinberg equilibrium.
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. 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.

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

| Characteristics | Controls n=212 | n=194 | EC | GCC | GAC | Controls n=212 | n=135 | GCC | GAC |
|----------------|---------------|-------|----|-----|-----|---------------|-------|-----|-----|
| Sex            |               |       |    |     |     |               |       |     |     |
| Male           | 141           | 127   | 0.049 | 0.824 | 144 | 0.096 | 0.756 | 91 | 0.030 | 0.862 |
| Female         | 71            | 67    | 0.874 | 0.203 | 68 | 0.396 | 0.456 | 44 |             |       |
| Mean age ± SD, years | 63±4.646 | 63±7.179 | - | 0.874 | | 64±9.070 | - | 0.396 | 63±6.852 | - | 0.456 |
| Marital status |               |       |    |     |     |               |       |     |     |
| Yes            | 209           | 190   | 0.836 | 0.489 | 208 | 0.685 | 134 | - | 0.147 |
| No             | 3             | 4     |     |     |     | 4             | 1     |     |     |
| Education      |               |       |    |     |     |               |       |     |     |
| ≤Primary school | 136          | 130   | 0.320 | 0.721 | 134 | 0.974 | 73 | - | 0.127 |
| Junior or senior | 73           | 64    |     |     |     | 75            | 58    |     |     |
| ≥College       | 3             | 0     |     |     |     | 3             | 4     |     |     |
| Occupation     |               |       |    |     |     |               |       |     |     |
| Labor          | 22            | 18    | 3.793 | 0.050 | 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, 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    |     |     |

*χ2 test were used to compare means of age; χ2 test was conducted if the total sample size was >40, and the minimum theoretical frequency was >5, otherwise; Fisher's exact probability test was performed. 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).

### 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
or gene-environment associations in this study, associations between \textit{CYP1A1} rs4646903, \textit{CYP2E1} rs2031920 and \textit{GSTM1} were detected, which was consistent with the aforementioned studies. However, in another meta-analysis, 11 studies about \textit{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 \textit{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 \textit{CYP1A1} rs4646903 variant allele, and a reduced risk of EC or GAC were detected.

To date, an increasing number of studies have investigated the associations between \textit{CYP1A1} rs4646903, \textit{CYP2E1} rs2031920 and digestive cancer risk (15,18,24,25). In a recent meta-analysis, seven articles reported on \textit{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, \textit{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 \textit{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 \textit{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 \textit{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 \textit{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 \textit{CYP1A1} rs4646903 variant allele, and a reduced risk of

Figure 2. Sequencing chromatogram of \textit{CYP1A1} rs4646903, \textit{CYP2E1} rs2031920 and \textit{GSTM1}. (A) Sequencing chromatogram of \textit{CYP1A1} rs4646903. The arrow points at the \textit{CYP1A1} rs4646903 SNP site. (Aa) Base at the SNP as a T (wild-type homozygous). (Ab) Base to be either a T or a C (heterozygous T/C). (Ac) Base to be a C (homozygous variant). (B) Sequencing chromatogram of \textit{CYP2E1} rs2031920. The arrow points at the \textit{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 \textit{GSTM1}. (Ca) \textit{GSTM1} present genotype. (Cb) \textit{GSTM1} null genotype. SNP, single nucleotide polymorphism; \textit{CYP1A1}, Cytochrome P4501A1; \textit{CYP2E1}, Cytochrome P4502E1; \textit{GSTM1}, Glutathione S-transferase mu 1.
Table III. Odds ratios and 95% Confidence Intervals of smoking, alcohol and GSTM1 genotypes in upper digestive tract cancer.

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

χ² test was conducted to compare the differences between groups. *P<0.05; **P<0.01; *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.

| Factors | Number (%) | Adjusted ORs of different modes of inheritance (95% CIs) |
|---------|------------|--------------------------------------------------------|
|         | TT (74 (34.9) CT (116 (54.7) CC (22 (10.4)) | P-value | 0.00 | 0.00 | 0.00 | 0.00 |
| Controls (n=212) | | | | | | | |
| EC (n=194) | | | | | | | |
| GCC (n=212) | | | | | | | |
| GAC (n=135) | | | | | | | |
| OR and P-values were calculated by multivariate unconditional logistic regression. *P<0.05; **P<0.01; †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; †, homogeneity wild genotype; ‡, heterogeneity variant genotype; +, 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.

| Factors | Number (%) | Adjusted ORs of different modes of inheritance (95% CIs) |
|---------|------------|--------------------------------------------------------|
|         | c1/c1 (118 (55.7) c1/c2 (84 (39.6) c2/c2 (10 (4.7)) | P-value | 0.00 | 0.00 | 0.00 | 0.00 |
| Controls (n=212) | | | | | | | |
| EC (n=194) | | | | | | | |
| GCC (n=212) | | | | | | | |
| GAC (n=135) | | | | | | | |
| OR and P-values were calculated by multivariate unconditional logistic regression. *P<0.05. †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; †, homogeneity wild genotype; ‡, heterogeneity variant genotype; ††, 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.

| Factors | Variant | Controls n=212 | OR (95% CI) | P-value | n=212 | OR (95% CI) | P-value | n=135 | OR (95% CI) | P-value |
|---------|---------|----------------|-------------|---------|--------|-------------|---------|--------|-------------|---------|
| Smoking | rs4646903 | No TT 41 32 | 1.00 (reference) | 0.011d | 29 1.00 (reference) | 0.001d | 19 1.00 (reference) | 0.049d |
|         |         | TT 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 (1.072-5.800) | 0.033 | 11 1.722 (0.639-4.637) | 0.282 |
|         | Yes TT 33 44 | 3.188 (1.482-6.857) | 0.003 | 47 4.193 (1.863-9.438) | 0.001 | 35 4.439 (1.846-10.674) | 0.001 |
|         |         | CT 37 46 | 2.597 (1.225-5.505) | 0.013 | 59 4.359 (1.979-9.601) | <0.001 | 34 3.503 (1.447-8.478) | 0.005 |
|         |         | CC 6 12 | 5.125 (1.551-16.943) | 0.007 | 17 8.618 (2.710-27.403) | <0.001 | 7 6.070 (1.580-23.325) | 0.009 |
| Smoking | rs2031920 | No c1/c1 73 44 | 1.00 (reference) | 0.002d | 49 1.00 (reference) | 0.001d | 40 1.00 (reference) | 0.017d |
|         |         | 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 (1.430-5.613) | 0.003 | 66 4.236 (2.147-8.359) | <0.001 | 42 2.818 (1.345-5.904) | 0.006 |
|         |         | c1/c2 29 57 | 6.345 (3.113-12.930) | <0.001 | 51 5.318 (2.546-11.106) | <0.001 | 29 3.300 (1.465-7.434) | 0.004 |
|         |         | c2/c2 6 3 | 3.185 (0.467-21.740) | 0.023 | 6 6.661 (1.202-36.901) | 0.030 | 5 7.621 (1.277-45.480) | 0.026 |
| Alcohol | rs4646903 | No TT 41 38 | 1.00 (reference) | 0.037d | 34 1.00 (reference) | 0.002d | 29 1.00 (reference) | 0.136d |
|         |         | 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 (1.122-15.155) | 0.033 | 16 6.820 (1.974-23.561) | 0.002 | 9 4.489 (1.185-17.002) | 0.178d |
| Alcohol | rs2031920 | No c1/c1 71 46 | 1.00 (reference) | 0.020d | 51 1.00 (reference) | 0.016d | 45 1.00 (reference) | 0.178d |
|         |         | 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 (0.406-1.539) | 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 (1.343-4.532) | 0.004 | 37 1.380 (0.702-2.714) | 0.351 |
|         |         | c1/c2 26 46 | 3.820 (1.913-7.629) | <0.001 | 44 3.070 (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 | No c1/c1 TT 46 37 | 1.00 (reference) | 0.060d | 49 1.00 (reference) | 0.976d | 29 1.00 (reference) | 0.998d |
|         |         | 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.

| Factors | Variant | Controls (n=194) | EC (n=212) | GCC (n=212) | P-value | P-value | P-value |
|---------|---------|------------------|------------|------------|---------|---------|---------|
|         |         | n=135            | n=212      | n=194      |         |         |         |
| c1/c2   | CT      | 46               | 44         | 41         | 0.833   | 0.866   | 0.842   |
|         | CC      | 4                | 10         | 5          | 0.912   | 0.696   | 0.711   |
| c2/c2   | CT      | 13               | 19         | 17         | 1.389   | 1.095   | 1.171   |
|         | CC      | 3                | 5          | 2          | 1.477   | 1.112   | 1.171   |

OR and P-values were calculated by multivariate unconditional logistic regression. *P<0.05; **P<0.001; *P<0.01.*

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 GSTT1/T1 both or either null genotypes were associated with increased risk, though this was not statistically significant (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 rs464642 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, LJJD, 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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