Human Genome Epidemiology (HuGE) Review

Glutathione S-Transferase M1 (GSTM1) and Glutathione S-Transferase T1 (GSTT1) Null Polymorphisms, Smoking, and Their Interaction in Oral Cancer: A HuGE Review and Meta-Analysis

Zhi-Jiang Zhang*, Ke Hao, Rong Shi, Genming Zhao, Guo-Xin Jiang, Yiqing Song, Xiaohui Xu, and Jin Ma

* Correspondence to Dr. Zhi-Jiang Zhang, Department of Epidemiology, School of Public Health, Shanghai Jiao Tong University, 227 Chongqing South Road, Shanghai, China (e-mail: zhang.zj@msn.com).

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The association between glutathione S-transferase M1 (GSTM1) and glutathione S-transferase T1 (GSTT1) null polymorphisms and oral cancer is not consistent across studies, and data on their interaction with smoking in oral cancer are lacking. The authors systematically searched PubMed and SciVerse Scopus for case-control studies examining the association between null genotypes of the GSTM1 and GSTT1 genes and oral cancer. Twenty-eight case-control studies published in English were identified. Summary odds ratios were derived via random-effects models. The summary odds ratio for the GSTM1 null genotype was 1.43 in Asians (95% confidence interval (CI): 1.14, 1.78; \( P < 0.01, I^2 = 73\% \)) and 0.98 in Caucasians (95% CI: 0.76, 1.28; \( P = 0.91, I^2 = 0\% \)). Case-only analysis of 6 studies (552 cases) showed an inverse multiplicative interaction between GSTM1 null polymorphisms and smoking (ever/high levels of smoking vs. never/low levels) (odds ratio (OR) = 0.51, 95% CI: 0.32, 0.82; \( P = 0.01, I^2 = 34\% \)). The GSTT1 null genotype was not significantly associated with oral cancer in Asians (OR = 1.07, 95% CI: 0.82, 1.38; \( P = 0.63, I^2 = 65\% \)) or Caucasians (OR = 1.04, 95% CI: 0.41, 2.65; \( P = 0.93, I^2 = 55\% \)). In conclusion, the GSTM1 null genotype may be associated with a higher risk of oral cancer in Asians but not in Caucasians, and this effect may be modified by smoking status. The GSTT1 null genotype may not be associated with oral cancer.

Oral cancer is one of the 10 most common cancers in the world (1). Most malignant oral cancers are squamous cell carcinoma, followed by adenocarcinoma and, rarely, other types. The World Health Organization predicts that a trend of increase in the incidence of oral cancer will continue worldwide for the next several decades. Despite advances in treatment for oral cancer, the 5-year survival rate remains poor, with survival being dependent on the stages at diagnosis because the majority of patients are diagnosed at stage III or IV (2). Hence, a better understanding of the risk factors for oral cancer may improve the secondary prevention strategies.

Previous studies have demonstrated that tobacco use is a strong risk factor for oral cancer (3–10). However, only a small proportion of persons who are exposed to tobacco products ultimately develop oral cancer. The differential susceptibility among tobacco users may result from polymorphisms in genes encoding the biotransformation enzymes, which are involved in the metabolism of tobacco and transform a procarcinogen into either a carcinogen or relatively harmless compounds. The glutathione S-transferases are a family of phase II xenobiotic metabolizing enzymes.

Abbreviations: CI, confidence interval; GSTM1, glutathione S-transferase M1; GSTT1, glutathione S-transferase T1; OR, odds ratio.

Editor’s note: This article is also available on the Web site of the Human Genome Epidemiology Network (http://www.cdc.gov/genomics/hugenet/default.htm).
Table 1. Odds Ratios for Oral Cancer Associated With the Glutathione S-Transferase M1 (GSTM1) or Glutathione S-Transferase T1 (GSTT1) Null Genotype in 28 Case-Control Studies

<table>
<thead>
<tr>
<th>First Author, Year (Reference No.)</th>
<th>Source of Controls</th>
<th>Ethnicity</th>
<th>Region</th>
<th>Tumor Site(s)</th>
<th>Genotype</th>
<th>No. of Cases</th>
<th>No. of Controls</th>
<th>% With Null Genotype</th>
<th>Hardy-Weinberg Equilibrium</th>
<th>Adjusted Odds Ratio</th>
<th>95% Confidence Interval</th>
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<tr>
<td>Deakin, 1996 (19)</td>
<td>Hospital patients</td>
<td>Caucasian</td>
<td>United Kingdom</td>
<td>Mouth</td>
<td>GSTM1</td>
<td>40</td>
<td>577</td>
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<td>54.8</td>
<td>NA</td>
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<td></td>
<td></td>
<td>GSTT1</td>
<td>34</td>
<td>509</td>
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<td>18.5</td>
<td>0.59</td>
<td>0.15, 1.73</td>
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<td>Park, 1997 (30)</td>
<td>Healthy persons and hospital patients</td>
<td>Caucasian</td>
<td>United States</td>
<td>Oral cavity</td>
<td>GSTM1</td>
<td>109</td>
<td>109</td>
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<td>53.2</td>
<td>NA</td>
<td>0.90</td>
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<td>Hung, 1997 (4)</td>
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<td>Asian</td>
<td>China</td>
<td>Oral cavity</td>
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<td>41</td>
<td>123</td>
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<td>57.7</td>
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<td>GSTT1</td>
<td>41</td>
<td>123</td>
<td>58.5</td>
<td>52.8</td>
<td>1.0</td>
<td>0.4, 2.5</td>
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<td>Caucasian</td>
<td>France</td>
<td>Oral cavity</td>
<td>GSTM1</td>
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<td>172</td>
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<td>Japan</td>
<td>Lip, oral cavity</td>
<td>GSTM1</td>
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<td>Hahn, 2002 (28)</td>
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Control for Confounding:
- Adjustment for age, ethnicity, cigarettes, alcohol, and betel quid
- Adjustment for age, gender, tobacco, and alcohol
- Matching on age and gender
- Matching on age and gender
- Matching on age, gender, and smoking
- Matching on region of origin; adjustment for age
- Matching on age, gender, smoking, and betel-chewing
- Matching on age, gender, alcohol, and raw vegetable and fruit intake
- Matching on age and gender
- Enrollment of smokers
<table>
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<th>Study</th>
<th>Type of participants</th>
<th>Region</th>
<th>Tissue</th>
<th>GSTM1</th>
<th>GSTT1</th>
<th>No.</th>
<th>Yes</th>
<th>Regression</th>
<th>Adjustment for age, gender, and tobacco dose/Exclusive enrollment of males who chew areca and smoked</th>
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</thead>
<tbody>
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<td>Majumder, 2005 (25)</td>
<td>Hospital patients</td>
<td>Asian India</td>
<td>Oral cavity</td>
<td>310</td>
<td>348</td>
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<td>33.6</td>
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<td>1.0 0.7, 1.4</td>
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<tr>
<td>Liu, 2005 (24)</td>
<td>Healthy persons and hospital patients</td>
<td>Asian China</td>
<td>Oral cavity</td>
<td>114</td>
<td>100</td>
<td>57.9</td>
<td>55.0</td>
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<td>0.81 0.53, 1.63</td>
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<td>Sugimura, 2006 (10)</td>
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<td>122</td>
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<td>Oral cavity</td>
<td>38</td>
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<td>38.2</td>
<td>NA</td>
<td>2.8 1.28, 5.98</td>
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<td>Sharma, 2006 (7)</td>
<td>Healthy persons</td>
<td>Asian India</td>
<td>Lip, mouth</td>
<td>40</td>
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<td>42.5</td>
<td>33.3</td>
<td>NA</td>
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<td>Anantharaman, 2007 (16)</td>
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<td>451</td>
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<td>72</td>
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<td>58.9</td>
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<td>Caucasian Spain</td>
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<td>Chen, 2010 (18)</td>
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<td>201</td>
<td>47.9</td>
<td>26.4</td>
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</tbody>
</table>

Abbreviations: GSTM1, glutathione S-transferase M1; GSTT1, glutathione S-transferase T1; NA, not applicable.
involved in catalyzing the conjugation reactions of reactive intermediates of electrophilic compounds with cytosolic glutathione. Based on sequence similarities, human cytosolic glutathione S-transferases are mainly coded for at 5 loci: GSTA (α), GSTT1 (θ), GSTM1 (μ), GSTPI (π), and GSTM3 (γ) (11). Polymorphisms in these genes, possibly by altering their expression and functional activities, may affect carcinogen activation/detoxification and DNA repair. Three alleles have been identified at the glutathione S-transferase M1 (GSTM1) locus: GSTM1*0, GSTM1*A, and GSTM1*B. Two major alleles have been identified at the glutathione S-transferase T1 (GSTT1) locus: GSTT1*1 and GSTT1*0. Previous studies showed that a homozygous deletion (0/0), or null genotype, at either the GSTM1 locus or the GSTT1 locus resulted in enzyme function loss, and thus it was hypothesized to be related to the susceptibility to oral cancer (12–14).

A number of case-control studies have investigated the relation of polymorphisms in these 2 genes to oral cancer but have generated equivocal results (3–10, 13, 15–33), and data on the interaction between either of the 2 genes and smoking are lacking. These studies were limited by small or moderate sample sizes. Meta-analysis can be used to pool data from these studies to obtain sufficient statistical power to detect the potential effect of small to moderate sizes associated with these polymorphisms. To our knowledge, meta-analyses have been conducted on the association between oral cancer and polymorphisms in GSTM1 (34) but, to date, not in GSTT1. Results from several new studies that examined GSTM1 or GSTT1 have been reported (4, 15–18, 25, 29, 32, 33). Therefore, we performed a systematic review and meta-analysis to investigate the associations between GSTM1 and GSTT1 null polymorphisms and oral cancer and to investigate the potential interactions between these genes and smoking.

MATERIALS AND METHODS

Study selection

The literature search was performed using PubMed (1966–July 2010) and SciVerse Scopus. The keywords used for searching were “oral cancer,” “mouth neoplasm,” “polymorphisms,” “glutathione S-transferases,” “GSTM1,” and/or “GSTT1.” We selected only case-control studies that had been published in English and investigated the association between GSTM1 or GSTT1 genotype (null genotype vs. wild type) and the risk of oral cancer. Oral cancer was defined as the presence of 1 or more tumors of the lip, oral cavity, or mouth. Studies that included cases of larynx or pharynx cancer were excluded. Because the etiology of lip cancer is not consistent with that of oral cancer at other sites in that sunlight exposure plays a role (35), we performed a sensitivity analysis by excluding studies with cases of lip cancer. We required that the sample size and the numbers of cases and controls with each genotype be available; publications that presented data allowing such results to be derived were also included. References from recent review articles were also checked for relevant articles. If there were multiple publications from the same study, the most complete or most recent publication was given precedence. The reviewers (Z.-J. Z., K. H.) determined the eligibility of studies by reading the title, abstract, or full text.

Data extraction and statistical analyses

Data on first author, publication year, source of controls, ethnicity, study region, tumor site, numbers of cases and controls, percentage of null genotypes, adjusted odds ratio and its 95% confidence interval, and covariates (if applicable) were extracted and tabulated in a standard format. The Q statistic and I² were calculated to assess heterogeneity between studies. An I² value less than 50% was considered to indicate low heterogeneity (36). Summary odds ratios were estimated via DerSimonian and Laird random-effects models (37). If there was heterogeneity between studies, we explored the source by testing the influence of each covariate (ethnicity (Asian, Caucasian, or nonspecified, as reported in the published primary study); source of controls (hospital patients, healthy population); Hardy-Weinberg equilibrium (i.e., the genotype distribution in the control population does not significantly deviate from the Hardy-Weinberg proportion: yes, no, not available); and publication year) in a meta-regression model. We assessed the influence of individual studies on the summary effect estimate by omitting 1 study at a time when recalculating the summary odds ratios. The influence of publication year was also assessed through cumulative meta-analysis. Publication bias was assessed by means of Begg’s funnel plot and Egger’s test (38).

We applied a case-only design in the meta-analyses to assess the multiplicative interactions between GSTM1 and GSTT1 null polymorphisms and smoking (ever/high levels of smoking vs. never/low levels) because this approach is more powerful than conventional case-control studies when testing for a possible multiplicative interaction under the assumption of independence between GSTM1 and GSTT1 null polymorphisms and smoking in the population (39–41). A 2-tailed P value less than 0.05 was considered significant. All analyses were performed using Stata, version 10.0 (StataCorp, College Station, Texas).

RESULTS

Of the 28 studies that met the criteria for entering the meta-analysis, 19 examined the GSTT1 polymorphism (4–7, 9, 10, 13, 15–26) and 27 examined the GSTM1 polymorphism (3–10, 13, 15–19, 21–33). Characteristics of the study design are shown in Table 1. The number of cases in the included studies varied from 30 to 456. Controls comprised hospital patients, healthy population controls, or both. In 4 studies, investigators specifically reported that the genotype distribution in controls did not deviate significantly from the Hardy-Weinberg proportion (10, 16, 24, 25).

Smoking

Data from 8 studies (3–10) comprising 938 oral cancer cases and 1,542 controls were pooled to calculate a summary odds ratio for smoking (odds ratio (OR) = 2.39, 95% confidence interval (CI): 1.82, 3.13; P < 0.01) (Figure 1). The results were not substantially changed after the exclusion
of 2 studies (5, 7) with cases of lip cancer (OR = 2.39, 95% CI: 1.66, 3.45; \( P < 0.01 \)). There was no evidence of heterogeneity between studies (\( Q \) statistic \( P = 0.15; I^2 = 35\% \)). There was no evidence of publication bias by visual examination of Begg’s funnel plot (data not shown) or test results from Egger’s test (\( P = 0.09 \)).

**GSTM1**

Data from 27 case-control studies comprising 2,780 oral cancer cases and 5,177 controls were pooled together for analysis of the *GSTM1* polymorphism. Heterogeneity between studies was suggested (\( Q \) statistic \( P < 0.01; I^2 = 68\% \)). We tested the influence of ethnicity, control sources, Hardy-Weinberg equilibrium, and publication year on the per-study effect size in meta-regression; none of these covariates were a significant source of heterogeneity (data not shown). After the hospital-based studies were excluded, heterogeneity was observed among the 15 remaining studies (3–9, 16, 18, 23, 26, 28, 29, 31, 33) with healthy controls (\( Q \) statistic \( P = 0.18; I^2 = 75\% \)). There was no evidence of publication bias by visual examination of Begg’s funnel plot (data not shown) or Egger’s test (\( P = 0.92 \)). The results were not substantially changed after the exclusion of 1 study (7) with cases of lip cancer (data not shown). When we excluded 1 study reporting smoking status as high/low (3) and categorized smoking status into ever and never smoking, the results were similar (OR = 0.52, 95% CI: 0.28, 0.96; \( P = 0.04 \)). Five of the 6 studies were carried out in Asian populations. After the exclusion of 1 study that was carried out in a population with nonspecified ethnicity (6), the multiplicative interaction between the *GSTM1* null polymorphisms and smoking in Asians was still statistically significant (OR = 0.46, 95% CI: 0.27, 0.78; \( P < 0.01 \)).

**GSTT1**

Data from 19 case-control studies comprising 2,073 oral cancer cases and 3,673 controls were pooled together for analysis of the *GSTT1* polymorphism. Heterogeneity was observed (\( Q \) statistic \( P < 0.01; I^2 = 73\% \)). Similar to the
GSTM1 analysis, we tested the influence of ethnicity, control sources, Hardy-Weinberg equilibrium, and publication year on per-study effect size in meta-regression; none of these covariates were a significant source of heterogeneity (data not shown). After the hospital-based studies were excluded, heterogeneity was observed in the 9 remaining studies (4–7, 9, 16, 18, 23, 26) with healthy controls ($Q$ statistic $P < 0.01$; $I^2 = 75\%$). There was no evidence of publication bias by visual examination of Begg’s funnel plot (data not shown) or test results from Egger’s test ($P = 0.11$).

We stratified the analysis by ethnicity. Among the 13 studies conducted in Asian populations (1,898 cases and 2,831 controls), moderate heterogeneity was observed ($Q$ statistic $P = 0.001$; $I^2 = 65\%$). The summary odds ratio was 1.07 (95% CI: 0.82, 1.38; $P = 0.63$). The results were not substantially changed after exclusion of the 2 studies (5, 7) with cases of lip cancer (data not shown). There was no heterogeneity between studies ($Q$ statistic $P = 0.91$; $I^2 = 0\%$). There was evidence of publication bias from Egger’s test ($P = 0.02$).

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DISCUSSION

In the present study, we examined the association between GSTM1 and GSTT1 null genotypes and oral cancer risk and assessed the multiplicative interaction between GSTM1, GSTT1, and smoking status. The main effect analysis suggested that the null genotype of GSTM1 may be associated with a higher risk of oral cancer in Asians but not in Caucasians. However, new investigations with more rigorous designs are required to address this question. Our results indicated that the GSTM1 polymorphism might modify the relation between smoking status and oral cancer risk. Few previous studies have taken this gene-smoking interaction into account. We did not find a significant association between the null genotype of GSTT1 and oral cancer risk in

A case-only analysis in 4 studies (347 cases) (6, 7, 10, 23) did not find a statistically significant multiplicative interaction between the GSTT1 null genotype and smoking status (ever vs. never) (OR = 1.04, 95% CI: 0.64, 1.71; $P = 0.87$). The results were not substantially changed after exclusion of 1 study (7) with cases of lip cancer (data not shown). There was no heterogeneity between studies ($Q$ statistic $P = 0.91$; $I^2 = 0\%$). There was evidence of publication bias from Egger’s test ($P = 0.02$).
Asians or Caucasians. Heterogeneity exists between studies, and we did not find a significant multiplicative interaction between the null genotype of GSTT1 and smoking status. Assessment of heterogeneity is necessary for most meta-analyses (42, 43). Meta-analyses might miss true effects in the presence of even modest between-study heterogeneity, because they are based on the assumption of etiologic homogeneity across studies (44). Heterogeneity could result from genotyping error, population stratification, selection bias, or population-specific gene-gene or gene-environment interaction, allelic heterogeneity, or chance (42, 45). The $I^2$ values surpassed the threshold of 50% in some of the subgroup analyses in the present study, indicating the presence of heterogeneity and insufficient power (44). However, evidence of replication was found for the associations between the null genotypes of GSTM1 and GSTT1 and oral cancer in the Caucasian and Asian populations (shown in Table 1), indicating the possibility of a modest genetic effect (46). Small sample sizes are the major weakness of most studies in the field. A meta-analysis pools data together to achieve a larger sample size. Random-effect calculation of summary effects estimates the mean effect under the assumption that the included studies are from a random sample of the relevant distribution of effects. Because of the presence of heterogeneity, however, the summary estimates provided in the present study would reflect only a crude analysis. We could not exclude the possibility of false-positive/-negative findings (47). Further studies need to focus on exploring the sources of heterogeneity.

The GSTM1 polymorphism is one of the most studied loci in relation to oral cancer risk. The homozygous deletion results in functional loss of the GSTM1 enzyme (13) and has been implicated in the genesis of several cancers, including lung cancer in Asians (48–50), breast cancer (51), and bladder cancer (52). The present study suggests that the GSTM1 null genotype is associated with a higher risk of oral cancer in Asians but not in Caucasians. The results are in agreement with the previous meta-analysis by Zhuo et al. (34). Eighteen articles (3, 5–10, 13, 19, 21–24, 26–28, 30, 31) were included in both our and their analysis. Nine relevant studies (4, 15–18, 25, 29, 32, 33) were included in our analysis but not in theirs, and 5 studies in their analysis (53–57) were not included in ours because 2 articles (53, 56) had been updated on the basis of the same population sources (25, 30), 2 articles (54, 55) did not separate pharyngeal cancer from oral cancer, and 1 study (57) was retrieved from a different database (China National Knowledge Infrastructure (http://www.global.cnki.net)). Although we pooled all published studies currently available on this topic, we believe our study is still far from conclusive, because in most previous studies regarding the association between the GSTM1 null genotype and oral cancer risk, investigators did not stratify the results according to smoking status. The results of our study suggest an inverse multiplicative interaction, (i.e., OR < 1), at least in Asian populations, between

![Graph showing summary estimate (odds ratio (OR) and 95% confidence interval (CI)) of the effect of interaction between the glutathione S-transferase M1 (GSTM1) null genotype and smoking (ever/high levels vs. never/low levels) on oral cancer risk. The squares indicate the ORs in the individual studies; each square’s size is proportional to the weight of the corresponding study in the meta-analysis. The diamond indicates the pooled OR. Horizontal lines, 95% CI.](https://academic.oup.com/aje/article-abstract/173/8/847/157767)
smoking status and the GSTM1 null polymorphisms. Although this significant gene-smoking interaction is reported herein for the first time and further data are required, it has to be taken into account when assessing the association between the GSTM1 null genotype and oral cancer risk. Data on other forms of tobacco use (e.g., chewing tobacco or bidis) were not fully collected or adjusted for. In addition, the sample sizes of the studies in Asian populations were small to modest, limiting their power to detect a probably modest effect.

The inverse interaction between the GSTM1 null polymorphisms and smoking status suggests that smoking is more detrimental to persons who carry the GSTM1*0 allele. It also suggests that GSTM1 may decrease oral cancer risk through mechanisms that are not specific to the detoxification of procarcinogens in tobacco smoke. Further studies are needed to investigate the underlying mechanisms. Previous studies investigating gene-smoking interaction showed an inverse effect of the interaction between smoking and the GSTM1 null polymorphisms on the risks of colorectal cancer (58), lung cancer (59), uterine cervical cancer (60), laryngeal cancer (61), and esophageal cancer (62), while a positive effect of the interaction (i.e., OR > 1) was observed for head and neck cancer (33, 63), upper aerodigestive tract cancers (64), and lung cancer (65). In the present study, we could not assess the effect of the interaction on an additive scale with the case-only design.

GSTM1 is known to be polymorphic in different ethnic groups. The homozygous deletion of this locus has been reported to be associated with loss of enzyme function. The null genotype of GSTT1 has been suggested to be associated with the risk of a number of cancers, including lung cancer in Asians (66), gastric cancer (67), leukemia (68), and hepatocellular carcinoma (69). However, previous meta-analyses showed that the GSTT1 polymorphism was not associated with the risk of nasopharyngeal cancer (70), head and neck cancer (71), esophageal cancer (72), breast cancer (73), or prostate cancer (74). In the present study, we did not find a significant association between the GSTT1 null genotype and oral cancer. Although we pooled data from 19 studies, small sample sizes may be a reason for the lack of statistical significance. In the context of complex diseases, it has been shown that the effect size of many risk alleles is modest and that tens of thousands of persons are needed to capture the signal (75).

The present study should be interpreted in light of a number of weaknesses. The study was a meta-analysis of case-control studies, some of which were hospital-based; thus, selection bias might exist. The definition of tobacco use and alcohol consumption varied between studies. No data were collected on secondhand smoke exposure. Authors of most included studies did not report whether the genotype distribution in control populations fitted the Hardy-Weinberg proportion. Adjustment for the magnitude of deviation from Hardy-Weinberg equilibrium tends to result in more significant heterogeneity among the included studies (76). The case-only analysis did not adjust for other covariates. Population stratification in genetic association studies may lead to biased or spurious results. The present study was based on published articles; therefore, publication bias may exist, though no evidence was suggested from either the funnel plots or Egger's tests.

To our knowledge, the present study is the first meta-analysis to date to report a multiplicative interaction between the GSTM1 polymorphisms and smoking status. In addition, our results suggest that there is no association between the GSTT1 null genotype and oral cancer risk, while the null genotype of GSTM1 may be associated with a higher risk of oral cancer in Asians but not in Caucasians. However, the interaction between the GSTM1 null polymorphism and smoking status was not accounted for in the present analysis because of the lack of stratification by smoking status in most of the included studies. Future studies with larger scales and more rigorous designs are needed to investigate the gene effects and the potential effect modification by smoking status, as well as other environmental factors.

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Author affiliations: Department of Epidemiology, School of Public Health, Shanghai Jiao Tong University, Shanghai, China (Zhi-Jiang Zhang, Jin Ma); Research Genetics, Rosetta Inpharmatics, Seattle, Washington (Ke Hao); Department of Preventive Medicine, School of Public Health, Shanghai Jiao Tong University, Shanghai, China (Rong Shi); Department of Epidemiology, School of Public Health, Fudan University, Shanghai, China (Genming Zhao); Department of Public Health Sciences, Karolinska Institute, Stockholm, Sweden (Guo-Xin Jiang); Division of Preventive Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts (Yiqing Song); and Department of Epidemiology and Biostatistics, College of Public Health and Health Professions, University of Florida, Gainesville, Florida (Xiaohui Xu).

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