

Association between CYP2E1 polymorphisms and risk of gastric cancer: An updated meta-analysis of 32 case-control studies

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Abstract. Previous studies suggested that *RsaI/PstI* and *DraI* polymorphisms on cytochrome P450 2E1 (CYP2E1) may be associated with susceptibility to gastric cancer (GC). However, this association remains ambiguous. A meta-analysis of previously published studies was performed in an attempt to elucidate this association. The odds ratio and 95% confidence interval were used to assess the strength of the association. In the overall analyses of *RsaI/PstI* and *DraI*, no association was identified. In the subgroup analyses, *RsaI/PstI* was identified to increase the risk of GC in the smoking population. In addition, in the previous studies of interactions with other genes, *RsaI/PstI* was revealed to be associated with increased GC risks when glutathione S-transferase- μ -1 or glutathione S-transferase θ -1 was null or *DraI* was homozygous wild-type. However, these stratified analyses were lacking credibility due to the limitation of correlational study numbers. In conclusion, CYP2E1 polymorphisms revealed no association with the risk of GC.

Introduction

For the past few years, the worldwide incidence of gastric cancer (GC) has decreased (1); however, GC remains the second leading cause of cancer-associated mortality worldwide (2). The incidence and mortality rate is rising in Eastern Asia (1,2), particularly in China (3). The growth of GC incidences is hypothesized to be caused by the interplay of environmental and genetic factors, which varies between area, gender, age and habitual behaviors (4-8). Specific variant alleles may modify the effects of environmental exposures and the gene-environment interactions may partly affect GC

incidence (5). In the last decade, more and more previous studies have focused on the association between polymorphisms and GC, however, only a few revealed an association with GC (4,7).

As an important metabolic enzyme, cytochrome P450 2E1 (CYP2E1) is critical in the metabolism of nitrosamines, benzene and vinyl chloride in the human body (9-11). Nitrosamine is considered as a pathogenic factor of GC (12); therefore, it is assumed that the variant alleles in CYP2E1 may affect the incidence of GC (13).

RsaI/PstI and *DraI* polymorphisms are regarded as the most frequent and powerful polymorphisms in CYP2E1 (14). *RsaI/PstI* polymorphisms, which are in complete linkage disequilibrium in the 5'-flanking promoter region of CYP2E1, are associated with higher transcription and increased enzyme activity (15). The *DraI* polymorphisms, however, are considered only to enhance transcription (14). The variant alleles in *RsaI/PstI* polymorphisms cause three genotypes, termed wild-type homozygous (C1C1), heterozygous (C1C2) and variant homozygous (C2C2) (15,16). *DraI* polymorphisms are divided into wild-type homozygous (DD), heterozygous (CD) and variant homozygous (CC) genotypes (17).

Studies concerning the association of *RsaI/PstI* polymorphisms and GC susceptibility have been performed in numerous previous studies, however, the results remain uncertain and controversial (18-45). However, investigations regarding *DraI* polymorphisms have rarely been performed. In the present study, 32 case-control studies (18-45) of 4,953 cases and 6,626 controls were screened from published papers between January 1995 and October 2014. These previous studies were used to calculate pooled statistics by meta-analysis, aiming to clarify the relevance of CYP2E1 polymorphisms and GC risk.

Materials and methods

Identification of previous studies. Data screening was performed in PubMed (<http://www.ncbi.nlm.nih.gov/pubmed>) and China National Knowledge Infrastructure database (<http://oversea.cnki.net/kns55/default.aspx>) between January 1995 and October 2014, without language limitation. The key words used for screening were 'CYP2E1', 'Cytochrome P450 2E1', 'polymorphism', 'gastric', 'neoplasm', 'cancer' and 'variation'. The titles and abstracts of each paper were browsed for preliminary screening. The references of retrieved papers were also examined to search for additional relevant studies.

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Key words: cytochrome P450 2E1, *RsaI/PstI*, *DraI*, gastric cancer, polymorphism, meta-analysis

Inclusion and exclusion criteria. The previous studies were selected using the following inclusion criteria: i) Case-control studies; ii) studies focusing on the relevance between CYP2E1 *RsaI/PstI* or *DraI* polymorphisms and GC susceptibility; iii) studies where detailed genotype frequencies were provided. Previous studies lacking Hardy-Weinberg equilibrium (HWE) were excluded. The titles and abstracts were reviewed for selection and the full-text papers were intensively read to confirm eligibility. Two reviewers were required to screen the studies independently, according to the criteria, and a third was involved in discussing any disagreement occurring between the previous two reviewers.

Data extraction. A form was designed to gather the following information: First author, year, country, ethnicity, genotyping method, source of control, numbers of different genotypes in cases/controls and HWE. The authors of the previous studies were contacted to confirm dubious information.

Statistical analysis. The meta-analysis focused on the associations between the CYP2E1 polymorphisms (*RsaI/PstI* and *DraI*) and GC susceptibility. The pooled odds ratios (ORs) were used to explain the correlation. In the previous studies of *RsaI/PstI* polymorphisms, ORs and their 95% confidence intervals (CIs) were calculated for the dominant model (C1C2 + C2C2 vs. C1C1) and allele frequency C2, vs. C1. An OR and 95% CI <1 indicated a significant difference between the cases and controls. In the subgroup analysis, the previous studies were grouped according to ethnicity, source of control, smoking and drinking status, and histology type. The ORs and 95% CIs of each group were also calculated to assess the influence of these factors on the association. In the previous studies of *DraI* polymorphisms, the ORs and 95% CIs were estimated for the dominant model (CD + CC vs. DD) and allele frequency (C vs. D).

In the meta-analysis, the I^2 value was used to confirm heterogeneity (46), with values <25, 25-50 and >50% indicating low, moderate and high heterogeneity, respectively. A χ^2 -based Q test was also used for the heterogeneity test (P_h), together with the random-effect model, in order to obtain a relatively conservative outcome (47). The significance of the pooled ORs and their 95% CIs were determined using the Z test. A Pearson's χ^2 test was used for assessing the HWE.

In order to elucidate the influence of each previous study included, influence analysis was performed by excluding each study in turn and analyzing the homogeneity and effect size for the remaining studies. Publication bias was assessed using Begg and Mazumdar's adjusted rank correlation test (48) and the Egger regression asymmetry test (49). Funnel plots were also used to illustrate the publication bias (50). All statistical calculations were performed using STATA 12.0 software (StataCorp LP, College Station, TX, USA).

Results

Searching results and characteristics of the previous studies included in the meta-analysis. The flow diagram of the study selection process is shown in Fig. 1. A total of 81 previous studies were identified in the database search, among which three studies were duplicated. A further 35 previous studies

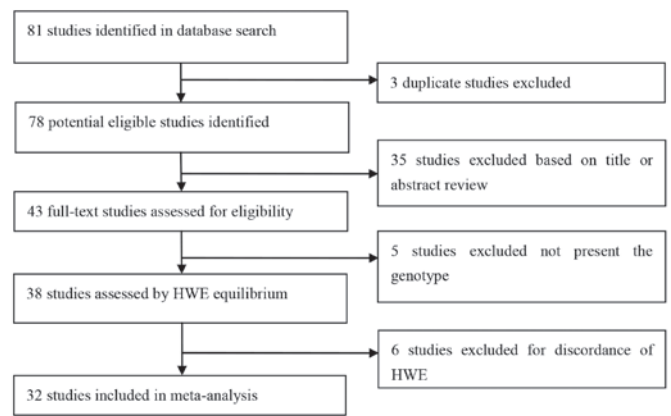


Figure 1. Flow diagram of included and excluded studies. HWE, Hardy-Weinberg equilibrium.

were excluded as a result of inconformity of case-control studies or irrelevance with the *RsaI/PstI* and *DraI* polymorphisms. The remaining 43 previous studies were read in full, among which five studies without detailed genotype frequencies were excluded. Following the exclusion of six previous studies for discordance of HWE, 32 previous studies were included in the meta-analysis (18-45). Of these, 26 were associated with *RsaI/PstI* polymorphisms, while the remaining six (20,24,27,33,35,43) were investigating *DraI* polymorphisms. Details of these previous studies are shown in Table I.

These previous studies were subgrouped according to ethnicity, source of control, smoking and drinking status, and histology type. Stratified analysis was performed in these subgroups. Previous studies concerning the interactions between *RsaI/PstI* and glutathione S-transferase- μ -1 (GSTM1) (36), glutathione S-transferase θ -1 (GSTT1) (40) or *DraI* (27,35) were listed and analyzed in order to identify more factors, which may influence the risk of GC (Table II).

Meta-analysis results. In the overall analysis of *RsaI/PstI* polymorphisms, the ORs and 95% CIs were 0.96 (0.82 and 1.12) in the dominant model (C1C2 + C2C2 vs. C1C1) and 1.02 (0.86 and 1.19) in gene frequency (C2 vs. C1; Table III).

The subgroup analyses of the clinical characteristics are shown in Table III. In the source of control subgroups, no significant differences were observed either in population-based studies or hospital-based studies. As for ethnicity, no significant differences were observed in any of the three subgroups (Fig. 2). In the smoking status subgroup, a significantly increased risk was observed in the smoking group [C1C2 / C2C2 vs. C1C1 = 1.56 (1.14 and 2.15), as shown in Fig. 3], while the non-smoking group revealed no significant difference. Subgroups of drinking status revealed no association with GC risk. In the previous studies, which assessed the interactions of different genes, *RsaI/PstI* was demonstrated to increase GC risks when GSTM1 was null [2.60 (1.13 and 5.99)]. A significantly increased risk was also observed in the previous studies investigating the interaction between *RsaI/PstI* and GSTT1. In the GSTT1 null group, a significant difference was observed between increased risk of GC and *RsaI/PstI* [0.22 (0.09 and 0.54)].

Table I. Characteristics of previous studies in the meta-analysis.

| Authors (year) | Country | Ethnicity | Genotyping method | Source | Cases | | | Control | | | HWE | Refs. |
|-------------------------------|------------|-----------|-------------------|--------|-------|------|------|---------|------|------|-----|-------|
| | | | | | C1C1 | C1C2 | C2C2 | C1C1 | C1C2 | C2C2 | | |
| <i>RsaI/PstI</i> | | | | | | | | | | | | |
| Ghoshal (2014) | India | Asian | PCR-RFLP | PB | 40 | 45 | 3 | 103 | 58 | 9 | Y | (25) |
| Yan (2013) | China | Asian | PCR-RFLP | HB | 77 | 39 | 4 | 79 | 36 | 5 | Y | (42) |
| Feng (2012) | China | Asian | PCR-RFLP | HB | 348 | 128 | 34 | 374 | 119 | 17 | Y | (27) |
| Malik <i>et al</i> (2009) | India | Asian | PCR-RFLP | HB | 88 | 20 | 0 | 177 | 17 | 1 | Y | (32) |
| Agudo <i>et al</i> (2006) | Britain | Caucasian | PCR-RFLP | PB | 226 | 13 | 0 | 880 | 39 | 1 | Y | (18) |
| Colombo <i>et al</i> (2004) | Brazil | Mixed | PCR-RFLP | HB | 89 | 11 | 0 | 134 | 16 | 0 | Y | (23) |
| Zhou <i>et al</i> (2003) | China | Asian | PCR-RFLP | PB | 85 | 45 | 15 | 140 | 75 | 14 | Y | (45) |
| Park <i>et al</i> (2003) | Korea | Asian | PCR-RFLP | PB | 80 | 33 | 7 | 94 | 48 | 3 | Y | (35) |
| Wu <i>et al</i> (2002) | China | Asian | PCR-RFLP | HB | 215 | 108 | 33 | 199 | 70 | 9 | Y | (33) |
| Tsukino <i>et al</i> (2002) | Japan | Asian | PCR-RFLP | HB | 71 | 42 | 7 | 88 | 58 | 12 | Y | (37) |
| Cai <i>et al</i> (2001) | China | Asian | PCR-RFLP | HB | 58 | 27 | 6 | 71 | 22 | 1 | Y | (21) |
| Qian <i>et al</i> (2001) | China | Asian | PCR-RFLP | PB | 88 | 47 | 7 | 88 | 68 | 8 | Y | (39) |
| Kato <i>et al</i> (1995) | Japan | Asian | PCR-RFLP | HB | 90 | 54 | 6 | 120 | 69 | 14 | Y | (28) |
| González <i>et al</i> (2004) | Costa Rica | Asian | PCR-RFLP | HB | 20 | 11 | 0 | 31 | 15 | 5 | Y | (19) |
| Boccia <i>et al</i> (2007) | Italy | Caucasian | PCR-RFLP | HB | 102 | 5 | - | 234 | 20 | - | Y | (20) |
| Gao <i>et al</i> (2002) | China | Asian | PCR-RFLP | PB | 58 | 31 | 9 | 121 | 62 | 13 | Y | (22) |
| Nan <i>et al</i> (2005) | Korea | Asian | PCR-RFLP | HB | 69 | 39 | - | 129 | 88 | - | Y | (26) |
| Kato <i>et al</i> (2011) | Japan | Asian | PCR-RFLP | HB | 280 | 186 | - | 340 | 213 | - | Y | (30) |
| Kato <i>et al</i> (1996) | Japan | Asian | PCR-RFLP | HB | 55 | 29 | - | 87 | 61 | - | Y | (29) |
| Malakar <i>et al</i> (2014) | India | Asian | PCR-RFLP | PB | 93 | 11 | 1 | 182 | 28 | 0 | Y | (31) |
| Nishimoto <i>et al</i> (2000) | Japan | Asian | PCR-RFLP | HB | 31 | 27 | 1 | 69 | 58 | 6 | Y | (34) |
| Suzuki <i>et al</i> (2004) | Japan | Asian | PCR-RFLP | HB | 107 | 38 | - | 112 | 65 | - | Y | (36) |
| Li and Xu (2007) | China | Asian | PCR-RFLP | HB | 25 | 10 | 6 | 17 | 16 | 8 | Y | (38) |
| Qian <i>et al</i> (2003) | China | Asian | PCR-RFLP | PB | 64 | 22 | 4 | 47 | 39 | 4 | Y | (40) |
| Wang <i>et al</i> (2005) | China | Asian | PCR-RFLP | HB | 33 | 14 | 1 | 22 | 23 | 3 | Y | (41) |
| Ye (2002) | China | Asian | PCR-RFLP | HB | 39 | 13 | 4 | 26 | 24 | 6 | Y | (44) |
| <i>DraI</i> | | | | | | | | | | | | |
| Yan (2013) | China | Asian | PCR-RFLP | HB | 70 | 42 | 8 | 70 | 46 | 4 | Y | (42) |
| Feng <i>et al</i> (2012) | China | Asian | PCR-RFLP | HB | 334 | 131 | 45 | 318 | 160 | 32 | Y | (27) |
| Wu <i>et al</i> (2002) | China | Asian | PCR-RFLP | HB | 195 | 120 | 41 | 158 | 100 | 20 | Y | (33) |
| Park <i>et al</i> (2003) | Korea | Asian | PCR-RFLP | PB | 78 | 35 | 7 | 85 | 45 | 8 | Y | (35) |
| Boccia <i>et al</i> (2007) | Italy | Caucasian | PCR-RFLP | HB | 92 | 15 | - | 227 | 27 | - | Y | (20) |
| Darazy <i>et al</i> (2011) | Lebanon | Asian | PCR-RFLP | PB | 12 | 1 | 0 | 66 | 4 | 0 | Y | (24) |

HWE, Hardy Weinberg equilibrium; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; PB, population-based; HB, hospital-based; C1C1, wild-type homozygous; C1C2, heterozygous; C2C2, variant homozygous.

In the meta-analysis of *DraI* polymorphisms, no significant risk of GC susceptibility was observed either in the dominant model (CD + CC vs. DD) or allele frequency (C vs. D). However, the interaction analysis of *RsaI/PstI* and *DraI* on CYP2E1 revealed that *RsaI/PstI* significantly increased the risk of GC when *DraI* was wild-type (DD) [1.55 (1.13 and 2.14)].

Heterogeneity between the previous studies. Heterogeneities of each comparison are shown in Table III. The results revealed that $I^2 = 61.4\%$ and $P_h < 0.001$. Compared with the overall analysis of *RsaI/PstI* polymorphisms, heterogeneities decreased in several subgroups (population-based controls group: $I^2 = 35.3\%$ and $P_h = 0.147$; Caucasian group: $I^2 = 41.1\%$ and $P_h = 0.192$).

In previous *DraI* polymorphism studies, the heterogeneity P-values were markedly higher compared with the critical value ($P=0.01$), noting that heterogeneities in the group of *DraI* polymorphisms were very little.

Sensitivity analysis. Influence analysis was performed by excluding studies one-by-one and analyzing the homogeneity and effect size for all of the remaining studies, aiming at examining the stability of the analysis. The results revealed that no individual study affected the pooled ORs significantly, in either the *RsaI/PstI* or *DraI* studies, confirming the stability of the analysis. This was associated with the high quality of the previous studies included.

Table II. Distribution of CYP2E1 *RsaI/PstI* and influential factors.

| Influential factors (Exposure + vs. exposure -) | No. (cases/controls) of CYP2E1 <i>RsaI/PstI</i> polymorphism | | | | Refs. |
|--|--|------------|-------------|------------|-------|
| | C1C1 | | C1C2 + C2C2 | | |
| | Exposure + | Exposure - | Exposure + | Exposure - | |
| Smoking (ever vs. never) | | | | | |
| Cai <i>et al</i> (2001) | 37/23 | 21/48 | 23/11 | 10/12 | (21) |
| Agudo <i>et al</i> (2006) | 151/503 | 79/403 | 9/18 | 4/22 | (18) |
| Boccia <i>et al</i> (2007) | 49/99 | 53/135 | 1/9 | 4/11 | (20) |
| Gao <i>et al</i> (2002) | 41/75 | 17/44 | 32/25 | 8/37 | (22) |
| Malakar <i>et al</i> (2014) | 73/105 | 20/77 | 20/11 | 8/1 | (31) |
| Zhou <i>et al</i> (2003) | 66/83 | 19/54 | 47/42 | 12/45 | (45) |
| Drinking (ever vs. never) | | | | | |
| Zhou <i>et al</i> (2003) | 33/33 | 49/107 | 23/22 | 36/66 | (45) |
| Suzuki <i>et al</i> (2004) | 48/32 | 51/51 | 17/13 | 20/34 | (36) |
| Malakar <i>et al</i> (2014) | 43/73 | 51/109 | 4/16 | 8/12 | (31) |
| Gao <i>et al</i> (2002) | 9/13 | 49/108 | 5/9 | 35/66 | (22) |
| Cai <i>et al</i> (2001) | 32/20 | 25/51 | 19/8 | 14/15 | (21) |
| Boccia <i>et al</i> (2007) | 68/123 | 32/111 | 5/10 | 0/10 | (20) |
| Histology type (intestinal vs. diffuse) | | | | | |
| Ghoshal <i>et al</i> (2014) | 27/103 | 8/103 | 23/67 | 20/67 | (25) |
| Kato <i>et al</i> (1996) | 27/87 | 28/87 | 17/61 | 12/61 | (29) |
| Wu <i>et al</i> (2002) | 98/199 | 99/199 | 49/79 | 46/79 | (33) |
| Nishimoto <i>et al</i> (2000) | 17/69 | 8/69 | 6/64 | 3/19 | (34) |
| Suzuki <i>et al</i> (2004) | 52/112 | 55/112 | 15/65 | 23/65 | (36) |
| GSTM1 (present vs. null) | | | | | |
| Suzuki <i>et al</i> (2004) | 67/22 | 45/30 | 26/5 | 39/10 | (36) |
| GSTT1 (present vs. null) | | | | | |
| Zhou <i>et al</i> (2003) | 22/30 | 42/17 | 14/21 | 12/22 | (45) |
| CYP2E1 <i>DraI</i> (DD vs. CD + CC) | | | | | |
| Park <i>et al</i> (2003) | 71/79 | 9/13 | 7/6 | 33/40 | (35) |
| Feng <i>et al</i> (2012) | 212/233 | 136/141 | 122/85 | 40/51 | (27) |

CYP2E1, cytochrome P450 2E1; GSTM1, glutathione S-transferase- μ -1; GSTT1, glutathione S-transferase θ -1; C1C1, wild-type homozygous; C1C2, heterozygous; C2C2, variant homozygous; DD, wild-type homozygous; CD, heterozygous; CC, variant homozygous.

Publication bias. Begg and Mazumdar's adjusted rank correlation test and the Egger regression asymmetry test were each used to examine the publication bias, as well as funnel plots. The results revealed that no publication bias was observed in *RsaI/PstI* (Fig. 4) and *DraI* studies. Publication bias occurred in the studies of *RsaI/PstI* [Egger's test: $P=0.033$ in the dominant model (C1C2 + C2C2, vs. C1C1)], while no publication bias was observed in the *DraI* studies.

Discussion

For previous studies of *RsaI/PstI* polymorphisms, the overall analysis revealed no association between mutant C2 and GC risk. However, in the subgroup analysis, mutant C2 in *RsaI/PstI* significantly increased GC risk in the smoking population and GSTM1- or GSTT1-null populations. In the *DraI* polymorphism studies, variant allele C revealed no association

with GC risk. In the interaction analysis, C2 in *RsaI/PstI* was revealed to increase GC risk when the *DraI* was not mutated.

The present study obtained two meta-analyses focusing on the association between *RsaI/PstI* polymorphisms and GC susceptibility (20,51). It was demonstrated that *RsaI/PstI* polymorphisms increased GC risk in the smoking population; however, no focus on the *DraI* polymorphisms or the interactions between two polymorphisms was provided. Therefore, the updated meta-analysis included 32 previous studies, in which 26 studies were on *RsaI/PstI* and the remaining six studies were on *DraI*. In our meta-analysis, more subgroups were made due to their potential influence on GC susceptibility. Furthermore, the present study demonstrated the interaction analysis between different gene polymorphisms. Previous studies on these interaction analyses may be insufficient; however, the results obtained may provide a guidance of which type of studies are required in the future.

Table III. Stratified analyses of polymorphisms in the CYP2E1 gene with gastric cancer risk.

| <i>RsaI/PstI</i> | No. (cases/controls) | C2 vs. C1 | | | | C1C2/C2C2 vs. C1C1 | | | |
|--------------------|----------------------|-------------------|---------|----------------|--------------------|--------------------|---------|----------------|--------------------|
| | | OR (95% CI) | P-value | P _h | I ² (%) | OR (95% CI) | P-value | P _h | I ² (%) |
| Overall | 3,727/5,510 | 1.02 (0.86, 1.19) | 0.850 | <0.001 | 61.4 | 0.96 (0.82, 1.12) | 0.574 | <0.001 | 56.2 |
| Source of controls | | | | | | | | | |
| PB | 1,027/2,124 | 1.02 (0.84, 1.25) | 0.811 | 0.147 | 35.3 | 0.96 (0.73, 1.28) | 0.828 | 0.032 | 54.4 |
| HB | 2,700/3,386 | 1.00 (0.79, 1.27) | 0.993 | <0.001 | 70.2 | 0.94 (0.78, 1.15) | 0.583 | 0.001 | 59.0 |
| Ethnicities | | | | | | | | | |
| Asian | 3,281/4,186 | 1.01 (0.85, 1.20) | 0.954 | <0.001 | 65.1 | 0.95 (0.81, 1.12) | 0.562 | <0.001 | 60.3 |
| Caucasian | 346/1,174 | 1.23 (0.65, 2.31) | 0.526 | - | - | 0.94 (0.44, 2.00) | 0.872 | 0.192 | 41.1 |
| Mixed | 100/150 | 1.03 (0.47, 2.27) | 0.936 | - | - | 1.04 (0.46, 2.33) | 0.934 | - | - |
| Smoking status | | | | | | | | | |
| Ever smoking | 549/1,014 | - | - | - | - | 1.56 (1.14, 2.15) | 0.006 | 0.376 | 6.3 |
| Never smoking | 255/889 | - | - | - | - | 1.23 (0.59, 2.60) | 0.571 | 0.018 | 63.3 |
| Drinking status | | | | | | | | | |
| Ever drinking | 305/372 | - | - | - | - | 0.91 (0.61, 1.37) | 0.676 | 0.752 | 0.0 |
| Never drinking | 371/740 | - | - | - | - | 1.08 (0.75, 1.54) | 0.663 | 0.238 | 26.2 |
| Histology type | | | | | | | | | |
| Intestinal | 331/906 | - | - | - | - | 0.84 (0.54, 1.32) | 0.456 | 0.045 | 58.9 |
| Diffuse | 315/906 | - | - | - | - | 1.05 (0.71, 1.58) | 0.798 | 0.131 | 43.6 |
| GSTM1 status | | | | | | | | | |
| Present | 93/27 | - | - | - | - | 1.71 (0.59, 4.99) | 0.328 | - | - |
| Null | 84/40 | - | - | - | - | 2.60 (1.13, 5.99) | 0.025 | - | - |
| GSTT1 status | | | | | | | | | |
| Present | 36/51 | - | - | - | - | 0.91 (0.38, 2.17) | 0.830 | - | - |
| Null | 54/39 | - | - | - | - | 0.22 (0.09, 0.54) | 0.001 | - | - |
| <i>DraI</i> status | | | | | | | | | |
| DD | 412/403 | - | - | - | - | 1.55 (1.13, 2.14) | 0.007 | 0.747 | 0.0 |
| CD + CC | 218/165 | - | - | - | - | 0.88 (0.57, 1.34) | 0.544 | 0.487 | 0.0 |

| <i>DraI</i> | No. (cases/controls) | C vs. D | | | | CD+CC vs. DD | | | |
|-------------|----------------------|-------------------|---------|----------------|--------------------|-------------------|---------|----------------|--------------------|
| | | OR (95% CI) | P-value | P _h | I ² (%) | OR (95% CI) | P-value | P _h | I ² (%) |
| Overall | 1,226/1,116 | 1.05 (0.91, 1.20) | 0.540 | 0.784 | 0.0 | 0.97 (0.82, 1.15) | 0.727 | 0.782 | 0.0 |

CYP2E1, cytochrome P450 2E1; OR, odds ratio; CI, confidence interval; PB, population-based; HB, hospital-based; GSTM1, glutathione S-transferase- μ -1; GSTT1, glutathione S-transferase θ -1; DD, wild-type homozygous; CD, heterozygous; CC, variant homozygous.

In the 26 studies of *RsaI/PstI* polymorphisms, no significant differences were observed in the homozygous dominant model (C1C2 + C2C2 vs. C1C1) and in C2 vs. C1, indicating the lack of association between the *RsaI/PstI* polymorphisms and the risk of GC.

In the subgroup analysis on source of controls, no statistically significant risks were observed in either groups with hospital-based controls or groups with population-based controls. Although hospital-based controls may not always be truly representative of the general population (52), differences were reflected between GC patients and those of healthy individuals. Population-based controls are an improved

representation of the entire population gene frequency compared with hospital-based controls, and provide a good reflection of gene frequency differences between GC patients and the overall population. Therefore, more case-control studies based on population-based controls will be performed in the future.

As for the subgroup analysis of ethnicity, no statistically significant differences were observed among groups of Asians, Caucasian or mixed. Although no statistically significant differences appeared in the subgroups, heterogeneity of the genotype frequencies existed in different ethnic groups clinically. The variant alleles C2 and C frequencies in CYP2E1 harbored in

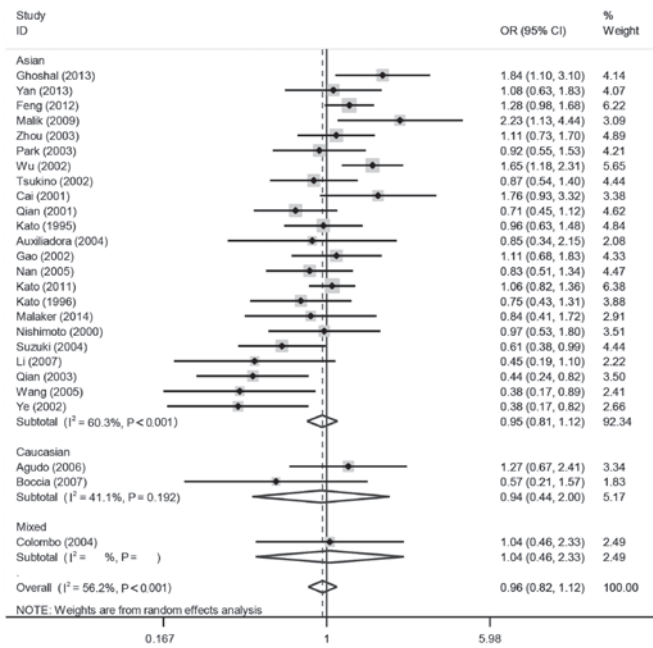


Figure 2. Forest plot of dominant comparison model (C1C2/C2C2 vs. C1C1) for subgroup comparison in *RsaI/PstI*. OR, odds ratio; CI, confidence interval.

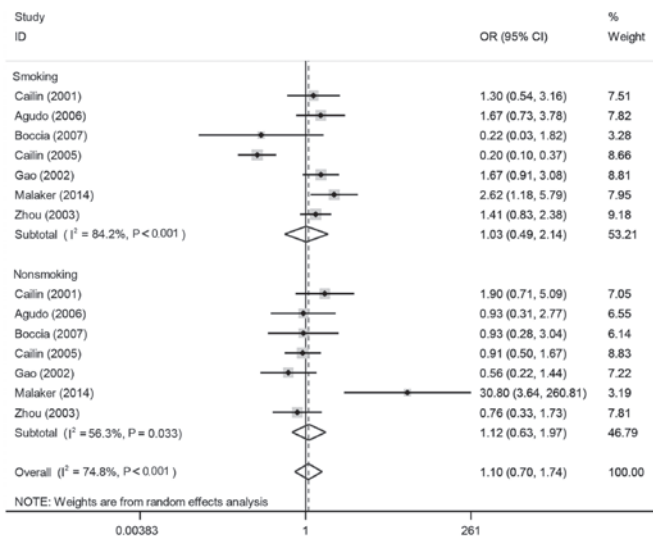


Figure 3. Forest plot of dominant comparison model for subgroup comparison (smoking status) in *RsaI/PstI*. OR, odds ratio; CI, confidence interval.

Asians are markedly higher compared with those in Caucasians or African-Americans (53-55). Similar cases were observed in several other polymorphisms (56). It is hypothesized that various living environments and diverse genotypes lead to different degrees of cancer susceptibility (8). A lack of association between GC risks and *RsaI/PstI* in Caucasians and mixed populations may be attributed to insufficiency of studies included and more studies of Caucasians are required in the future.

In the smoking subgroup, mutant C2 was demonstrated to be associated with increased GC risk. A previous study (57) revealed that smoking is a risk of cancer. Tobacco smoke contains many carcinogens, including benzopyrene and nitrosamine. CYP2E1 is critical in the metabolism of nitrosamines,

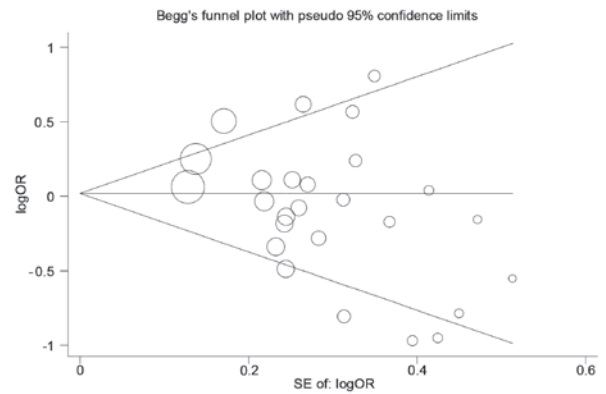


Figure 4. Funnel plot for publication bias in dominant model (C1C2/C2C2 vs. C1C1) in *RsaI/PstI* polymorphisms. (Begg's P-value = 0.108, Egger's P-value = 0.033). OR, odds ratio; SE, standard error.

benzene and vinyl chloride in the human body. Therefore, the interaction between smoking and CYP2E1 polymorphisms may magnify the GC incidence. Previous studies focusing on the interaction between smoking status and CYP2E1 were few, and more credible results depended on more studies being included (18,20-22,31,45).

In the drinking status subgroup, no significant association was observed between *RsaI/PstI* polymorphisms and GC risk. Alcohol can directly stimulate the gastric mucosa and damage gastric mucosal, making the gastric mucosal epithelium more susceptible to carcinogens (58). In addition, the stimulation of alcohol activated the function of CYP2E1 and in this resulted in an increased GC susceptibility with a synergistic effect. More studies involving an interaction between drinking and CYP2E1 polymorphisms may assist in obtaining a positive result.

According to the pathological type, GC can be divided into intestinal and diffuse types. Tumor cells in the intestinal type are normally confined to the lining of the stomach, while the diffuse type has a tendency to widely spread. The subgroup analysis may assist in understanding how differences in CYP2E1 affects the two types of GC. However, the result revealed no significant difference between the intestinal and diffuse groups.

Six previous *DraI* polymorphism studies (20,24,27,33,35,43), showed that no significant association was observed between *DraI* polymorphisms and GC risk. However, in consideration of the fact that only six studies were included in the research regarding *DraI* polymorphisms, the reliability of the results should be treated with caution, as more case-control tests are required to support the results.

Tumor incidence is often a combination of multiple factors. The interaction of multiple genes increases the impact on GC susceptibility compared with a single gene. Notably, negative association between a gene and cancer susceptibility does not mean that the gene has no impact on cancer risk. In the previous studies, which involved *RsaI/PstI* polymorphisms and other genes, it was revealed that the *RsaI/PstI* polymorphism significantly increases GC risk when GSTM1 or GSTT1 were in a null status. GSTT1 and GSTM1 convert carcinogens in the body into an inactive state, therefore, detoxifying them. However, when GSTT1 and GSTM1 are mutated into a null state, their

detoxification functions are lost, which increases cancer susceptibility (59). Additionally, mutant genotype C2 in *RsaI/PstI* has a suppressive effect in this process and GC risk was increased by such an interaction theoretically. Statistically, no significant result was obtained. However, the results were based on limited research data and the credibility was questionable. Further studies are required to improve the result in the future.

The two polymorphisms on an identical gene may lead to a synergistic effect or antagonism. When analyzing the interaction of *RsaI/PstI* and *DraI* polymorphisms, the mutant C2 in *RsaI/PstI* was revealed to increase GC risk when the *DraI* was without mutation (DD). This result may reveal that the two polymorphisms are working antagonistically. An *RsaI/PstI* mutation may increase cancer risks, while *DraI* functions with the opposite effect. Previous studies (17,60) on *DraI* polymorphisms and cancer susceptibility have revealed that *DraI* was more likely to be a risk factor of cancer, which is contrary to our assumption. By contrast, limited data may bring the contingency and must be treated with caution.

The heterogeneities in the subgroups of Caucasians and population-based controls decreased compared with the overall analysis of the *RsaI/PstI* polymorphisms, meaning that the source of controls and ethnic groups are undoubtedly factors for the formation of heterogeneity.

Publication bias occurred in the studies of *RsaI/PstI*, most probably due to several reasons: Previous studies with negative results are more difficult to publish compared with those with positive results; authors prefer to write articles with positive results as opposed to negative results. The existence of publication bias led to our cautious attitude to the positive results obtained in the present meta-analysis.

Certain limitations were observed in this meta-analysis. Firstly, only published results were included, which actually contributed to publication bias, causing the results to be treated with a conservative attitude. Secondly, several previous studies were excluded since they provided no detailed genotypic frequencies, therefore adding selection bias to a certain extent. Thirdly, more studies focusing on *RsaI/PstI* and *DraI* polymorphisms in the same cases and controls were included; however, the authors provided no matched genotype frequencies of the two genetic loci, which resulted in exploring the interaction between them with limited data. Finally, the lack of the sample size influenced the credibility in several subgroup analyses and gene interaction studies. More studies focusing on large-scale samples with multi-variables are required in the future. In conclusion, a lack of association was observed between the risk of GC and CYP2E1 *RsaI/PstI* or *DraI* polymorphisms.

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