Functional $PstI/RsaI$ Polymorphism in $CYP2E1$ Is Associated with the Development, Progression and Poor Outcome of Gastric Cancer

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Abstract

**Background:** Cytochrome P450 2E1 ($CYP2E1$), an ethanol-inducible enzyme, has been shown to metabolically activate various carcinogens, which is critical for the development and progression of cancers. It has demonstrated that $CYP2E1$ polymorphisms alter the transcriptional activity of the gene. However, studies on the association between $CYP2E1$ polymorphisms ($PstI/RsaI$ or $DraI$) and gastric cancer have reported conflicting results. Thus, the aim of the present study was to investigate whether $CYP2E1$ polymorphisms is associated with the development and progression of gastric cancer and its prognosis in Chinese patients.

**Methods:** A case-control study was conducted in which $CYP2E1$ $PstI/RsaI$ and $DraI$ polymorphisms were analyzed in 510 Chinese patients with gastric cancer and 510 age- and sex-matched healthy controls by PCR-RFLP. Odds ratios were estimated by multivariate logistic regression, and the lifetime was calculated by Kaplan-Meier survival curves. In addition, a meta-analysis was also conducted to verify the findings.

**Results:** For $CYP2E1$ $PstI/RsaI$ polymorphism, C2C2 homozygotes ($OR = 2.15; CI: 1.18–3.94$) and C2 carriers ($OR = 1.48; CI: 1.13–1.96$) were associated with an increased risk of gastric cancer when compared with C1C1 homozygotes. Both C1C2 and C2C2 genotypes were associated with advanced stage, but not the grade of gastric cancer. Moreover, C2C2 genotype was identified as an independent marker of poor overall survival for gastric cancer. However, there was not any significant association between $CYP2E1$ $DraI$ polymorphism and the risk of gastric cancer. In the meta-analysis, pooled data from 13 studies confirmed that the $CYP2E1$ $PstI/RsaI$ polymorphism was associated with a significantly increased risk of gastric cancer.

**Conclusion:** $CYP2E1$ $PstI/RsaI$ polymorphism is associated with increased risk of development, progression and poor prognosis of gastric cancer in Chinese patients. Pooled data from 13 studies, mainly in Asian countries, are in agreement with our findings.

Background

Gastric cancer, one of the most common cancers and the second leading cause of cancer death worldwide, remains an important public health problem. Studies have shown that 989,600 new gastric cancer cases occurred in 2008 and that 738,000 patients die annually of this disease [1–2]. There is a considerable geographic variation in the gastric cancer incidence, with higher rates in Asia and some parts of South America. Gastric cancer is one of the most prevalent malignant tumors in China, accounting for 38% of worldwide cases every year [3–4]. Although it is well known that environmental factors, dietary habits, and Helicobacter pylori infection are associated with the risk of gastric cancer, the host genetic factor is also believed to be important in gastric carcinogenesis [5]. Genetic susceptibilities could be explained, in part, by single nucleotide polymorphisms (SNPs) of susceptible genes [6–7]. Therefore, determination and understanding of genetic and molecular factors involved in gastric cancer development and prognosis may help identify novel genetic biomarkers and highlight potential avenues of investigation for targeted therapies.

Cytochrome P450 2E1 ($CYP2E1$), which is located on chromosome 10q26.3, is an 11.7 kb gene consisting of 9 exons and 8 introns,
and encoding a 493 amino acid protein [8]. CYP2E1 is an ethanol-inducible enzyme that metabolically activates various carcinogens, such as benzene, vinyl chloride and N-nitrosamines [9-10]. N-nitrosamines are present in tobacco smoke, and activation of N-nitrosamines has been linked to the development of various cancers, including gastric cancer [11-12]. Functional CYP2E1 gene polymorphisms that alter the transcriptional activity of the gene and thus its substances such as N-nitrosamines would influence the susceptibility of cancers. Two genetic polymorphisms in the 5’-flank region (identified by RsaI is -1053C>T (rs2031920) and PstI is -1293G>C (rs6138267), respectively), which are in close linkage disequilibrium, have been reported to alter transcriptional activity of the gene [13-14]. The individuals with predominant homozygous allele (C1/C1), the heterozygote and the rare homozygote, respectively [15-17]. Another important polymorphism detectable with DraI polymorphism with the risk of lung cancer [18], oral cancer [19], and pancreatic cancer [20].

Recently, a few studies on the association between the CYP2E1 polymorphism and gastric cancer have also been published, but those studies have yielded contradictory results [21-33]. Moreover, there has been no report on the association between CYP2E1 polymorphism and survival of patients with gastric cancer. Therefore, the aim of this study was to investigate whether CYP2E1 polymorphism is associated with the development and progression of gastric cancer and its prognosis in Chinese patients. In addition, we also carried out a meta-analysis of selected high quality studies published between 1990 and 2011, in order to reveal more precise association between CYP2E1 polymorphism and gastric cancer.

**Materials and Methods**

**Study Population**

The study included 510 patients who were admitted for gastric cancer treatment to the First Affiliated Hospital of Nanjing Medical University between May 2006 and September 2008 and 510 age- and sex-matched healthy controls. All subjects were unrelated ethnic Han Chinese and residents in Jiangsu Province. All cases were newly diagnosed and histologically confirmed without previous chemotherapy or radiotherapy. The pathological stage of gastric cancer was classified according to the tumor-lymph node-metastasis (TNM) classification system into stage I (T1-T2N0M0), stage II (T1-T2N1M0 or T3N0M0), stage III (T3N1M0, T1-T3N2M0, TanyN3M0, or T4NanyM0), or stage IV (T4NanyM1) [34]. Tumor grade was grouped into low (well differentiated), intermediate (moderately differentiated), or high grade (poor differentiated) according to the World Health Organization (WHO) grade classification [35]. The healthy controls were recruited from individuals living in the same residential areas who took part in routine medical examination at the same hospital with normal findings during the examination and were age- (±5 years) and sex-matched to the cases.

The study was approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University, and the number of the document was 2008(1101). Written informed consent was obtained from all subjects.

**DNA Extraction and Genotyping of CYP2E1**

Whole blood was collected into EDTA-coated tubes and centrifuged for 15 min, and the buffy coat layer was isolated. Genomic DNA was extracted from 200 ml of buffy coat using a Qiagen QIAamp DNA Blood Mini kit (Qiagen Inc., Valencia, CA). Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used for gene analysis. The primer structure and restriction enzyme are shown in Table 1. Genotyping was performed without knowledge of the case/control status. The gel images were read independently by two research assistants. If a consensus was not reached on the tested genotypes, then the genotyping was repeated independently until a consensus was reached. To validate the RFLP method, 100 (50 from cases and 50 from controls) samples were selected randomly for direct sequencing with an ABI PRISM Dye Terminator sequencing Kit (Applied Biosystems, Foster City, Calif) with the samples loaded onto an ABI 3700 sequencer. The concurrence rate of these two methods was 99%.

**Statistical Analysis**

Hardy-Weinberg equilibrium of alleles was assessed by chi-square test. Comparison of age between cases and controls was assessed by the Mann-Whitney U test. The difference in the distribution of genotypes between cases and controls was determined using chi-square test, and the association between the CYP2E1 polymorphisms and gastric cancer risk was estimated by odds ratio (OR) with the 95% confidence interval (CI). Logistic regression was used to control for selected potential confounders (sex, age, and smoking habit) and to estimate crude and adjusted OR and 95% CI. Cumulative overall survival curves were constructed using the method of Kaplan-Meier and the difference was evaluated by the log-rank test. All data analyses were done using SPSS software (version 11.0, Chicago, IL, USA). A P value of <0.05 was considered statistically significant.

**Meta-analysis**

The electronic databases PubMed, Embase and Web of Science, were searched for studies eligible for inclusion in the present meta-analysis, using the terms: “CYP2E1”, “P4502E1”, “polymorphism”, “gastric” and “cancer or carcinoma or tumor or neoplasm”. An upper date limit of December 5, 2011 was applied, while a lower date limit was 1990. All published English language papers with full text matching the eligible criteria were retrieved. The citations in identified articles and in review articles were also examined. When the same patient population was included in more than one publication, only the most recent or most complete one was included in the meta-analysis. Inclusion criteria included: (a), case-control study on the association between the CYP2E1 polymorphism and gastric cancer; (b), English publication; (c), sufficient published data available for estimating an OR with 95% CI; and (d), the genotypes of the controls consistent with the Hardy-Weinberg equilibrium distribution. Information was carefully and independently extracted from all eligible publications by two of the authors according to the inclusion criteria listed above. Disagreement was resolved by discussion between the two authors. If the two authors did not reach a consensus, a third author was consulted and a final decision was made by the majority of the votes. The following data were collected from each study: first author’s name, publication date, ethnicity, study design, pathological types of gastric cancer, the total numbers of cases and controls, and information on CYP2E1 (PstI/RsaI and/or DraI) polymorphisms. Then we used the Review Manager 4.2 software (The Cochrane Collaboration, Oxford, United Kingdom) for meta-analysis.
Characteristics of Study Population
The demographic and pathological characteristics of patients and controls are listed in Table 2. There were no significant differences in demographic data between cases and controls. However, 51.6% of cases were smokers whereas the rate was 44.1% for the controls (OR = 1.55, 95% CI: 1.05–1.73, P = 0.017). Of the patients with gastric cancer, 41 (8.0%), 181 (35.5%), and 160 (31.8%) had low-, intermediate-, and high-grade tumors, respectively, and 56 (11.0%), 116 (22.7%) and 231 (45.3%) had low-, intermediate-, and high-grade tumors, respectively.

Association between CYP2E1 Polymorphisms and Gastric Cancer Risk
The genotype frequencies of the polymorphisms in the controls were consistent with the Hardy-Weinberg equilibrium distribution (P = 0.055 for PstI, and P = 0.056 for DraI). Table 3 shows the frequency distribution of CYP2E1 PstI or DraI genotypes and the estimated ORs (95% CI) for gastric cancer. In CYP2E1 PstI restriction analysis, there was a significant difference in the distribution of genotypes between the case and control groups. Individuals with the C1C2 or C2C2 genotype had a significantly elevated risk of developing gastric cancer compared with the C1C1 homozygotes, with an adjusted OR (95% CI) of 1.35 (1.01–1.80) and 2.15 (1.18–3.94), respectively. Moreover, C2 carriers (C1C2 or C2C2) had an adjusted OR (95% CI) of 1.48 (1.13–1.96), compared with the C1C1 homozygotes. However, in CYP2E1 DraI restriction analysis, there was no significant difference between the case and control group in the distribution of genotypes. The adjusted OR (95% CI) for TA, AA, and A carriers were 0.76 (0.58–1.01), 1.34 (0.83–2.17), and 0.85 (0.65–1.10), respectively, compared with the TT homozygotes. When the CYP2E1 PstI and DraI genotypes were analyzed together, the individuals with C2C2/AA, had a significantly elevated risk of gastric cancer, with an adjusted OR (95% CI) of 2.66 (1.27–5.57), compared with the C1C1/TT genotype (Table 3).

Association between CYP2E1 Polymorphisms and Gastric Cancer Disease Status
In CYP2E1 PstI restriction analysis, both C1C2 and C2C2 genotypes were associated with advanced stage, but not the grade of gastric cancer (Table 4). The frequencies of C2C2 genotype were 21.9%, 24.9%, 31.5%, and 44.0% in stages I, II, III, and IV tumors, respectively, whereas the frequencies of C2C2 genotype were 4.9%, 4.4%, 11.1%, and 24.0%, respectively. C2C2 genotype was associated with the advanced stage of gastric cancer, among all of the three subgroup analyses (i.e. III vs. I; III+ IV vs. I; III+ IV vs. I+ II), and the adjusted ORs (95% CI) were 5.17 (1.05–25.34), 4.80 (1.03–22.45), and 4.38 (1.92–9.97), respectively, compared with the C1C1 homozygotes. In addition, C1C2 genotype was associated with the advanced stage of gastric cancer only in the subgroup analysis comparing stages III+ IV with stages I+ II (adjusted OR = 1.89; CI: 1.18–3.03), compared with the C1C1 homozygotes. However, no association between CYP2E1 RsaI polymorphism and gastric cancer grade was detected (Table 4).

Association between CYP2E1 Polymorphisms and Gastric Cancer Survival
Overall, patients with gastric cancer were followed up for a median (range) of 39 (3–72) months. Kaplan-Meier survival curves (Figure 1A) and log-rank test show that CYP2E1 PstI polymorphism was associated with the poor overall survival of gastric cancer. C1C2 or C2C2 genotype had a markedly poor overall survival, compared with the C1C1 genotype (P<0.001). The median estimated cumulative survival was significantly lower in...
C1C2 or C2C2 carriers (28 months; 95% CI: 22.1–33.9 months, or 23 months; 95% CI: 14.0–32.0 months, respectively), compared with C1C1 carriers (44 months; 95% CI, 38.6–49.4 months). However, the survival was not significantly associated with the CYP2E1 Dra polymorphism (Figure 1B).

### Table 3. Associations between CYP2E1 polymorphisms and gastric cancer risk.

| Polymorphism of CYP2E1 | Case | Control | Crude OR (95% CI) | Adjusted* OR (95% CI) |
|------------------------|------|---------|------------------|----------------------|
|                        | No.  | %      | No.  | %      |                      |                      |
| **Pst genotypes**      |      |         |      |        |                      |                      |
| C1C1                   | 348  | 68.2    | 374  | 73.3    | 1.00                 | 1.00                 |
| C1C2                   | 128  | 25.1    | 119  | 23.3    | 1.31 (0.98–1.74)     | 1.35 (1.01–1.80)     |
| C2C2                   | 34   | 6.7     | 17   | 3.3     | 2.21 (1.21–4.03)     | 2.15 (1.18–3.94)     |
| C1C2+C2C2              | 162  | 31.8    | 136  | 26.7    | 1.42 (1.09–1.86)     | 1.49 (1.13–1.96)     |
| **Dra genotypes**      |      |         |      |        |                      |                      |
| TT                     | 334  | 65.5    | 318  | 62.3    | 1.00                 | 1.00                 |
| TA                     | 131  | 25.7    | 160  | 31.4    | 0.78 (0.59–1.03)     | 0.76 (0.58–1.01)     |
| AA                     | 45   | 8.8     | 32   | 6.3     | 1.33 (0.83–2.16)     | 1.34 (0.83–2.17)     |
| TA+AA                  | 176  | 34.5    | 192  | 37.6    | 0.86 (0.66–1.11)     | 0.85 (0.65–1.10)     |
| **Pst and Dra genotypes** |   |         |      |        |                      |                      |
| C1C1/TT                | 212  | 41.6    | 233  | 45.7    | 1.00                 | 1.00                 |
| C1C2/TA                | 83   | 16.3    | 77   | 15.1    | 1.20 (0.83–1.72)     | 1.13 (0.79–1.63)     |
| C2C2/AA                | 26   | 5.1     | 11   | 2.2     | 2.62 (1.26–5.44)     | 2.66 (1.27–5.57)     |

*Adjusted for sex, age and smoking habit.

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### Table 4. Association with CYP2E1 Rsal/Pstl polymorphism and progression of gastric cancer.

| Total | C1C1 | C1C2 | C2C2 |
|-------|------|------|------|
| No.   | No.  | No.  | No.  |
|       | %    | %    | %    |
| Tumor stage |
| I     | 41   | 30   | 73.2 | 9    | 21.9 | 4.9 |
| II    | 181  | 128  | 70.7 | 45   | 24.9 | 8   |
| III   | 162  | 93   | 57.4 | 51   | 31.5 | 13  |
| IV    | 25   | 8    | 32.0 | 11   | 44.0 | 24  |
| Adjusted* OR (95% CI) |
| III vs. I | 1.00 | 2.29 (0.94–5.58) | 5.17 (1.05–25.54) |
| III+ IV vs. I | 1.00 | 2.30 (0.98–5.38) | 4.80 (1.03–22.45) |
| III+ IV vs. II | 1.00 | 1.89 (1.18–3.03) | 4.38 (1.92–9.97) |
| Tumor grade |
| Low   | 56   | 39   | 69.6 | 13   | 23.2 | 4   |
| Intermediate | 116  | 79   | 68.1 | 20   | 17.2 | 17  |
| High  | 231  | 152  | 65.8 | 67   | 20.3 | 12  |
| Adjusted* OR (95% CI) |
| Intermediate vs. Low | 1.00 | 0.59 (0.26–1.35) | 1.88 (0.58–6.14) |
| High vs. Low          | 1.00 | 0.50 (0.20–1.26) | 0.65 (0.19–2.24) |

*Adjusted for sex, age and smoking habit.

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### Table 5. Association with CYP2E1 Dra polymorphism and progression of gastric cancer.

| Total | TT | TA | AA |
|-------|----|----|----|
| No.   | No. | No. | No. |
|       | %   | %   | %   |
| Tumor stage |
| I     | 41  | 25  | 61.0 | 12   | 29.3 | 4   |
| II    | 181 | 122 | 67.4 | 38   | 21.0 | 12  |
| III   | 162 | 98  | 60.5 | 52   | 32.1 | 12  |
| IV    | 25  | 15  | 60.0 | 7    | 28   | 3   |
| Adjusted* OR (95% CI) |
| III vs. I | 1.00 | 1.00 (0.45–2.20) | 0.90 (0.25–3.17) |
| III+ IV vs. I | 1.00 | 0.98 (0.45–2.14) | 0.93 (0.28–3.11) |
| III+ IV vs. II | 1.00 | 1.37 (0.86–2.19) | 0.75 (0.37–1.52) |
| Tumor grade |
| Low   | 56  | 39  | 69.6 | 11   | 19.7 | 6   |
| Intermediate | 116  | 75  | 64.7 | 31   | 26.7 | 10  |
| High  | 231 | 171 | 74.0 | 34   | 14.7 | 26  |
| Adjusted* OR (95% CI) |
| Intermediate vs. Low | 1.00 | 1.39 (0.62–3.09) | 1.02 (0.33–3.12) |
| High vs. Low          | 1.00 | 0.71 (0.33–1.54) | 1.08 (0.41–2.81) |

*Adjusted for sex, age and smoking habit.

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Meta-Analysis on the Association between CYP2E1 Polymorphisms and Gastric Cancer Risk

A total of 13 publications met the inclusion criteria [21–33]. Of these studies, one study [33] was excluded because the same data was available in a later publication [27]. With the pooled data from those previous studies and our current investigation, this meta-analysis included 2937 cases and 3602 controls. The characteristics of these studies are provided in Table 6. There...
was a statistically significant association between C2C2 genotype (OR = 1.73; 95% CI: 1.26–2.38; \( P = 0.0008 \)) and gastric cancer risk (Figure 2A). However, there was no significant association between C2 carriers and gastric cancer risk. The pooled ORs for C2 carriers were 1.01 (95% CI: 0.80–1.28; \( P = 0.93 \)), compared with the homozygous wild-type genotype (C1/C1) (Figure 2B). Prior to the present study, there were only two previous studies evaluating the association between \textit{CYP2E1} \textit{DraI} polymorphism and gastric cancer risk [21,29]. Because the samples of these studies were too small to generate a sufficient power, we did not conduct the meta-analysis on this polymorphism. Nevertheless, all the three studies reported that there was no significant association between \textit{DraI} polymorphism and gastric cancer risk.

**Discussion**

In the present case-control study, for \textit{CYP2E1} \textit{PstI} polymorphism, we observed that both C2 carriers and C2C2 genotypes were significantly associated with gastric cancer risk and poor clinical prognosis. However, we did not find any significant association between \textit{CYP2E1} \textit{DraI} polymorphism and both gastric cancer risk and clinical prognosis. In addition, our meta-analysis also confirmed that the \textit{CYP2E1} \textit{PstI} polymorphism, but not \textit{DraI}, was associated significantly with the risk of gastric cancer, which provided further evidence indicating an association between this functional polymorphism and gastric cancer susceptibility.

Gastric cancer is a multistep process in which genetic and environmental factors interact in the development of cancer. Interindividual genetic differences in susceptibility to chemical carcinogens are among the most important host factors in human cancer [36–38]. It has been proposed that various host factors affect susceptibility to cancer, even following the same exposure to environmental carcinogenic factors [39–41]. Of special interest is \textit{CYP2E1} whose polymorphisms are related to substantial interindividual variation in metabolizing carcinogens and cancer risks [42–44]. Our results indicating the association between the

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**Figure 1.** Kaplan-Meier survival curves for gastric cancer patients with \textit{CYP2E1} \textit{PstI}/\textit{RsaI} (A) and \textit{DraI} (B) polymorphisms. (A), C1C2 or C2C2 genotype had a markedly poor overall survival, compared with C1C1 genotype (\( P < 0.001 \)); (B), The survival was not significantly associated with the \textit{CYP2E1} \textit{DraI} polymorphism. doi:10.1371/journal.pone.0044478.g001
CYP2E1 polymorphism and the risk of gastric cancer are biologically plausible. CYP2E1 catalyzes oxidation and DNA adduct formation of some low-molecular carcinogens of gastric cancer [45–46]. It has been revealed that the PstI and RsaI restriction sites are in the transcription-regulation region of CYP2E1 [14]. A tenfold increase in gene expression of the homozygous C2/C2 genotype of CYP2E1 using PstI or RsaI digestion has been reported [14]. In contrast, the polymorphism detected by DraI digestion of CYP2E1 is located in intron 6, and no functional significance of this polymorphism exists. This may explain why the PstI/RsaI polymorphism of CYP2E1 conferred a greater risk of gastric cancer in this study. Our meta-analysis provided further evidence indicating an association between CYP2E1 PstI/RsaI polymorphism and gastric cancer susceptibility. It is agreed with the previous meta-analysis in 2007 [47], which reported that CYP2E1 PstI/RsaI polymorphism may be a risk factor for gastric cancer in Asians. Another interesting finding in the present study was the association of the CYP2E1 PstI/RsaI polymorphism with gastric cancer stage, which may mirror the substantial role of this polymorphism in the progression. We also reported, for the first time, that this polymorphism affected gastric cancer survival. Recently, studies have demonstrated that CYP2E1 plays an important role in tumor progression, and may be used as a prognostic indicator. For instance, Vaclavikova et al. observed that increased CYP2E1 expression was associated with an invasive breast cancer, and suggested its potential role as a breast cancer prognosis marker [48]. Tsunedomi et al. also found that the expression of CYP2E1 was associated with the progression of hepatitis C virus-associated hepatocellular carcinoma [49]. In addition, CYP2E1 positivity is closely correlated with a poor survival of patients with non-small cell lung carcinoma, and the expression of CYP2E1 in bronchial epithelium has a prognostic potential [50]. Conversely, an animal study demonstrated that blockade of cytochrome P450 significantly reduced capillary formation and tumor size in glial tumors formed by injection of rat glioma 2 (RG2) cells, and also resulted in an increased animal survival time [51]. Furthermore, epidemiologic studies have also indicated that the CYP2E1 PstI/RsaI polymorphism is associated with cancer progression and prognosis [52,53]. CYP2E1 C2C2 genotype is significantly associated with advanced clinical stages, and also associated with tumor recurrence, since it is important for determining the parameters associated with tumor progression and poor outcomes in patients with head and neck squamous cell carcinoma [52]. Haque AK et al. observed that CYP2E1 wild-type allele was significantly associated with better survival of non-small cell lung carcinoma and the expression of p53 [53]. However, it remains unclear whether CYP2E1 PstI/RsaI polymorphism is associated with the differentiation of cancer. In our study, we found there was no significant association with the differentiation of gastric cancer.
Table 6. Characteristics of gastric cancer case-control studies included in meta-analysis on the association between CYP2E1 polymorphisms and gastric cancer.

| Study               | Year | Population | Case Control | Case Control |
|---------------------|------|------------|--------------|--------------|
| Park et al          | 2003 | Koreans   | 80           | 33           | 7            | 94           | 48           | 3            |
| Cai et al           | 2001 | Chinese    | 58           | 27           | 6            | 71           | 22           | 1            |
| Nishimoto et al     | 2000 | Japanese  | 209          | 38           | 1            | 241          | 75           | 8            |
| Tsukino et al       | 2002 | Chinese    | 71           | 42           | 7            | 88           | 58           | 12           |
| Gao et al           | 2002 | Chinese    | 58           | 31           | 9            | 121          | 62           | 13           |
| Colombo et al       | 2004 | Brazilian  | 89           | 11           | 0            | 134          | 16           | 0            |
| Nan et al           | 2005 | Korean     | 268          | 148          | 348          | 121          | 41           |
| Suzuki et al        | 2004 | Japanese  | 107          | 38           | 112          | 65           |
| Wu et al            | 2002 | Chinese    | 215          | 108          | 33           | 199          | 70           | 9            |
| Cai et al           | 2005 | Chinese    | 96           | 57           | 6            | 77           | 110          | 5            |
| Malik et al         | 2009 | Indian     | 88           | 20           | 0            | 177          | 17           | 1            |
| Kato et al          | 2011 | Japanese  | 280          | 186          | 340          | 213          |
| Current study       |      | Chinese    | 348          | 128          | 34           | 374          | 119          | 17           |
| I restriction analysis |    | TT         | TA           | AA           | TT           | TA           | AA           |
| Park et al          | 2003 | Koreans   | 35           | 7            | 78           | 45           | 8            | 85           |
| Wu et al            | 2002 | Chinese    | 195          | 120          | 41           | 158          | 100          | 20           |
| Current study       |      | Chinese    | 334          | 131          | 45           | 348          | 121          | 41           |

*C1C2>C2C2.
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There were a few limitations in the present study. First, data on the cancer stage and differentiation status were unknown for a few patients, which may bring some bias to the results indicating the association between the polymorphism and cancer status. Second, although we carried out a meta-analysis, in order to further confirm our results, only published studies were included in it. This may have limited the power of the pooled results.

In conclusion, CYP2E1 PstI/RsaI polymorphism is associated with development and progression of gastric cancer and poor prognosis of patients with gastric cancer. However, there was no significant association between CYP2E1 DraI polymorphism and the risk of gastric cancer. Future investigation in this area should aim to elucidate the underlying mechanisms between CYP2E1 PstI/RsaI polymorphism and gastric cancer.

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**Author Contributions**

Conceived and designed the experiments: ZX GZ ZC WER. Performed the experiments: JF XP JY HX. Analyzed the data: JF XP WER ZC. Contributed reagents/materials/analysis tools: GZ. Wrote the paper: JF.
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