Lack of any Association between Insertion/Deletion (I/D) Polymorphisms in the Angiotensin-converting Enzyme Gene and Digestive System Cancer Risk: a Meta-analysis

Jin-Fei Liu1, Hao-Jun Xie2, Tian-Ming Cheng1*

Abstract

Objective: To investigate the association between the gene polymorphisms of angiotensin-converting enzyme (ACE) and digestive system cancer risk. Method: A search was performed in Pubmed, Medline, ISI Web of Science and Chinese Biomedical (CBM) databases, covering all studies until Sep 1st, 2013. Statistical analysis was performed by using Revman5.2 and STATA 12.0. Results: A total of 15 case-control studies comprising 2,390 digestive system cancer patients and 9,706 controls were identified. No significant association was found between the I/D polymorphism and digestive cancer risk (OR =0.93, 95%CI = (0.75, 1.16), \(P=0.53\) for DD+DI vs. II). In the subgroup analysis by ethnicity and cancer type, no significant associations were found for the comparison of DD+DI vs. II. Results from other comparative genetic models also indicated a lack of associations between this polymorphism and digestive system cancer risks. Conclusions: This meta-analysis suggested that the ACE D/I polymorphism might not contribute to the risk of digestive system cancer.

Keywords: ACE - digestive system cancer - meta-analysis - polymorphism

Introduction

The angiotensin-converting enzyme (ACE) is a major component of the renin-angiotensin system (RAS) and plays a crucial role in the regulation of circulatory homeostasis. Much evidence indicates that ACE associated with the pathology of carcinoma (Abali et al., 2002, Bauvois et al., 2004). ACE is differentially expressed in several malignancies (Bauvois et al., 2004) and influences tumor cell proliferation, tumor cell migration, angiogenesis, and metastatic behavior (Abali et al., 2002; Yoshiji et al., 2002a, b). Epidemiologic studies have also indicated that ACE inhibitors might decrease the risk and mortality rate of cancers (Lever et al., 1998). The human ACE gene is located on chromosome 17q23, and many polymorphisms have been identified (Kitsios and Zintzaras, 2009). The polymorphism is characterized by the presence or absence of a 287-bp Alu repetitive sequence, which results in three genotypes: II, DI and DD (Haiman, 2003). Insertion (I) or deletion (D) polymorphism of ACE gene has functional relevance, since the carriers of D allele have higher ACE activity (Rigat et al., 1990).

Recent years, many studies investigated the role of this polymorphism in the etiology of digestive system cancer (Rocken et al., 2005; Kupcinskas et al., 2011; Yuan et al., 2013). However, the observed associations of these studies were inconsistent, and a single study might be insufficient to detect a possible small effect of the polymorphism on digestive system cancer. We conducted a meta-analysis combining all eligible case-control studies to estimate the association between this polymorphism and digestive system cancer risks.

Materials and Methods

Publication search

We searched literatures in Pubmed database, Web of Science database, Medline database, Chinese Biomedical database(CBM) to identify articles that evaluated the associations between polymorphisms in ACE gene and digestive system cancer risks (Last search was updated on Sep 1st, 2013). The search terms were used as follows: ‘digestive system neoplasms or digestive cancer or biliary tract neoplasms or liver neoplasms or pancreatic neoplasms or esophageal neoplasms or stomach neoplasms or intestinal neoplasms’ and ‘peptidyl-dipeptidase A or angiotensin-converting enzyme or ACE’ in combination with ‘polymorphism, genetic or polymorphism, single nucleotide or variant or mutation’. The languages were limited to English and Chinese. The following inclusion criteria were used in the meta-analysis: (1) the study should evaluate the I/D polymorphism in ACE gene and digestive system cancer risk, (2) the study should be a case-control study.
design, (3) enough information had to be provided to calculate the odds ratio (OR) with 95% confidence interval (CI), (4) the distribution of genotypes in the control groups should be consistent with Hardy-Weinberg equilibrium (HWE). Accordingly, the following exclusion criteria were also used: (1) abstracts and reviews, (2) studies in which the genotype frequencies were not reported, (3) repeated or overlapped publications, (4) animal studies.

Data extraction
Data were independently checked and extracted by two investigators. The following items were collected from each study: first author’s name, year of publication, country of origin, ethnicity, genotyping methods, cancer type, total number of cases and controls, genotype distributions in cases and controls.

Statistical analysis
For each case-control study, the HWE of genotypes in the control group was assessed by using Pearson’s $X^2$ test. Combined ORs for the association between ACE with digestive system cancer were generated using additive, dominant and recessive inheritance. The significance of the pooled OR was determined by the Z-test and $P$ value less than 0.05 was considered as statistically significant. Heterogeneity among studies was assessed by a $X^2$ based Q- and I² - statistic. Heterogeneity was considered significant for $P$ value less than 0.10. When the $P$ value of heterogeneity was greater than 0.10, the fixed-effects model was used, otherwise, the random-effects model was used. To evaluate the ethnicity-specific, cancer type-specific effects, subgroup analyses were performed by ethnic group (‘Caucasian’ and ‘Asian’) and cancer types. Publication bias was assessed by using Begg’s funnel plots and Egger’s test. Sensitivity analysis was performed to assess the stability of the results by sequentially excluding each study (Zhang et al., 2011). All analyses were performed using the software Revman5.2 and STATA 12.0.

Results

Studies selection and characteristics in the meta-analysis
There were 61 results relevant to the search words in the selected databases (Figure 1). After reading the titles and abstracts, 36 potential articles were included for full-text view. Further screening of these articles, 18 of them were excluded for being not relevant to cancer risk with ACE gene polymorphism and not healthy controls. Thus, 18 articles were left for data extraction. 2 case-control studies were excluded for the genotypes in control group not consistent with HWE (Srivastava et al., 2010; Su et al., 2013), and 1 case-control study was excluded for data duplicated (Zhou et al., 2010). Thus, a total of 15 case-control studies in 15 articles were finally identified. The characteristics of included case-control studies are summarized in Table 1. Genotype and allele distributions for each case-control study are shown in Table 2. There were 7 studies of Asians (Goto et al., 2005; Sugimoto et al., 2006; Hou et al., 2010; Hibi et al., 2011; Liu et al., 2011; Ji et al., 2012; Yuan et al., 2013), 8 of Europeans (Ebert et al., 2005; Rocken et al., 2005; Nikiteas et al., 2007; Rocken et al., 2007; van der Knaap et al., 2008; Toma et al., 2009; Lukic et al., 2011). In this meta-analysis, the most studied cancers were gastric cancer and colorectal cancer, the genotype methods are a classic PCR assays.

Meta-analysis results
As shown in Figure 2, heterogeneity of DD+DI vs. II for all studies was analyzed and the value of $X^2$ was 39.8 with 24 degrees of freedom and $P$ value less than 0.01 in a random-effects model. Additionally, I-square value is another
Table 2. Distribution of ACE Genotype and Allele among Gastrointestinal Cancer Patients and Controls

<table>
<thead>
<tr>
<th>Study</th>
<th>Case</th>
<th>Control</th>
<th>Case</th>
<th>Control</th>
<th>HWE for control group</th>
</tr>
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<tr>
<td></td>
<td>II</td>
<td>ID</td>
<td>DD</td>
<td>II</td>
<td>ID</td>
</tr>
<tr>
<td>Rocken et al., 2005</td>
<td>24</td>
<td>57</td>
<td>32</td>
<td>41</td>
<td>95</td>
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<tr>
<td>Ebert et al., 2005</td>
<td>6</td>
<td>46</td>
<td>36</td>
<td>33</td>
<td>72</td>
</tr>
<tr>
<td>Goto et al., 2005</td>
<td>76</td>
<td>98</td>
<td>28</td>
<td>209</td>
<td>189</td>
</tr>
<tr>
<td>Sugimoto et al., 2006</td>
<td>54</td>
<td>53</td>
<td>12</td>
<td>50</td>
<td>60</td>
</tr>
<tr>
<td>Rocken et al., 2007</td>
<td>37</td>
<td>69</td>
<td>35</td>
<td>41</td>
<td>95</td>
</tr>
<tr>
<td>Nikiteas et al., 2007</td>
<td>15</td>
<td>27</td>
<td>50</td>
<td>6</td>
<td>44</td>
</tr>
<tr>
<td>Vander et al., 2008</td>
<td>34</td>
<td>97</td>
<td>45</td>
<td>1332</td>
<td>3006</td>
</tr>
<tr>
<td>Toma et al., 2009</td>
<td>25</td>
<td>50</td>
<td>33</td>
<td>30</td>
<td>73</td>
</tr>
<tr>
<td>Hou et al., 2010</td>
<td>17</td>
<td>11</td>
<td>2</td>
<td>9</td>
<td>16</td>
</tr>
<tr>
<td>Liu et al., 2011</td>
<td>71</td>
<td>138</td>
<td>32</td>
<td>95</td>
<td>158</td>
</tr>
<tr>
<td>Kupcinis et al., 2011</td>
<td>27</td>
<td>59</td>
<td>28</td>
<td>62</td>
<td>110</td>
</tr>
<tr>
<td>Lukic et al., 2011</td>
<td>24</td>
<td>17</td>
<td>4</td>
<td>30</td>
<td>72</td>
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<tr>
<td>Hibi et al., 2011</td>
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<td>26</td>
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<tr>
<td>Yuan et al., 2013</td>
<td>59</td>
<td>214</td>
<td>16</td>
<td>84</td>
<td>211</td>
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Table 3. Summary of Different Comparative Results

<table>
<thead>
<tr>
<th>Variables</th>
<th>N</th>
<th>Cases/Controls</th>
<th>DD+DI vs. II</th>
<th>DD vs. DI+II</th>
<th>DD vs. II</th>
<th>D vs. I</th>
<th>DI vs. II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(OR (95%CI))</td>
<td>(OR (95%CI))</td>
<td>(OR (95%CI))</td>
<td>(OR (95%CI))</td>
<td>(OR (95%CI))</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>2390/9706</td>
<td>0.93</td>
<td>0.53</td>
<td>0.84</td>
<td>0.4</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.75,1.16)</td>
<td>(0.67,1.17)</td>
<td>(0.60,1.16)</td>
<td>(0.60,1.11)</td>
<td>(0.60,1.11)</td>
</tr>
<tr>
<td>Subgroup by ethnicity</td>
<td>7</td>
<td>1513/2550</td>
<td>1.03</td>
<td>0.79</td>
<td>0.8</td>
<td>0.46</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.84,1.25)</td>
<td>(0.43,1.46)</td>
<td>(0.46,1.33)</td>
<td>(0.77,1.16)</td>
<td>(0.47,1.19)</td>
</tr>
<tr>
<td>Subgroup by cancer type</td>
<td>8</td>
<td>769/7006</td>
<td>0.87</td>
<td>0.54</td>
<td>0.98</td>
<td>0.83</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>(0.57,1.35)</td>
<td>(0.80,1.20)</td>
<td>(0.56,1.38)</td>
<td>(0.77,1.15)</td>
<td>(0.57,1.36)</td>
</tr>
<tr>
<td>Subgroup by ethnicity</td>
<td>6</td>
<td>1218/2359</td>
<td>1.17</td>
<td>0.27</td>
<td>1.08</td>
<td>0.53</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.88,1.56)</td>
<td>(0.85,1.38)</td>
<td>(0.80,1.79)</td>
<td>(0.91,1.29)</td>
<td>(0.90,1.53)</td>
</tr>
<tr>
<td>Subgroup by cancer type</td>
<td>6</td>
<td>788/6785</td>
<td>0.82</td>
<td>0.25</td>
<td>0.9</td>
<td>0.32</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.58,1.15)</td>
<td>(0.73,1.11)</td>
<td>(0.61,1.10)</td>
<td>(0.82,1.06)</td>
<td>(0.31,1.03)</td>
</tr>
</tbody>
</table>

Figure 2. Meta-analysis with a Random-effects Model for the Association Between Digestive System Cancer Risk and the ACE I/D Polymorphism (DD+DI vs II)

Figure 3. Meta-analysis with a Random-effects Model for the Association Between Digestive System Cancer Risk and the ACE I/D Polymorphism (DD+DI vs II): Subgroup Analysis by Ethnicity

index of the test of heterogeneity. In Figure 2, the I-square was 65%, suggesting the presence of heterogeneity. Thus, the random-effects model was chosen to synthesize the data. OR was 0.93 (95%CI = 0.75-1.16) and the test for overall effect Z value was 0.62 (P=0.53). The results suggested that the variant D allele carriers (DI+DD) do not have a significant increased risk of digestive system cancer compared with those individuals without D allele (II). Summary of the results of other genetic comparisons are listed in Table 3.

Subgroup analyses were performed after stratifications of the data by ethnicity and cancer types. In the subgroup analysis by ethnicity (Figure 3), no significant increased risks were found in European and Asians. In the subgroup analysis by cancer types (Figure 4), no significant increased risks were found in colorectal cancer and gastric cancer.

Publication bias
Begg’s funnel plot and Egger’s test were performed to assess the publication bias of the literatures. The shape...
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Selection of Studies on the ACE I/D Polymorphism

Figure 5. Begg’s Funnel Plot for Publication Bias in Subgroup Analysis by Cancer Types

Risk and the ACE I/D Polymorphism (DD+DI vs II):

Figure 4. Meta-analysis with a Random-effects Model for the Association Between Digestive System Cancer

between this polymorphism and digestive system cancer studies have been published to investigate the associations about the digestive system tumors. Recently several new association between the ACE polymorphism and cancer recently conducted a meta-analysis and found there no risk (Zhang et al., 2011), but there were a few studies to a change in the plasma ACE level. Zhang et al. have

Discussion

The angiotensin-converting enzyme (ACE) is a major component of the renin-angiotensin system (RAS) and plays a crucial role in the regulation of circulatory homeostasis. Much evidence indicates that ACE associated with the pathology of carcinomas. ACE is differentially expressed in several malignancies and influences tumor cell proliferation, tumor cell migration, angiogenesis, and metastatic behavior. This polymorphism is based on insertion or deletion of a 287-bp Alu sequence, leading to a change in the plasma ACE level. Zhang et al. have recently conducted a meta-analysis and found there no association between the ACE polymorphism and cancer risk (Zhang et al., 2011), but there were a few studies about the digestive system tumors. Recently several new studies have been published to investigate the associations between this polymorphism and digestive system cancer risks; the results were inconsistent and conflict. In order to resolve this issue, we conducted a meta-analysis of 15 case-control studies, including 2390 cases and 9706 controls, to evaluate the associations between the ACE I/D polymorphism and digestive system cancer risks.

Our results indicated that the ACE I/D polymorphism was not associated with digestive system cancer risks. Taking into account the property of genetic background, we conducted subgroup analysis by ethnicity. This meta-analysis included two subgroups: ‘Caucasian’ and ‘Asian’. In this meta-analysis, there no significant association between this polymorphism and digestive system cancer risk in any sub-populations. Interestingly, this polymorphism and digestive system cancer risks in Asians and Caucasian were inversely related, although they were not statistically significant. These results may indicate that this polymorphism may produce different effects in different populations. Further studies are needed to validate these findings for different ethnic groups. In another subgroup analysis by digestive system cancer types, we found that this polymorphism was not related to increased risks in both sub-cancer types (gastric and colorectal).

Taking into account the limitation of studies in each subgroup, these null associations may be due to chance because studies with small sample size may have insufficient statistical power to detect a slight effect. Additional, cancer is multifactorial disease with complex etiology, for which interplay of various genetic and non-genetic factors is characteristic. Lukic et al. found that ACE I/D polymorphism may play a role in the development of pancreatic cancer through interaction with other genetic and environmental factors (Lukic et al., 2011), so cofounding factors should be considered in future studies. ACE may not affect the incidence of digestive system cancer, however, it may affect the progression of digestive system cancer. Rocken et al. have found that the gene polymorphism influence the metastatic behavior of gastric cancer (Rocken et al., 2005). Thus, future studies are warranted to identify the associations between ACE polymorphism and the risk of progression and metastasis of digestive system cancer.

Heterogeneity is one of the important issues when performing meta-analysis. The heterogeneity between studies existed in overall comparisons. After subgroup analysis by ethnicity and cancer types, we found that the heterogeneity was effectively decreased or removed in Asians, gastric cancer and colorectal cancer subgroup, suggesting that certain effects of genetic variants are cancer specific and ethnic specific. In addition, in the sensitivity analysis which sequentially excluding individual studies, statistically similar results were obtained. Furthermore, we did not detect a publication bias by Egger’s funnel plots and Begg’s test in the present study. All these indicated the stability and reliability of the meta-analysis results in our study.

This study has several limitations. First, only published studies in Chinese and English which were included by the selected databases were included for data analysis, some potential studies could be missed. Second, due to lack of original data, we could not evaluate the potential
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interactions of gene-gene and gene-environment, so the calculated ORs and P values may have deviations from the true biological situation. Third, this meta-analysis included data only from Europeans, and Asians. Fourth, Meyer and Vashishtha have reported that PCR amplification of ACE I/D polymorphism using only flanking primer pairs would misclassify 4-5% of the ID genotype as the DD genotype and a second PCR should performed to confirm the DD genotype (Meyer and Vashishtha, 1995). However, only a small portion of included studies performed a second PCR, indicating the possibility of imprecise results of the meta-analysis. Fifth, in the subgroup analysis by ethnicity and cancer type, the numbers of studies analyzed were small, and the statistical power was low that caution should be taken in interpreting these results.

In conclusion, these results suggest that there is no significant association between the D/I polymorphism of ACE gene and digestive system cancer risk.

Acknowledgements

The authors declare that they have no competing interests.

References