



Quantitative assessment of the association between the angiotensin-converting enzyme gene insertion/deletion polymorphism and digestive system cancer risk

J. Wang*, S. Yang*, F.H. Guo, X. Mao, H. Zhou, Y.Q. Dong, Z.M. Wang and F. Luo

Department of General Surgery, HuaShan Hospital,
Fudan University School of Medicine, Shanghai, China

*These authors contributed equally to this study.

Corresponding authors: Z.M. Wang / F. Luo

E-mail: wang19701010@126.com / luofen0@126.com

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ABSTRACT. The angiotensin-converting enzyme (*ACE*) gene insertion/deletion (*I/D*) polymorphism has been reported to be associated with digestive system cancer; however, the results from previous studies have been conflicting. The present study aimed to investigate the association between the *ACE I/D* polymorphism and the risk of digestive system cancer using a meta-analysis of previously published studies. Databases were systematically searched to identify relevant studies published prior to December 2014. We estimated the pooled OR with its 95%CI to assess the association. The meta-analysis consisted of thirteen case-control studies that included 2557 patients and 4356 healthy controls. Meta-analysis results based on all the studies showed no significant association between the *ACE I/D* polymorphism and the risk of digestive system cancer (DD vs II: OR = 0.85, 95%CI = 0.59-1.24; DI vs II: OR = 0.94, 95%CI = 0.78-1.15; dominant model: OR = 0.96, 95%CI = 0.81-

1.15; recessive model: OR = 1.06, 95%CI = 0.76-1.48). Subgroup analyses by race and cancer type did not detect an association between the *ACE* I/D polymorphism and digestive system cancer risk. However, when the analyses were restricted to smaller studies (N < 500 patients), the summary OR of DI vs II was 0.80 (95%CI = 0.66-0.97). Our analyses detected a possibility of publication bias with a misestimate of the true association by smaller studies. Overall, meta-analysis results suggest the *ACE* I/D polymorphism might not be associated with susceptibility to digestive system cancer. Further large and well-designed studies are needed to confirm these conclusions.

Key words: ACE; I/D polymorphism; Digestive system cancer; Genetic polymorphism

INTRODUCTION

Digestive system cancers are very common worldwide and are major causes of cancer-related deaths globally (Parkin et al., 2005). These malignancies often exhibit aggressive clinical behavior characterized by local invasiveness and early metastasis. Despite much investigation, the exact mechanism of carcinogenesis is not yet fully elucidated. Inflammation, smoking, alcohol, and low-nutrition intake are known to be the main exogenous contributors to the risk of digestive system cancer (Zhao et al., 2014). Although these factors have been documented to influence the risk of digestive system cancer, not all individuals with these features develop the disease, even though they are exposed in the same environment as those that do. This indicates that other factors might contribute to digestive system cancer pathogenesis. With the developments in molecular biology, researchers have provided strong evidence that genetic factors are important in the pathogenesis of cancer (Thompson et al., 2009; Fujimori et al., 2012).

The renin-angiotensin system (RAS) has been implicated in the regulation of blood pressure and cardiovascular homeostasis (van der Knapp et al., 2008). Angiotensin II, the main biologically active peptide of the RAS, is converted from angiotensin I via the angiotensin-converting enzyme (ACE). ACE is differentially expressed in several carcinomas and might affect tumor cell proliferation, migration, and angiogenesis (Röcken et al., 2005). The *ACE* gene is located on 17q23 in humans (Jeunemaitre et al., 1992). There is a common insertion/deletion (I/D) polymorphism in this gene characterized by the presence or absence of a 287-bp Alu repetitive sequence in intron 16 (Castellon and Hamdi, 2007). Homozygotes for the D allele have the highest ACE plasma levels, homozygotes for the I allele have the lowest, and ID heterozygotes have intermediate levels (Rigat et al., 1990).

To date, many studies have shown that the *ACE* I/D polymorphism might be closely associated with the risk of cancers, including lung and breast cancer (Cheon et al., 2000; Namazi et al., 2010). In addition, several studies have found an association between the *ACE* I/D polymorphism and digestive system cancer risk. However, the published results remain controversial, possibly due to small sample sizes, low statistical power, and clinical heterogeneity (Ammar et al., 2012). In the present study, therefore, we performed a meta-analysis to examine whether the *ACE* I/D polymorphism was associated with the risk of digestive system cancer based on all eligible published case-control studies.

MATERIAL AND METHODS

Study selection

A computerized literature search was carried out in the US National Library of Medicine PubMed database, along with ISI Web of Knowledge, Medline, Embase, and Google Scholar Search databases (updated to December 2014) by two investigators independently to collect articles on case-control studies examining the association of the *ACE* I/D polymorphism with digestive system cancer. References of the retrieved articles were also screened for original studies (J.W. and S.Y.). The following search terms were used: “ACE”, “digestive system cancer”, “polymorphism”, “single nucleotide polymorphism”, and “genetic polymorphism”. The reference lists of major textbooks, reviews, and included articles were identified through manual searches to find other potentially eligible studies.

Inclusion and exclusion criteria

Studies included in this meta-analysis met the following criteria: 1) case-control studies that addressed patients with digestive system cancer and healthy controls; 2) studies on the association of the *ACE* I/D polymorphism and susceptibility to digestive system cancer; and 3) studies that included sufficient genotype data for extraction. Studies were excluded if they were: 1) not a case-control study; 2) duplicates of previous publications; 3) based on incomplete data; or 4) meta-analyses, case reports, letters, reviews, or editorial articles.

Quality assessment

The quality of these studies was also evaluated independently by the same two investigators according to the predefined quality assessment rules shown in Table 1 (Jiang et al., 2012). The criteria covered the representativeness of cases, source of controls, ascertainment of digestive system cancer, total sample size, quality control of genotyping methods, and Hardy-Weinberg equilibrium (HWE) in the control population. Disagreements were resolved by consensus. The total score ranged from 0 (worst) to 15 (best). Papers scoring <10 were classified as “low quality” and those scoring ≥ 10 as “high quality”.

Data extraction

Information was extracted from all eligible publications independently by 2 investigators according to the inclusion criteria listed above. Disagreements were settled by discussion among all reviewers. The following characteristics were collected from each study: first author, year of publication, country, nationality, numbers of patients and controls, gene polymorphisms, and evidence of HWE.

Statistical analysis

The Fisher exact test (<http://ihg.gsf.de/cgi-bin/hw/hwa1.pl>) was used to test whether the distributions of genotypes among controls was in accord with HWE. The strength of the associations

between the *ACE I/D* polymorphism and the susceptibility to digestive system cancer were estimated by OR and 95%CI under homozygote comparison (DD vs II), heterozygote comparison (DI vs II), dominant (DD+DI vs II), and recessive (II+DI vs DD) models between groups. Heterogeneity was tested by using a Q-test. A fixed effect model was used to pool the data when the P value of the Q-test was ≥ 0.05 ; otherwise, a random effect model was selected. We also performed sensitivity and subgroup analyses to explore the reason of heterogeneity. Subgroup analysis was performed to test the effect of cancer type, country, and sample size. Potential publication bias was evaluated using the funnel plot and the Begg test. Statistical analysis was conducted using the STATA statistical package version 12.0 (STATA, College Station, TX, USA).

Table 1. Scale for quality assessment.

Criteria	Score
Source of patients	
Selected from population or cancer registry	3
Selected from hospital	2
Selected from pathology archives, but without description	1
Not described	0
Source of controls	
Population-based	3
Blood donors or volunteers	2
Hospital-based (cancer-free patients)	1
Not described	0
Specimens from patients used to determine genotypes	
White blood cells or normal tissues	3
Tumor tissues or exfoliated cells of tissue	0
Hardy-Weinberg equilibrium in controls	
Hardy-Weinberg equilibrium	3
Hardy-Weinberg disequilibrium	0
Total sample size	
≥ 1000	3
≥ 500 but < 1000	2
≥ 200 but < 500	1
> 0 but < 200	0

RESULTS

Characteristics of included studies

The literature search identified a total of 71 potentially relevant papers. Of these, 54 were excluded because of obvious irrelevance by reading the titles and abstracts, leaving 17 articles for full publication review. Of these, 4 were excluded for a variety of reasons. Finally, a total of 13 studies were included in our meta-analysis, which included 2557 patients with cancer and 4356 controls (Goto et al., 2005; Sugimoto et al., 2006; Nikiteas et al., 2007; Röcken et al., 2005, 2007; Toma et al., 2009; Vairaktaris et al., 2009; Hibi et al., 2011; Kupcinkas et al., 2011; Liu et al., 2011, 2012; Yuan et al., 2013; Zha et al., 2014). A flow diagram of the study identification and selection is shown in Figure 1. All the studies were published between 2005 and 2014. The genotype distributions among the controls of all studies were consistent with HWE. All included studies were of high quality as the quality score assessment of each one was higher than or equal to 10 points. All the articles were written in English. There were 7 studies of Asians and 6 of Caucasians. The types of cancers in these studies included gastric cancer (N = 5), colorectal cancer (N = 4), oral cancer (N = 2), and hepatocellular carcinoma (HCC, N = 2). The main characteristics of the studies included are summarized in Table 2.

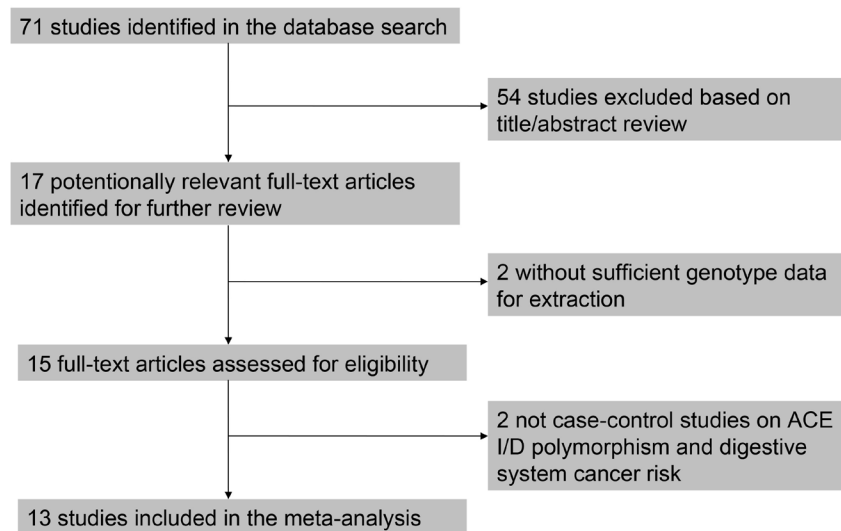


Figure 1. Flow diagram of study identification. ACE I/D = angiotensin-converting enzyme gene insertion/deletion (polymorphism).

Table 2. Characteristics of the studies included for meta-analysis.

Study included	Area	Race	Cancer type	Patients/ Controls	Genotypes for patients			Genotypes for controls			HWE test	Quality scores
					II	ID	DD	II	ID	DD		
Goto (2005)	Japan	Asian	Gastric	202/454	76	98	28	209	189	56	0.20	13
Rocken (2005)	Germany	Caucasian	Gastric	113/189	24	57	32	41	95	53	0.90	10
Sugimoto (2006)	Japan	Asian	Gastric	119/132	54	53	12	50	60	22	0.58	10
Nikiteas (2007)	Greece	Caucasian	Colorectal	92/102	15	27	50	6	44	52	0.40	10
Rocken (2007)	Germany	Caucasian	Colorectal	141/189	37	69	35	41	95	53	0.90	10
Toma (2009)	Romania	Caucasian	Colorectal	108/150	25	50	33	30	73	47	0.86	10
Vairaktaris (2009)	Greece	Caucasian	Oral	160/153	30	70	60	9	66	78	0.30	11
Liu (2011)	China	Asian	Colorectal	241/299	71	138	32	95	158	46	0.14	14
Hibi (2011)	Japan	Asian	Gastric	582/1740	252	255	75	745	791	204	0.79	13
Kupcinskas (2011)	Germany	Caucasian	Gastric	114/238	27	59	28	62	110	66	0.24	11
Liu (2012)	China	Asian	Oral	186/120	78	50	58	57	54	9	0.43	13
Yuan (2013)	China	Asian	HCC	289/384	59	214	16	84	211	89	0.05	11
Zha (2014)	China	Asian	HCC	210/206	50	103	57	57	115	34	0.06	12

HWE = Hardy-Weinberg equilibrium; I = insertion; D = deletion; HCC = hepatocellular carcinoma.

Quantitative synthesis

A summary of the meta-analysis findings of the association between the ACE I/D polymorphism and digestive system cancer risk is shown in Figure 2 and Table 3. The combined results based on all studies showed that the ACE I/D polymorphism was not associated with digestive system cancer risk in the population as a whole (DD vs II: OR = 0.85, 95%CI = 0.59-1.24; DI vs II: OR = 0.94, 95%CI = 0.78-1.15; dominant model: OR = 0.96, 95%CI = 0.81-1.15; recessive model: OR = 1.06, 95%CI = 0.76-1.48). Sensitivity analysis was performed by successively deleting one single study from the overall pooled analysis to check the influence of the removed data. However, the results revealed that no single study changed the between-study heterogeneities (Figure 3).

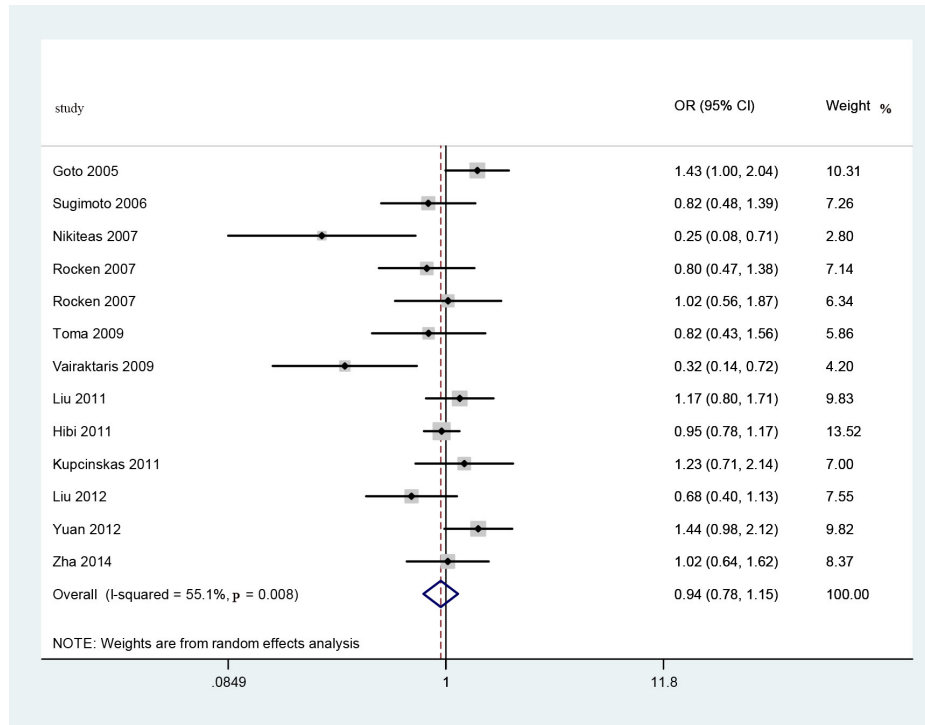


Figure 2. Meta-analysis of the association between the ACE I/D polymorphism and digestive system cancer risk (DI vs II). ACE I/D = angiotensin-converting enzyme gene insertion/deletion.

Table 3. Summary of different comparative results.

Variable	N	Homozygous co-dominant		Heterozygous co-dominant		Dominant model		Recessive model	
		DD vs II	P _{het} ^a	DI vs II	P _{het} ^a	DD+DI vs II	P _{het} ^a	II+DI vs DD	P _{het} ^a
All	13	0.85 (0.59-1.24)	0.000	0.94 (0.78-1.15)	0.008	0.96 (0.81-1.15)	0.015	1.06 (0.76-1.48)	0.000
Ethnicity									
Asian	7	1.07 (0.62-1.85)	0.000	1.02 (0.79-1.32)	0.011	1.08 (0.95-1.22)	0.378	0.98 (0.53-1.81)	0.000
Caucasian	6	0.66 (0.42-1.02)	0.050	0.71 (0.46-1.10)	0.027	0.70 (0.46-1.06)	0.023	1.17 (0.95-1.44)	0.471
Cancer type									
Gastric	5	1.05 (0.84-1.30)	0.364	0.96 (0.68-1.35)	0.019	1.04 (0.90-1.20)	0.242	0.99 (0.81-1.21)	0.477
Colorectal	4	0.77 (0.55-1.07)	0.508	0.78 (0.48-1.26)	0.051	0.88 (0.68-1.13)	0.121	1.08 (0.83-1.39)	0.842
Oral	2	1.05 (0.05-20.10)	0.000	0.50 (0.24-1.03)	0.125	0.60 (0.13-2.71)	0.001	0.57 (0.06-5.40)	0.000
HHC	2	0.70 (0.09-5.05)	0.000	1.26 (0.93-1.69)	0.259	1.15 (0.86-1.52)	0.834	1.65 (0.18-15.50)	0.000
Sample size									
≥500	4	0.80 (0.43-1.47)	0.000	1.12 (0.97-1.29)	0.114	1.08 (0.94-1.24)	0.326	1.28 (0.95-1.56)	0.000
<500	9	0.88 (0.52-1.50)	0.000	0.80 (0.66-0.97)	0.078	0.83 (0.62-1.11)	0.015	0.91 (0.62-1.35)	0.000

HHC = hepatocellular carcinoma; ^aP value of the Q-test for heterogeneity.

Subgroup analysis

Considering the potential impact of confounding factors on the overall results, we further performed subgroup analyses. When stratified according to ethnicity, the ACE I/D polymorphism was not found to be associated with digestive system cancer in Asians or Caucasians (Table

2). When stratified according to cancer type, the *ACE* I/D polymorphism was not found to be associated with gastric cancer, colorectal cancer, oral cancer, or HCC. Further subgroup analysis using sample size also detected no significant association with sample size ≥ 500 . However, when limiting the analysis to the study sample size < 500 , we detected significant association between *ACE* I/D polymorphism and digestive system cancer (DI vs II: OR = 0.80, 95%CI = 0.66-0.97).

Publication bias

A funnel plot and the Begg test were used to assess the publication bias of the sampled studies. The results demonstrated no evidence of publication bias in our study, suggesting that the publication bias was low in the present meta-analysis (all $P > 0.05$).

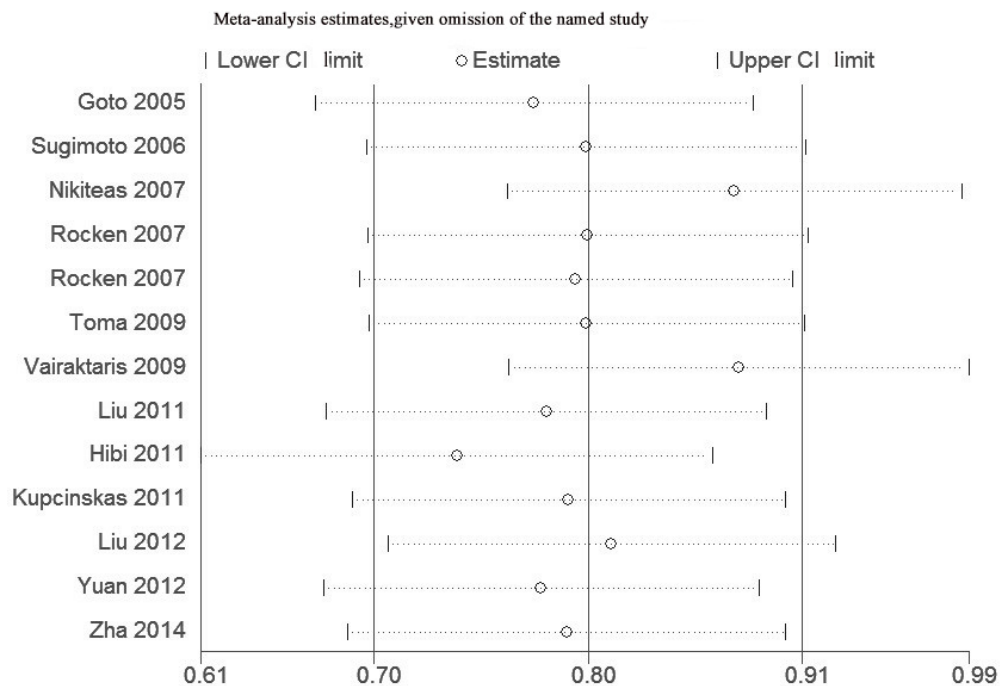


Figure 3. One-way sensitivity analysis of the pooled ORs and 95% CIs for the *ACE* I/D polymorphism, from sequential omission of each dataset in the meta-analysis (DI vs II). *ACE* I/D = angiotensin-converting enzyme gene insertion/deletion.

DISCUSSION

The *ACE* I/D polymorphism has been found to influence serum ACE activity, and DD carriers have increased ACE levels (Rigat et al., 1990). Some studies have shown that the *ACE* I/D polymorphism was associated with several cancer types including lung and breast cancer by influencing tumor cell proliferation, tumor cell migration, and angiogenesis (Haiman et al., 2003). However, to our knowledge, the relationship between the *ACE* I/D polymorphism and the risk of digestive system cancer has not been completely clarified. The most likely reason for the

inconsistencies among these studies is that they are single case-control studies with small sample sizes. Therefore, we conducted this meta-analysis to combine the same type of studies to increase the sample size and statistical power, and thereby obtain a more authentic result.

The present meta-analysis, including 2557 patients and 4356 controls from 13 case-control studies, explored the association between the *ACE I/D* polymorphism and digestive system cancer risk. The results of the present meta-analysis revealed that the *ACE I/D* polymorphism is not associated with increased or decreased risk of digestive system cancer in the overall population. Because of the difference in genetic backgrounds and the environments in which the subjects lived, we performed an ethnicity-specific subgroup analysis, and detected no significant association among Asians or Caucasians, in line with the overall data. Similarly, in the subgroup analysis based on cancer type, significantly increased and decreased cancer risks were not observed for gastric cancer, colorectal cancer, oral cancer, or HCC. However, as the present meta-analysis also involved several studies with a small sample size, there might have been a selective bias for the association between the *ACE I/D* polymorphism and digestive system cancer development. Accordingly, following stratification by sample size (<500), this meta-analysis detected significant association, suggesting a possibility of publication bias in the smaller studies. Therefore, caution should be exercised when considering this conclusion.

The present study has some limitations. First, in this meta-analysis, the primary articles only provided data on Caucasians and Asians. Data regarding other ethnicities, such as Africans, were not available. Second, subgroup analyses on age, gender, smoking, alcohol, and other factors (such as *Helicobacter pylori* infection, an important risk factor for digestive system cancer) were not conducted in the present study due to insufficient relevant data available in the primary studies. Third, the sample sizes for a proportion of studies included were relatively small; the matching criteria for the cases and controls were also not strict. Thus, bias might exist. Further investigations with larger sample sizes and strict matching criteria are needed to address the possible associations. Finally, as only published English studies were included in this study, publication and potential language biases might have occurred.

In conclusion, our meta-analysis indicates that the *ACE I/D* polymorphism is not associated with risk of digestive system cancer. Owing to the above-mentioned limitations, the findings should be verified by further research in the near future.

Conflicts of interest

The authors declare no conflict of interest.

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