RESEARCH ARTICLE

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 Liu[1], Hao-Jun Xie[2], Tian-Ming Cheng[1]*

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

Asian Pac J Cancer Prev, **14 (12)**, 7271-7275

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

[1]*Guangdong Provincial Key Laboratory of Gastroenterology, Department of Gastroenterology,* [2]*Department of Respiratory and Critical Care Medicine, Nanfang Hospital, Southern Medical University, Guangzhou, Guangdong, China *For correspondence: chengtm@fimmu.com*

Table 1. Characteristics of Case-control Studies Included in Meta-analysis

Study	Year	Country	Ethnicity	Cancer type	No. (Cases/Controls)	Genotyping method
Rocken et al., 2005	2005	Germany	Caucasian	Gastric	113/189	PCR
Ebert et al., 2005	2005	Germany	Caucasian	Gastric	88/145	PCR
Goto et al., 2005	2005	Japan	Asian	Gastric	202/454	PCR
Sugimoto et al., 2006	2006	Japan	Asian	Gastric	119/132	PCR
Rocken et al., 2007	2007	Germany	Caucasian	Colorectal	141/189	PCR
Nikiteas et al., 2007	2007	Greece	Caucasian	Colorectal	92/102	PCR
Vander et al., 2008	2008	Netherlands	Caucasian	Colorectal	176/6015	PCR
Toma et al., 2009	2009	Romanian	Caucasian	Colorectal	108/150	PCR
Hou et al., 2010	2010	China	Asian	Colon	30/30	PCR
Liu et al., 2011	2011	China	Asian	Colorectal	241/299	PCR
Kupcinskas et al., 2011	2011	Germany	Caucasian	Gastric	114/238	PCR
Lukic et al., 2011	2011	Serbia	Caucasian	Pancreatic	45/128	PCR
Hibi et al., 2011	2011	Japan	Asian	Gastric	582/1201	PCR
Ji et al., 2012	2012	China	Asian	Esophageal	50/50	PCR
Yuan et al., 2013	2013	China	Asian	Hepatocellular	289/384	PCR

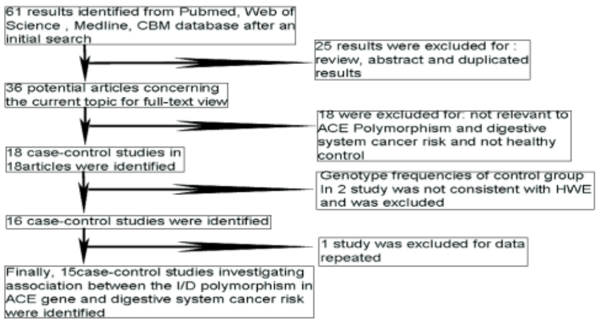

Figure 1. Flow Chart of Selection Process for Eligible Articles

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 Person'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 I^2- 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 control. 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; Kupcinskas et al., 2011; 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 $P<0.01$ 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

Study	Case			Control			Case		Control		HWE for control group	
	II	ID	DD	II	ID	DD	I	D	I	D	X^2	P
Rocken etal.,2005	24	57	32	41	95	53	105	121	177	201	0.02	0.90
Ebert et al., 2005	6	46	36	33	72	40	58	118	138	152	0.00	0.96
Goto et al., 2005	76	98	28	209	189	56	250	154	607	301	1.64	0.20
Sugimoto et al., 2006	54	53	12	50	60	22	161	77	160	104	0.31	0.58
Rocken et al., 2007	37	69	35	41	95	53	143	139	177	201	0.02	0.90
Nikiteas et al., 2007	15	27	50	6	44	52	57	127	56	148	0.70	0.40
Vander et al., 2008	34	97	45	1332	3006	1677	165	187	5670	6360	0.05	0.83
Toma et al., 2009	25	50	33	30	73	47	100	116	133	167	0.03	0.86
Hou et al., 2010	17	11	2	9	16	5	45	15	34	26	0.22	0.64
Liu et al., 2011	71	138	32	95	158	46	280	202	348	250	2.21	0.14
Kupcinskas et al., 2011	27	59	28	62	110	66	113	115	234	242	1.35	0.24
Lukic et al., 2011	24	17	4	30	72	26	65	25	132	124	2.04	0.15
Hibi et al., 2011	252	255	75	530	537	134	759	405	1597	805	0.01	0.91
Ji et al., 2012	17	11	22	15	26	9	45	55	56	44	0.15	0.70
Yuan et al., 2013	59	214	16	84	211	89	332	246	379	389	3.77	>0.05

Table 3. Summary of Different Comparative Results

Varibles	N	Cases/Controls	DD+DI vs. II		DD vs. DI+II		DD vs. II		D vs. I		DI vs. II	
			OR (95%CI)	P*	OR (95%CI)	p*	OR (95%CI)	p*	OR (95%CI)	p*	OR (95%CI)	p*
Total	15	2390/9706	0.93 (0.75,1.16)	0.53	0.89 (0.67,1.17)	0.4	0.84 (0.60,1.16)	0.29	0.94 (0.82,1.08)	0.41	0.82 (0.60,1.11)	0.19
Subgroup by ethnicity												
Asian	7	1513/2550	1.03 (0.84,1.25)	0.79	0.8 (0.43,1.46)	0.46	0.79 (0.46,1.33)	0.37	0.95 (0.77,1.16)	0.59	0.75 (0.47,1.19)	0.22
Caucasian	8	769/7006	0.87 (0.57,1.35)	0.54	0.98 (0.80,1.20)	0.83	0.88 (0.56,1.38)	0.58	0.94 (0.77,1.15)	0.54	0.88 (0.57,1.36)	0.56
Subgroup by cancer type												
gastric	6	1218/2359	1.17 (0.88,1.56)	0.27	1.08 (0.85,1.38)	0.53	1.2 (0.80,1.79)	0.38	1.08 (0.91,1.29)	0.36	1.18 (0.90,1.53)	0.23
colorectal	6	788/6785	0.82 (0.58,1.15)	0.25	0.9 (0.73,1.11)	0.32	0.82 (0.61,1.10)	0.18	0.94 (0.82,1.06)	0.3	0.57 (0.31,1.03)	0.06

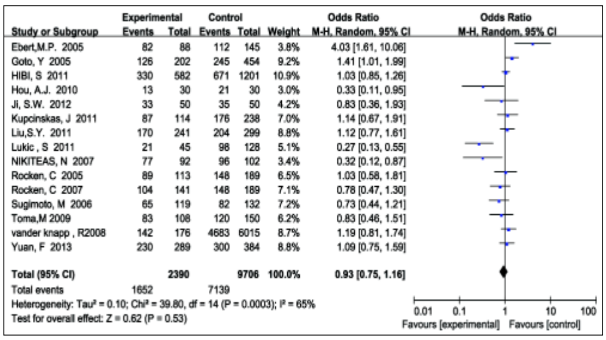

Figure 2. Meta-analysis with a Random-effects Model for the Association Between Digestive System Cancer Risk and the ACE I/D Polymorphism (DD+DI vs II)

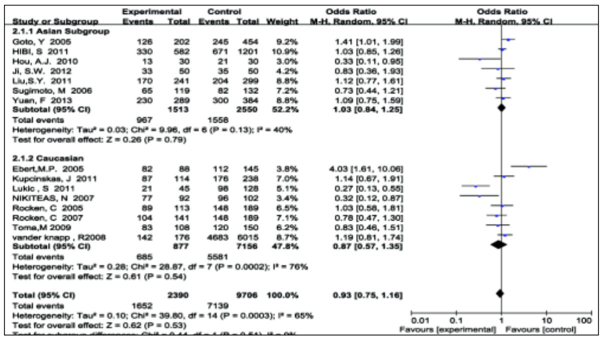

Figure 3. Meta-analysis with a Random-effects Model for the Association Between Digestive System Cancer Risk and the ACE I/D Polymorphism (DD+DI vs II): Subgroup Analysis by Ethnicity

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

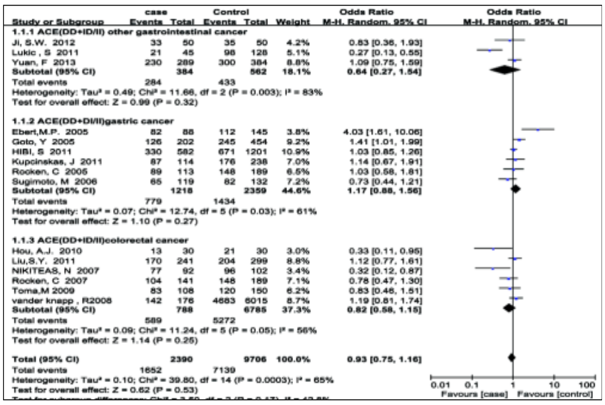

Figure 4. Meta-analysis with a Random-effects Model for the Association Between Digestive System Cancer Risk and the ACE I/D Polymorphism (DD+DI vs II): Subgroup Analysis by Cancer Types

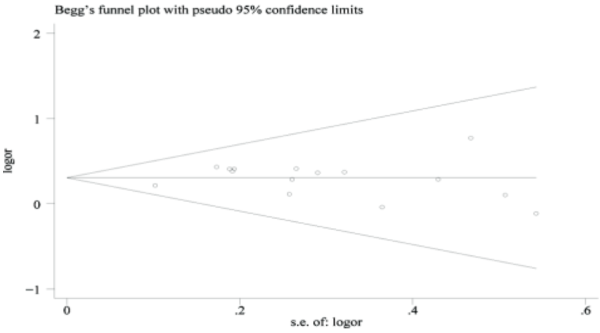

Figure 5. Begg's Funnel Plot for Publication Bias in Selection of Studies on the ACE I/D Polymorphism (DD+DI vs II)

of the funnel plots seemed approximately symmetrical (DD+DI vs. II) and the Egger's test did not show any evidence of publication bias (t =0.11 and P= 0.91 for DD+DI vs. II) (Figure 5).

Sensitivity analysis

Sensitivity analysis was analyzed as previous study (Zhang, 2011). Briefly, after excluding each case-control study for DD+DI vs. II comparison, statistically similar results were obtained, suggesting the results of this meta-analysis are stable.

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. Futhermore, 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 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 caner 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 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

Abali H, Gullu IH, Engin H, et al (2002). Old antihypertensives as novel antineoplastics: angiotensin-I-converting enzyme inhibitors and angiotensin II type 1 receptor antagonists. *Med Hypotheses*, **59**, 344-8.

Bauvois B (2004). Transmembrane proteases in cell growth and invasion: new contributors to angiogenesis? *Oncogene*, **23**, 317-29.

Ebert MP, Lendeckel U, Westphal S, et al (2005). The angiotensin I-converting enzyme gene insertion/deletion polymorphism is linked to early gastric cancer. *Cancer Epidemiol Biomarkers Prev*, **14**, 2987-9.

Goto Y, Ando T, Nishio K, et al (2005). The ACE gene polymorphism is associated with the incidence of gastric cancer among *H. pylori* seropositive subjects with atrophic gastritis. *Asian Pac J Cancer Prev*, **6**, 464-7.

Haiman CA, Henderson SO, Bretsky P, Kolonel LN, Henderson BE (2003). Genetic variation in angiotensin I-converting enzyme (ACE). and breast cancer risk: the multiethnic cohort. *Cancer Res*, **63**, 6984-7.

Hibi S, Goto Y, Ando T, et al (2011). No association between angiotensin I converting enzyme (ACE). I/D polymorphism and gastric cancer risk among Japanese. *Nagoya J Med Sci*, **73**, 169-75.

Hou AJ, Zhou L, Hu Y, et al (2010). The ACE gene polymorphism of angiotensin-converting enzyme in 30 cases of colon cancer patients(chinese). *Chinese J Ethnomed Ethnoph*, 99-100.

Ji SW, Mao XD, Liu JM, et al (2012). The gene polymorphism of angiotensin-converting enzyme in patients with esophageal cancer. *Acta Universitatis Medicinalis Anhui*, **34**, 35-8 (in Chinese).

Kitsios G, Zintzaras E (2009). ACE (I/D). polymorphism and response to treatment in coronary artery disease: a comprehensive database and meta-analysis involving study quality evaluation. *BMC Med Genet*, **10**, 50.

Kupcinskas J, Wex T, Bornschein J, et al (2011). Lack of association between gene polymorphisms of Angiotensin converting enzyme, Nod-like receptor 1, Toll-like receptor 4, FAS/FASL and the presence of Helicobacter pylori-induced premalignant gastric lesions and gastric cancer in Caucasians. *BMC Med Genet*, **12**, 112.

Lever AF, Hole DJ, Gillis CR, et al (1998). Do inhibitors of angiotensin-I-converting enzyme protect against risk of cancer? *Lancet*, **352**, 179-84.

Liu SY, Sima X, Wang CH, Gao M (2011). The association between ACE polymorphism and risk of colorectal cancer in a Chinese population. *Clin Biochem*, **44**, 1223-6.

Lukic S, Nikolic A, Alempijevic T, et al (2011). Angiotensin-converting enzyme gene insertion/deletion polymorphism in patients with chronic pancreatitis and pancreatic cancer. *Dig Surg*, **28**, 258-62.

Meyer BR, Vashishtha A (1995). Angiotensin-converting-enzyme genotype and ischemic heart disease. *N Engl J Med*, **333**, 458-459, 459-460.

Nikiteas N, Tsigris C, Chatzitheofylaktou A, Yannopoulos A (2007). No association with risk for colorectal cancer of the insertion/deletion polymorphism which affects levels of angiotensin-converting enzyme. *In Vivo*, **21**, 1065-8.

Rigat B, Hubert C, Alhenc-Gelas F, et al (1990). An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest*, **86**, 1343-6.

Rocken C, Lendeckel U, Dierkes J, et al (2005). The number of lymph node metastases in gastric cancer correlates with the angiotensin I-converting enzyme gene insertion/deletion polymorphism. *Clin Cancer Res*, **11**, 2526-30.

Rocken C, Neumann K, Carl-McGrath S, et al (2007). The gene polymorphism of the angiotensin I-converting enzyme correlates with tumor size and patient survival in colorectal cancer patients. *Neoplasia*, 9, 716-22.

Srivastava K, Srivastava A, Mittal B (2010). Angiotensin I-converting enzyme insertion/deletion polymorphism and increased risk of gall bladder cancer in women. *Dna Cell Biol*, **29**, 417-22.

Su ZH, Wang RH, Yang SX, et al (2013). Study of ACE gene polymorphism and disease susceptibility in hui-chinese gastric cancer in Qinghai province(chinese). *J Qinghai Med College*, **34**, 1-6.

Sugimoto M, Furuta T, Shirai N, et al (2006). Influences of chymase and angiotensin I-converting enzyme gene polymorphisms on gastric cancer risks in Japan. *Cancer Epidemiol Biomarkers Prev*, **15**, 1929-34.

Toma M, Cimponeriu D, Apostol P, et al (2009). Lack of association between ACE ID polymorphism and colorectal cancer in Romanian patients. *Chirurgia (Bucur)*, **104**, 553-6.

van der Knaap R, Siemes C, Coebergh JW, et al (2008). Renin-angiotensin system inhibitors, angiotensin I-converting enzyme gene insertion/deletion polymorphism, and cancer: the Rotterdam Study. *Cancer*, **112**, 748-57.

Yoshiji H, Kuriyama S, Fukui H (2002a). Perindopril: possible use in cancer therapy. *Anticancer Drug*, **13**, 221-8.

Yoshiji H, Kuriyama S, Fukui H (2002b). Angiotensin-I-converting enzyme inhibitors may be an alternative anti-angiogenic strategy in the treatment of liver fibrosis and hepatocellular carcinoma. Possible role of vascular endothelial growth factor. *Tumour Biol*, **23**, 348-56.

Yuan F, Zhang LS, Li HY, et al (2013). Influence of angiotensin I-converting enzyme gene polymorphism on hepatocellular carcinoma risk in China. *Dna Cell Biol*, **32**, 268-73.

Zhang Y, He J, Deng Y, et al (2011). The insertion/deletion (I/D). polymorphism in the Angiotensin-converting enzyme gene and cancer risk: a meta-analysis. *BMC Med Genet*, **12**, 159.

Zhou L, Hu Y, Gao HF, et al (2010). Polymorphism of angiotensin I-converting enzyme gene in peripheral blood of colon cancer patients(chinese). *Cancer Res Prev Treat*, **37**, 1040-3.