RESEARCH ARTICLE

Insertion/deletion (I/D) in the Angiotensin-converting Enzyme Gene and Breast Cancer Risk: Lack of Association in a Meta-analysis

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Abstract

Purpose: Breast cancer is an important cause of cancer-related death in women. Numerous studies have evaluated the association between the insertion/deletion (I/D) polymorphism in the angiotensin-converting enzyme (ACE) gene and breast cancer risk. However, the specific association is still controversial rather than conclusive. Therefore, we performed a meta-analysis of related studies to address this controversy. Methods: PubMed, EMBASE, Google Scholar and the Chinese National Knowledge Infrastructure databases were systematically searched to identify relevant studies. A meta-analysis was performed to examine the association between the I/D polymorphism in the ACE gene and susceptibility to breast cancer. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated. Results: 10 separate studies of 7 included articles with 10,888 subjects on the relation between the I/D polymorphism in the ACE gene and breast cancer were analyzed by meta-analysis, and our results showed no association between the I/D polymorphism in the ACE gene and breast cancer in total population and different populations. No publication bias was found in the present study. Conclusions: The ACE I/D polymorphism may not be associated with breast cancer risk. Further large and well-designed studies are needed to confirm this conclusion.

Keywords: Breast cancer - ACE - gene polymorphism - meta-analysis

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Introduction

Breast cancer is among the leading chronic conditions affecting adult women. Excluding skin cancers ,breast cancer is the most common malignancy among women,accounting for nearly one in three cancers diagnosed among women in the United States. It is the second leading cause of cancer death (Smigal et al., 2006). Risk factors for breast cancer include getting older,being overweight,using hormone replacement therapy (also called menopausal hormone therapy), taking birth control pills, drinking alcohol, not having children or having first child after age 35 or having dense breasts (Setiawan et al., 2009). In addition, genomewide association studies provide evidence that genetic factors are important in the pathogenesis of breast cancer (Wacholder et al., 2010).

The renin–angiotensin system (RAS) is implicated in the regulation of blood pressure and cardiovascular homeostasis. Angiotensin II (Ang II), the main biologically active peptide of the RAS, is converted from angiotensin I (Ang I) via angiotensin I-converting enzyme (ACE).ACE is differentially expressed in several carcinomas and may affect tumor cell proliferation,migration and angiogenesis (Rocken et al., 2005). The human ACE gene contains many polymorphisms,which is located on chromosome 17q23, and the 287-bp Alu insertion/deletion (I/D, rs4646994) polymorphism in intron 16 is widely studied (Haiman et al., 2003), and ACE I/D polymorphism has been related to ACE levels (Villard et al., 1996).

Over the past few years more and more studies show that the ACE I/D polymorphism may be closely associated with breast cancer risk, and the incidence of breast cancer is much higher with the ACE DD genotype (Gonzalez-Zuloeta et al., 2005). However,the published results have been inconsistent. Meta-analysis can be a useful tool in detecting an association that could otherwise remain masked in the sample size studies, especially in those evaluating rare allele frequency polymorphisms (Attia et al., 2003), which has recently become an important part of genetic research mainly to reconcile previously conducted studies that gave inconsistent results.In the present study, we investigated whether or not the ACE I/D polymorphism is associated with breast cancer risk by performing a meta-analysis of the data from the literature.

Materials and Methods

Literature review

A comprehensive systematic search was conducted by two independent reviewers (Xin-hong Pei and Hui-xiang

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Li). PubMed, EMBASE, Google Scholar and the Chinese National Knowledge Infrastructure databases searches were performed to retrieve papers linking ACE I/D polymorphism and susceptibility to breast cancer available by October 2012 without language restrictions using the key words 'breast cancer', 'angiotensin-converting enzyme/ACE', 'I/D' and 'gene polymorphism' for relevant citations.The reference lists of major textbooks,review articles and included articles were identified through manual searches to find other potentially eligible studies.

Inclusion and exclusion criteria

Studies were included in the meta-analysis if they met the following criteria: i) case-control studies that addressed breast cancer cases and healthy controls; ii) studies that evaluated the association between ACE I/D polymorphism and breast cancer risk and iii) sufficient genotype data for calculation of odds ratio(OR) with confidence interval(CI). Studies were excluded when: i) not case-control studies that evaluated the association between ACE I/D polymorphism and breast cancer risk; ii) case reports, letters, reviews, meta-analysis, and editorial articles and iii) Raw data did not provide adequate document.

Data extraction

Two reviewers (Xin-hong Pei and Hui-xiang Li) independently extracted the data and information from each included article, and discussed to reach a consensus when disagreements occurred. The following information was extracted from the eligible studies: name of the first author, year of publica¬tion, Area, ethnicity,number of cases and controls, genotype distribution of subjects with and without breast cancer,and evidence of Hardy-Weinberg equilibrium (HWE) in controls.

Statistical analysis

We assessed HWE in the controls for each study using x^2 test and a $P<0.05$ was considered as significant disequilibrium.The association of ACE I/D polymorphism with breast cancer was estimated by calculating pooled OR and 95% CI under a homozygote comparison (DD vs II), a heterozygote comparison (DD vs ID), a dominant model (II+ID vs DD) and a recessive model (DD+ID vs II). I^2 test was performed to evaluate whether the variation was due to heterogeneity or by chance, I^2 values of 25, 50 and 75% were defined as low, moderate and high estimates, respectively (Wang et al., 2012). If heterogeneity was found among the studies, the pooled OR was estimated by the fixed-effects model ($P>0.10$ or $I^2<50\%$). Otherwise, the random-effects model was used to estimate the pooled OR. Subgroup analysis based on ethnicity was used to explore the diversity among different studies. Because only one study was performed in African patients, the result of subgroup analysis could not be reliable for Africans. Begg's test was used to measure publication bias ($P<0.05$ was considered a significant publication bias). Sensitivity analysis was performed by removing the studies not in HWE.Analyses were performed in Stata 12.0 (StataCorp, College Station, TX, USA). All the P values were two-sided.

Results

Eligible studies

There were 48 articles relevant to the search words (Figure 1). Through screening the title and reading the abstract and the entire article, 10 eligible studies of 7 included articles were selected for further analysis (Haiman et al., 2003; Koh et al., 2003; Yaren et al., 2007; van et al., 2008; Alves et al., 2009; Namazi et al., 2010; Mendizabal-Ruiz et al., 2011), containing the population studies of 7 Caucasian (Haiman et al., 2003C; Haiman et al., 2003D; Yaren et al., 2007; van et al., 2008; Alves et al., 2009; Namazi et al., 2010; Mendizabal-Ruiz et al., 2011), 2 Asians (Haiman et al., 2003B; Koh et al., 2003) and 1 South Africa (Haiman et al., 2003A). Of the 10 retrospective studies,6 studies used population-based controls (Haiman et al., 2003; Koh et al., 2003; van et al., 2008), and 4 studies used hospital-based controls (Yaren et al., 2007; Alves et al., 2009; Namazi et al., 2010; Mendizabal-Ruiz et al., 2011). The genotype distributions

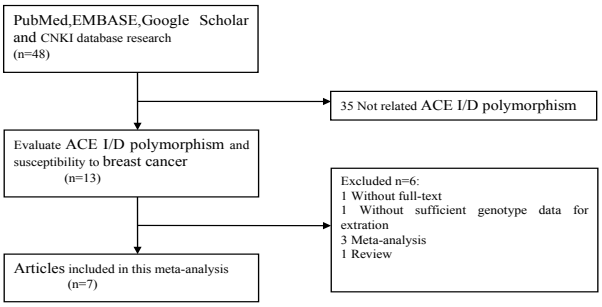

Figure 1. Flow Diagram of Study Searching and Selection Process

Table 1. Characteristics of the Included Studies for Meta-analysis

Study included	Year	Area	Race	Cases/Controls	Genotypes for cases			Genotypes for controls			HWE test
					II	ID	DD	II	ID	DD	
Koh et al	2003	Singapore	Asian	124/464	59	46	19	205	220	39	0.06
Haiman et al A	2003	USA	African	257/631	62	118	77	100	310	221	0.61
Haiman et al B	2003	USA	Asian	284/357	119	128	37	154	160	43	0.88
Haiman et al C	2003	USA	Caucasian	249/652	73	127	49	189	301	162	0.06
Haiman et al D	2003	USA	Caucasian	292/402	79	129	84	91	187	124	0.2
Yaren et al	2007	Turkey	Caucasian	57/52	2	24	31	7	12	33	0.01
Van der et al	2008	Netherlands	Caucasian	153/6015	32	67	54	1332	3006	1677	0.83
Alves et al	2009	Brazil	Caucasian	101/307	20	20	61	53	113	141	0
Mendizabal-Ruiz et al	2010	Mexico	Caucasian	63/288	4	6	53	74	151	63	0.4
Namazi et al	2010	Iran	Caucasian	70/70	8	42	20	7	34	29	0.51

Table 2. Summary ORs and 95% CI of the Insertion/deletion (I/D) Polymorphism in the Angiotensin-converting Enzyme Gene and Breast Cancer Risk

Subgroup	Genetic model	Sample size		Type of model	Test of heterogeneity		Test of association		Test of publication bias	
		Case	Control		I^2	P	OR	95% CI	z	P
Overall	DD vs II	1650	9238	Random	79.5%	0.00	1.22	0.81-1.84	0.00	1.00
	DD vs ID			Random	87.4%	0.00	1.35	0.87-2.09	0.00	1.00
	Dominant model			Random	89.2%	0.00	0.76	0.49-1.17	0.00	1.00
	Recessive model			Random	58.2%	0.00	0.98	0.77-1.23	0.00	1.00
Caucasians	DD vs II	985	7786	Random	81.9%	0.00	1.40	0.80-2.48	0.00	1.00
	DD vs ID			Random	90.7%	0.00	1.40	0.74-2.65	0.00	1.00
	Dominant model			Random	92.0%	0.00	0.71	0.38-1.32	0.00	1.00
	Recessive model			Random	56.8%	0.00	1.12	0.81-1.55	0.00	1.00
Asians	DD vs II	665	1452	Fixed	5.9%	0.30	1.31	0.88-1.93	0.00	1.00
	DD vs ID			Random	71.8%	0.06	1.54	0.72-3.29	0.00	1.00
	Dominant model			Random	57.6%	0.13	0.70	0.39-1.25	0.00	1.00
	Recessive model			Fixed	0.0%	0.47	0.98	0.76-1.25	0.00	1.00
consistent with HWE	DD vs II	1492	8879	Random	83.1%	0.00	1.18	0.74-1.88	0.00	1.00
	DD vs ID			Random	88.3%	0.00	0.39	0.86-2.25	0.00	1.00
	Dominant model			Random	91.0%	0.00	0.73	0.44-1.21	0.00	1.00
	Recessive model			Random	61.1%	0.01	0.96	0.75-1.22	0.00	1.00

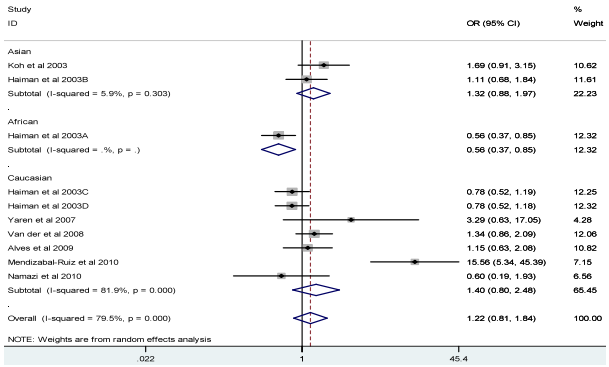

Figure 2. The Association of ACE I/D Polymorphism with the Risk for Breast Cancer(DD vs II)

among the controls of all studies were consistent with HWE except for Yaren et al and Alves et al. The main characteristics of included studies are listed in Table 1.

Meta-analysis

The results of meta-analysis were calculated in DD vs II (OR=1.22, 95% CI 1.81-1.84), DD vs ID(OR=1.35, 95% CI 0.87-2.09), a dominant model (OR=0.76, 95% CI 0.49-1.17) and a recessive model (OR=0.98,95% CI 0.77-1.23). These results suggested that ACE I/D polymorphism might not be related to the increased risk of breast cancer in total population (Figure 2). When stratified for ethnicity, we detected no significant association in Caucasians (DD vs II: OR=1.40, 95% CI 0.80-2.48; DD vs ID: OR=1.40, 95% CI 0.74-2.65; a dominant model: OR=0.71, 95% CI 0.38-1.32 and a recessive model:OR=1.12,95% CI 0.81-1.55) and in Asians (DD vs II: OR=1.31, 95% CI 0.88-1.93; DD vs ID: OR=1.54, 95% CI 1.72-3.29; a dominant model: OR=0.70, 95% CI 0.39-1.25 and a recessive model:OR=0.98,95% CI 0.76-1.25). Sensitivity analysis was performed with controls in the HWE and the results were not altered, indicating the results of meta-analysis were statistically significant (Table 2).

Publication bias

The funnel plot and Begg's test was used to assess the publication bias. Results showed that there was no publication bias (Table 2). The results implied that the publication bias was low in the present meta-analysis.

Discussion

ACE had an important role in the regulation of blood pressure and cardiovascular homeostasis.In addition,ACE is also influence tumor cell proliferation,migration,angio genesis and inflammation (Ager et al., 2008), and studies showed that ACE inhibitor could lower the breast cancer risk (Lever et al., 1998), suggesting that ACE may be involved in cancer development and progression. In recent years,more evidences indicated that the ACE I/D polymorphism is related to ACE plasma leves (Rigat et al., 1990; Sayed-Tabatabaei et al., 2004), the result suggest that the ACE I/D polymorphism may be implicated in the carcinogenesis. Koh et al. (2003) were the first to report that women with DD genotypes had decreased risk of breast cancer using a case-control study. The study included 189 incident breast cancer cases and 671 female control subjects, and all participants were Chinese. For this study is in a Chinese population,and it may be not suitable for other ethnic group. Later, several studies, but not all, have confirmed the association of ACE I/D polymorphism with increased risk for breast cancer. However, these observed associations above used a small number of samples,the results may reflect chance observations rather than true associations.To help clarify the controversial finding, the meta-analysis is performed to obtain a more competitive result by combining the comparable studies, increasing the sample size and statistical power. Our meta-analysis quantitatively assessed the association between ACE I/D polymorphism and breast cancer. Finally 10 case-control studies involving a total of 1650 breast cancer cases and 9238 healthy controls were included,and the results of our meta-analysis did not show any significant association between ACE I/D polymorphism and breast cancer risk. Considering the result may be affected by ethnicity,we performed a race-related subgroup analysis,and no significant associations were found in Asians and Europeans.For few study performed in Africans,further

studies are necessary to validate in Africans.

Potential function of ACE I/D polymorphism might be affected via gene-gene and gene-environment interactions. the effects of ACE I/D polymorphism might have limited impact on breast cancer. For many gene polymorphisms contribute to RAS activity, the gene-gene interactions and linkage disequilibrium with breast cancer risk should be further investigated. Evidence suggest that the ACE I/D polymorphism and green tea are related to the risk of breast cancer (Yuan et al., 2005). But one study could not included in the meta-analysis, further studies of ACE I/D polymorphism and green tea interactions should be taken into consideration to investigate the possible relationships.

There were some limitations in our meta-analysis. Firstly, because of incomplete raw data or publication limitations, some relevant studies could not be included in our analysis. Secondly, our systematic review was based on unadjusted data, as the genotype information stratified for the main confounding variables was not available in the original papers and also the confounding factors addressed across the different studies were variable. Finally, our analysis did not consider the possibility of gene-gene or gene-environment interactions between the ACE I/D polymorphism and breast cancer risk.

In conclusion, our meta-analysis suggests no association between ACE I/D polymorphism and breast cancer risk. As few studies are available in this field and current evidence remains limited, it should be emphasized that there is a necessity to conduct large-scale studies with adequate methodological quality in order to come to a definitive conclusion.

Acknowledgements

The author(s) declare that they have no competing interests.

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