**AGER** genetic polymorphisms increase risks of breast and lung cancers

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Received September 14, 2015
Accepted November 15, 2015
Published December 22, 2015
DOI http://dx.doi.org/10.4238/2015.December.22.2

**ABSTRACT.** We evaluated the associations between three common polymorphisms in the *AGER* gene and the risks of breast (BC) and lung (LC) cancer using meta-analysis. A systematic electronic search of the literature was conducted to identify all potential correlation studies in Embase, Web of Science, Cochrane Library, CINAHL, PubMed, CISCOM, China BioMedicine (CBM), and China National Knowledge Infrastructure (CNKI) databases. Five case-control studies that investigated the correlation of *AGER* gene polymorphisms with BC and LC were included in the meta-analysis, representing 4337 subjects. An increased frequency of the *AGER* rs1800625 T>C polymorphism was observed in patients with either BC or LC. We found that the frequencies of *AGER* rs1800624 T>A and rs2070600 G>A variants were positively related to the risks of BC and LC under allelic models, but that these relationships were not detected under dominant models. Disease-stratified results under allelic models demonstrated that the frequencies of the *AGER* rs1800625 T>C and rs2070600 G>A polymorphisms were positively correlated with the susceptibility to LC, while the same correlations were not found in BC. Further subgroup analysis...
by genotyping method indicated that the rs1800624 T>A variant was associated with increased risks of BC and LC under a dominant model in both non-polymerase chain reaction-restriction fragment length polymorphism (non-PCR-RFLP) and PCR-RFLP subgroups. In conclusion, these data indicated that common polymorphisms in the AGER gene might increase the risks of BC and LC.

**Key words:** AGER; Breast cancer; Lung cancer; Polymorphisms; Meta-analysis

**INTRODUCTION**

Breast cancer (BC) derived from breast tissue associated with a lump in the breast, abnormal fluid from the nipple, or a red patch of skin remains the second-most fatal disease in women worldwide (Feijs et al., 2013). The highest incidence rate occurs in developed counties, whereas the rate in China has increased from 23 to 31% within the last 10 years (Liang et al., 2014). Furthermore, in the United States, there were an estimated 207,000 new cases of BC in 2010 and nearly 40,000 deaths that were attributed to this disease (Massa et al., 2012). It has been reported that the observed improvement in the number of BC survivors has been due to more accurate predictions of prognosis, which are used to select therapeutic direction (Choi et al., 2012). There are various risk factors for BC including dietary habits, estrogen levels, family history, menophania and menopausal periods, and genetic predisposition (Girschik et al., 2013). Furthermore, evidence has demonstrated that the morbidity and mortality of BC are strongly correlated with patient obesity as measured by body mass index, and are affected by dietary imbalance and physical activity (Wang et al., 2013). Lung cancer (LC) is regarded as another malignancy with worldwide influence, especially in industrialized countries where constitutes 20% mortality from all cancer types (Al-Alao et al., 2013). In spite of its prevalence among both men and women, the highest incidence of LC occurs in individuals between 50 to 70 years of age (Demirci et al., 2013). The majority of patients with LC are diagnosed at an advanced stage, and the 5-year survival rate remains poor, at only approximately 10-15% (Feng et al., 2014). The etiologic factors for LC primarily consist of environmental and hereditary factors including cigarette smoking, which also seriously affects airway obstruction and breathing (Sundar et al., 2014). In addition, previous studies have demonstrated the effect of advanced glycosylation end-product receptor (AGER) gene polymorphism on the susceptibility to BC and LC (Tesarová et al., 2007; Boor et al., 2010).

AGER, a cell surface protein, is a multi-ligand receptor of the immunoglobulin superfamily that is expressed in combination with certain active ligands including advanced glycosylation end-products (AGEs), amyloid-β protein, and several members of the S100 family (Gao et al., 2010). The formation of the AGER complex primarily includes components such as non-enzymatic glycation proteins and lipoproteins, and its protein structure is comprised of an extracellular domain and a single-transmembrane domain with a short cytosolic tail (Fang et al., 2010). The human AGER gene is located on chromosome 6p21.3, has a total length of 3.27 kb, and contains 11 exons (Vasků et al., 2002). AGER expression is induced by excess oxidative stress and increased inflammatory responses after tissue injury, and its expression has been suggested to have devastating effects on tissue reorganization (Tiszlavicz et al., 2009). It has further been suggested that AGER expression might have great promise
as a marker for detecting cancers such as prostate, colorectal (CRC), BC, and LC (Wang et al., 2012; Pan et al., 2014). A previous study has shown that several variant forms of the AGER gene exist, such as T-429C, T-374A, A2184G, and G1704T; of these, Gly82Ser polymorphisms of AGE were shown to be involved in the poorly differentiated CRC, which means that the specific mutation can be used to predict the diagnosis and prognosis of CRC (Qian et al., 2014). Furthermore, AGER overexpression has been correlated with maintenance of autophagy function and suppression of apoptosis, which together can provoke pancreatic malignant cells to survive, proliferate, and infiltrate (Kang et al., 2012). Various effects of AGER polymorphic variants have been observed in the LC, and the AGER T-429C variant in the promoter region has been associated with disease severity and damage to lung function (Beucher et al., 2012). In addition, some reports have speculated that genetic polymorphisms in the AGER gene might also be implicated in the pathogenesis of BC by controlling the activation and expression of AGER to stimulate the invasion and migration of BC tumor cells (Tesarová et al., 2007; Hashemi et al., 2012). Accordingly, scholars have suggested that the risk of development of BC and LC might be attributed to various mutations in the AGER gene related to tumor progression (Wang et al., 2012). However, the published reports have reached conflicting conclusions (Tesarová et al., 2007; Pan et al., 2014). To address these inconsistencies, we performed this meta-analysis to determine the role of AGER gene polymorphisms in the susceptibility to BC and LC.

MATERIAL AND METHODS

Data sources and key words

In this study, we ascertained papers published prior to May 31, 2014, which assessed the correlation of AGER genetic mutations with the susceptibility to BC and LC, through the application of computerized databases [Embase, Web of Science, Cochrane Library, CINAHL, PubMed, CISCOM, China BioMedicine (CBM), and China National Knowledge Infrastructure (CNKI)] utilizing the selected common key words (“advanced glycosylation end-product receptor” or “AGE receptor” or “RAGE” or “receptor for advanced glycation end products” or “amphoterin receptor” or “AGER”) and (“polymorphism, genetic” or “polymorphisms” or “variants” or “SNP” or “mutation” or “genetic variants”) and (“breast neoplasms” or “BC” or “breast tumor” or “breast carcinoma” or “mammary cancer” or “mammary carcinoma” or “mammary neoplasms” or “mammary neoplasms” or “mammary neoplasms” or “mammary neoplasms” or “mammary carcinoma”) and (“lung neoplasms” or “LC” or “lung carcinoma” or “pulmonary carcinoma” or “pulmonary cancer” or “pulmonary carcinoma” or “pulmonary cancer” or “pulmonary neoplasms” or “lung tumor” or “pulmonary tumor”). No restriction was set regarding the language of the article. We also further scanned the bibliographies of related papers manually to identify additional potentially relevant papers.

Study selection

We searched for all human-associated case-control studies providing available genotypic data for single nucleotide polymorphisms (SNPs) in the AGER gene, and including patients with BC or LC, which might have resulted from AGER genetic variants. We extracted only studies that supplied the sample number and sufficient information about AGER genetic polymorphisms, and excluded those articles with incomplete, unavailable, or inappropriate clinicopathological data, or those regarding BC or LC diagnoses that were not
confirmed by histopathological examinations. In addition, only the studies including the minimum number of patients (>70), and containing control groups conforming to Hardy-Weinberg equilibrium (HWE) were enrolled. However, when the extracted studies had subjects that overlapped more than 50% between two or more papers, we enrolled the study whose population was the most comprehensive. At the same time, after careful reexamination, only the latest or most complete study was included when the extracted studies were published by the same authors.

Data extraction

In order to reduce the bias and enhance the credibility, two investigators (WHZ and YFZ) extracted information from the retrieved papers according to the selection criteria separately, and arrived at a consensus on all the items through discussion and reexamination. The following relevant data were extracted from eligible Studies: surname of first author, time of publication, country, ethnicity, age, gender, study type, study design, number of samples, source of controls, disease type, genotyping method for AGER genetic variants, available genotype, genotype and mutation frequencies, and HWE evidence in controls.

Quality assessment

To decide whether the study in question was of high quality, the two investigators used the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) quality score system to assess the studies independently (da Costa et al., 2011). According to the STROBE scores, the enrolled articles were divided into 3 levels: low (0-19), moderate (20-29), and high quality (30-40). Any discrepancies in the STROBE scores of the enrolled publications were resolved through discussion with a third reviewer.

Statistical analysis

Under allelic [mutant (M) allele versus wild-type (W) allele] and dominant (WW + WM versus MM) models, the summary ORs with 95%CIs were used to calculate the effect size for each study with the utilization of the Z-test, which was aggregated utilizing the STATA software, version 12.0 (Stata Corp., College Station, TX, USA) by two investigators separately. Cochran’s Q-statistic and $I^2$ tests were also used to quantify heterogeneity among studies. A random-effect model (DerSimonian and Laird method) was applied for the evidence of significant heterogeneity ($P < 0.05$ or $I^2 > 50\%$), whereas a fixed-effect model (Mantel-Haenszel method) was applied when there was no statistical heterogeneity ($P > 0.05$ or $I^2 < 50\%$) (Peters et al., 2006; Jackson et al., 2012). One-way sensitivity analysis was carried out to assess if the results could have been affected significantly through sequential deletion of a single study in our meta-analysis to reflect the influence on the overall results. A funnel plot was constructed to assess publication bias, which might affect the accuracy of the estimates. The symmetry of the funnel plot was further assessed by the Egger linear regression test (Zintzaras and Ioannidis, 2005).

RESULTS

Studies included

Our present meta-analysis included a total of 5 case-control studies published between...
2007 and 2014 that provided information on the correlation of SNPs in the AGER gene with BC or LC susceptibility (Tesarová et al., 2007; Hashemi et al., 2012; Wang et al., 2012; Pan et al., 2013, 2014). The demographic information on adult subjects with BC or LC, and other characteristics and the methodological quality of the extracted studies included in our present meta-analysis are listed in Table 1. There were 4 studies in Asian populations and the remaining study was in a Caucasian population, including 4337 subjects altogether (2081 patients and 2256 healthy controls). The countries where the studies were performed were China (N = 3), Iran (N = 1), and the Czech Republic (N = 1). The detection method for AGER genetic polymorphisms in this meta-analysis included polymerase chain reaction (PCR)-ligase detection reaction (N = 2), PCR-restriction fragment length polymorphism (N = 2), and hexaprimer amplification refractory mutation system PCR (N = 1). The SNPs in the AGER gene examined in this meta-analysis were rs1800625 T>C, rs1800624 T>A, and rs2070600 G>A. The study selection procedure is displayed in Figure 1. Initially, a total of 373 papers were selected from the 9 databases through screening the titles and key words. This was followed by exclusion of the duplicates (N = 3), letters, reviews, or meta-analyses (N = 76), non-human studies (N = 87), and the studies not related to the research topics (N = 90); the remaining studies (N = 117) were reviewed and an additional 109 studies were excluded because they were not case-control or cohort studies (N = 27), not relevant to the AGER gene (N = 38), or not relevant to BC or LC (N = 44). After the remaining 8 candidate publications were further reviewed, 5 studies were enrolled in the final analysis, and 3 were excluded for not supplying enough information.

AGER genetic mutations in BC or LC

As shown in Figure 2, the pooled ORs for the AGER rs1800625 T>C mutation revealed an increased frequency of the AGER rs1800625 T>C polymorphism in patients with BC or LC (allelic model: OR = 1.25, 95%CI = 1.13-1.38, P < 0.001; dominant model: OR = 1.31, 95%CI = 1.12-1.53, P = 0.001). The pooled ORs for AGER rs1800624 T>A and rs2070600 G>A variants suggested a positive relationship between the frequency of AGER rs1800624 T>A and rs2070600 G>A mutations and the occurrence of BC and LC under an allelic model (rs1800624 T>A: OR = 1.18, 95%CI = 1.07-1.30, P = 0.001; rs2070600 G>A: OR = 1.18, 95%CI = 1.07-1.31, P = 0.001), but the same relationship was not detected under a dominant model (all P > 0.05; Figure 2). Subgroup analysis based on disease type under an allelic model demonstrated that the frequencies of rs1800625 T>C and rs2070600 G>A polymorphisms in the AGER gene were positively correlated with the susceptibility to LC (rs1800625 T>C: OR = 1.27, 95%CI = 1.14-1.43, P < 0.001; rs2070600 G>A: OR = 1.24, 95%CI = 1.11-1.37, P < 0.001), while the same correlation was not found in the BC subgroup (both P > 0.05; Figure 3). In addition, subgroup analysis by disease type suggested that the frequency of the AGER rs1800624 T>A variant in patients with both BC and LC was higher than that in the controls (both P < 0.05; Figure 3). Further subgroup analysis based on the genotyping method under a dominant model indicated that the frequencies of AGER rs1800625 T>C and rs1800624 T>A mutations were positively associated with the susceptibility to BC and LC by using both PCR-RFLP and non-PCR-RFLP (all P < 0.05; Figure 3). However, the rs2070600 G>A variant in the AGER gene was not observed to be obviously correlated with the risk of BC and LC using either non-PCR-RFLP or PCR-RFLP methods (both P > 0.05; Figure 3).
Table 1. Main characteristics and methodological quality of eligible studies.

<table>
<thead>
<tr>
<th>First author</th>
<th>Year</th>
<th>Country</th>
<th>Ethnicity</th>
<th>Source of controls</th>
<th>Total Patients</th>
<th>Control Patients</th>
<th>Gender (M/F)</th>
<th>Age (years)</th>
<th>Genotyping methods</th>
<th>Gene</th>
<th>SNP</th>
<th>STROBE</th>
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<tr>
<td>Pan H</td>
<td>2014</td>
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<td>PB</td>
<td>1013</td>
<td>509</td>
<td>504</td>
<td>0/509</td>
<td>0/504</td>
<td>55.6 ± 10.1</td>
<td>56.3 ± 9.3</td>
<td>PCR-LDR</td>
</tr>
<tr>
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<td>China</td>
<td>Asian</td>
<td>PB</td>
<td>1622</td>
<td>819</td>
<td>803</td>
<td>531/288</td>
<td>520/283</td>
<td>57.4 ± 10.5</td>
<td>57.0 ± 9.7</td>
<td>PCR-LDR</td>
</tr>
<tr>
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<td>562</td>
<td>764</td>
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<td>440/324</td>
<td>-</td>
<td>-</td>
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<tr>
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<td>Iran</td>
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<td>PB</td>
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<td>93</td>
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<td>0/93</td>
<td>45.3 ± 1.8</td>
<td>43.3 ± 13.0</td>
<td>H-ARMS-PCR</td>
</tr>
<tr>
<td>Tesarova P</td>
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<td>Czech</td>
<td>Caucasian</td>
<td>PB</td>
<td>212</td>
<td>120</td>
<td>92</td>
<td>0/120</td>
<td>0/92</td>
<td>61.2 ± 11.9</td>
<td>56.2 ± 9.2</td>
<td>PCR-RFLP</td>
</tr>
</tbody>
</table>

M = male; F = female; SNP = single nucleotide polymorphism; STROBE = Strengthening the Reporting of Observational Studies in Epidemiology; PB = population-based; PCR-LDR = polymerase chain reaction-ligase detection reaction; PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism; H-ARMS-PCR = hexaprimer amplification refractory mutation system PCR.
Figure 1. Flow chart of the study selection procedure. Five case-control studies were included.

Figure 2. Forest plot of the associations between AGER genetic mutations and susceptibility to breast or lung cancer.
Figure 3. Subgroup analyses for the associations between AGER genetic mutations and susceptibility to breast (BC) or lung (LC) cancer.

Sensitivity analysis and publication bias

A leave-one-out sensitivity analysis suggested that the overall statistical significance did not change when any single study was omitted. Therefore, the current meta-analysis data was determined to be relatively stable and credible (Figure 4). The graphical funnel plots of the 5 studies for AGER rs1800625 T>C, rs1800624 T>A, and rs2070600 G>A mutations presented as symmetrical, and the Egger test suggested no publication bias (all P > 0.05; Figure 5).
Figure 4. Sensitivity analyses for the associations between AGER genetic mutations and susceptibility to breast or lung cancer.

Figure 5. Funnel plots of the associations between AGER genetic mutations and susceptibility to breast or lung cancer.
DISCUSSION

Based on numerous previous studies, this meta-analysis examined the relationship between various polymorphisms in the AGER gene including rs1800625 T>C, rs1800624 T>A, and rs2070600 G>A with BC and LC. Our findings illustrated that the AGER polymorphisms rs1800625 T>C, rs1800624 T>A, and rs2070600 G>A can be considered as genetic candidates to predict early diagnoses of BC and LC. Research has demonstrated that the combination of multi-ligands and AGER acts as the mediator to stimulate the production of key proliferation molecules including nuclear factor-κB, p21ras, cdc42/rac, and mitogen-activated protein kinases, which are involved in the cell growth, proliferation, invasion, and infiltration of tumors through the induction of their downstream effects (Logsdon et al., 2007; Sparvero et al., 2009). Another study demonstrated that the discovery of soluble AGER (sAGER) secreted from cells serves to bind with AGER ligands and is capable of suppressing AGER activities to prevent disadvantageous effects (Jing et al., 2010b). It has therefore been proposed that AGER expression might manifest in the pathogenesis of cancer proliferation and metastasis and might even be helpful for the clinical application of diagnosing multiple types of cancers (Riehl et al., 2009). It has further been demonstrated that sAGER can forcefully inhibit the production of tissue metalloproteinases, resolutely decreasing the speed of tumor cell aggression and migration into other tissues (Jing et al., 2010a; Kostova et al., 2010). Therefore, a potential function of AGER variation (rs1800625 T>C, rs1800624 T>A, rs184003 G>T, and rs2070600 G>A) might be to influence the bioavailability of AGER; this might in turn imply that AGER might represent a potential breast cancer-susceptibility gene (Pan et al., 2014). Furthermore, an additional study has shown that specific genetic variants of AGER might lead to amplified inflammatory responses, and thereby might play a substantial role in affecting transcriptional process, consequently leading to an increased risk of BC incidence (Hashemi et al., 2012). The well-established genetic mutants in the AGER gene identified in published reports include -429T/C (rs1800625 T>C), -374T/A (rs1800624 T>A), and 82G/S (rs2070600 G>A), all of which are responsible for the acceleration of malignant cell proliferation, invasion, and infiltration in patients with LC (Wang et al., 2012). Several published studies were found to be in agreement with our results that the rs1800625 T>C, rs1800624 T>A, and rs2070600 G>A polymorphisms of the AGER gene might potentially contribute to the pathologies of LC and BC (Tesarová et al., 2007; Pan et al., 2013).

To exclude other noted factors that might have impacted the accuracy and normalization of our study, we conducted a subsequent stratified analysis. With regard to disease type, subgroup analysis suggested that rs1800625 T>C and rs2070600 G>A polymorphisms in the AGER gene under an allelic model were significantly correlated with LC predisposition, while such connection was not found in the BC subgroup. Regarding the genotype detection method, our results revealed that there were evident associations of the frequencies of the AGER polymorphisms rs1800625 T>C and rs1800624 T>A in the risk for BC and LC under a dominant model with either PCR-RFLP or non-PCR-RFLP, but such association was not observed for AGER rs2070600 G>A.

There were some specific limitations in this current meta-analysis. First, only five articles regarding the genetic polymorphisms of rs1800625 T>C, rs1800624 T>A, and rs2070600 G>A in the AGER gene were included in this study. Such restricted literature size might limit or restrict the generalizability of the findings to other populations. Second, this study might have included selective publication and language biases derived from the fact that the screened
references of papers published in languages other than English and Chinese were not included.

In summary, our study supported the existence of positive associations between the rs1800625 T>C, rs1800624 T>A, and rs2070600 G>A variants in the AGER gene and increased risks of BC and LC.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

We would like to acknowledge the helpful comments on this paper received from our reviewers.

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