

Original Article

-374T/A polymorphism of the receptor for advanced glycation end products is associated with decreased risk of breast cancer in a Chinese population

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Received February 3, 2015; Accepted April 3, 2015; Epub June 15, 2015; Published June 30, 2015

Abstract: Purpose: we aimed to investigate the receptor for advanced glycation end products (RAGE) -374T/A polymorphism and breast cancer risk in a Chinese population. Methods: The study subjects included 188 women with histologically confirmed breast cancer and 210 controls. The RAGE genotypes were determined using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) assay. Pearson's χ^2 test was used to test the association between cases and controls and genotype frequencies. The association between the polymorphism and risk of breast cancer was estimated by odds ratio (OR) and 95% confidence interval (95% CI). Results: The AA genotype was significantly higher in breast cancer patients than in controls (37.77% vs. 28.10%, $P = 0.002$). Furthermore, the A allele frequency was significantly higher in the case group than in the control group (55.32% vs. 42.14%, $P < 0.001$). With the TT genotype as reference, the adjusted OR for AA homozygous carriers reached to 0.36 (95% CI: 0.17-0.88; $P = 0.03$). Under the dominant model of inheritance, the TA+AA genotype was associated with significantly decreased risk for breast cancer (adjusted OR = 0.38, 95% CI = 0.27-0.87; $P = 0.02$). The A allele carriage also presented a lower risk for breast cancer (adjusted OR = 0.42; 95% CI, 0.33-0.91; $P = 0.04$). Conclusion: Our findings suggest that the polymorphic variants of RAGE-374T/A may have an influence on breast cancer risk among Chinese women.

Keywords: Receptor for advanced glycation end products, breast cancer, polymorphisms, risk

Introduction

Breast cancer is the most common form of cancer in females and is the second cause of cancer related mortality in the world [1]. Asian countries have witnessed greatest increase of the globally rising breast cancer burden during the last several decades [2].

The receptor for advanced glycation end products (RAGE) is a member of the immunoglobulin superfamily of cell surface molecules and a receptor for advanced glycation end products (AGEs) [3]. It has been shown to participate in several important pathologic responses, including Alzheimer's disease, diabetes, inflammation, and cancer. RAGE has previously been suggested to stimulate growth, survival, and metastatic spread of cancers [4]. To date, sev-

eral genetic variants have been identified in RAGE gene, including T-429C, T-374A, G1704T, and A2184G [5]. Several studies have suggested that polymorphisms within regulation elements and/or ligand-binding regions of RAGE gene may affect the expression or function of RAGE in a given milieu [5, 6]. Previously, researchers have investigated the RAGE -374T/A polymorphism and breast cancer risk, however, their results were not consistent [7-9]. Therefore, in the present study, we investigated RAGE -374T/A polymorphism in 188 patients with breast cancer and 210 healthy women.

Materials and methods

Subjects

The study was approved by the ethics committee of Affiliated Hospital of Weifang Medical

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Table 1. Characteristics of the sample population

Characteristic	Cases (N = 188)		Controls (N = 210)		P value
	N	%	N	%	
Age (years)					
< 60	82	43.62	102	48.57	0.365
≥ 60	106	56.38	108	51.43	
Smoking status					
Ever	156	82.98	177	84.29	0.786
Never	32	17.02	33	15.71	
Alcohol drink					
Ever	134	71.28	138	65.71	0.238
Never	54	28.72	72	34.29	
Menopausal status					
Premenopausal	76	40.43	92	43.81	0.419
Postmenopausal	112	59.57	118	56.19	

Table 2. Genotype and allele frequencies of RAGE -374T/A polymorphism in studied groups

Genotype	Cases (N = 188)		Controls (N = 210)		P value
	N	%	N	%	
TT	51	27.13	92	43.81	0.002
TA	66	35.11	59	28.10	
AA	71	37.77	59	28.10	
Alleles					
T	168	44.68	243	57.86	< 0.001
A	208	55.32	177	42.14	

University and informed consent was obtained from all subjects participated the study. The study subjects included 188 women with histologically confirmed breast cancer. All cases of breast cancer were recruited from March 2006 to February 2014 at the Department of Thyroid & Breast Surgery, Affiliated Hospital of Weifang Medical University. Clinicopathologic variables were obtained from the medical records of the breast cancer patients. All cases were classified according to the tumor-node-metastasis (TNM) classification criteria of International Union Against Cancer. Differentiation grade was classified according to WHO classification. 210 controls were selected from the inpatients admitted to the hospital during the same period, having no history or diagnosis of any cancer and genetic disease. They were matched with the cases on age, smoking status, and alcohol use.

Genotyping

DNA was extracted from peripheral whole blood using a Qiagen DNA Isolation Kit (Qiagen, Valencia, CA, USA). For amplification of the region containing the -429T/C polymorphism, the following primers were used: forward primer 5' GGG GCA GTT CTC TCC TCACT 3' and reverse primer 5' GGT TCA GGC CAG ACT GTTGT 3'. Polymerase chain reaction (PCR) amplification was conducted in a 25 µL volume containing 100 ng of genomic DNA and 12.5 pmol of each primer. Annealing temperature was 59.5°C and final extension occurred at 72°C for 7 min. Restriction analysis was performed with all PCR product using 3 units of restriction nucleases, MfeI overnight at 37°C. The restriction products were directly separated by electrophoresis in 3 percent agarose gel, and visualized in ultraviolet (UV) light after ethidium bromide staining. Digestion with MfeI revealed fragments of 215 and 35 bp for the wild-type allele -374T and 250 bp for the mutated allele -374A.

Statistical analysis

Statistical analysis was performed using commercial software (SPSS for Windows, 18.0, SPSS Inc, Chicago, IL, USA). Statistical comparisons were made between the cases and controls using Fisher's exact test. To investigate whether the genotype was in Hardy-Weinberg equilibrium, distribution of the observed and expected genotype frequencies were compared using a χ^2 test. Pearson's χ^2 test was used to test the association between cases and controls and genotype frequencies. The association between the polymorphism and risk of breast cancer was estimated by odds ratio (OR) and 95% confidence interval (95% CI). A P-value less than 0.05 was considered statistically significant.

Results

Clinical characteristics of the two groups

The clinical characteristics of all participants are summarized in **Table 1**. There was no significant difference between the groups regarding age ($P = 0.365$), smoking status ($P = 0.786$), alcohol drink ($P = 0.238$), and menopausal status ($P = 0.419$).

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Table 3. Association of RAGE -374T/A polymorphism with breast cancer risk

	Patients	Controls	OR (95% CI) ¹	P value
General genotype				
TT	51	92	1.00 (Reference)	
TA	66	59	0.45 (0.31-1.19)	0.11
AA	71	59	0.36 (0.17-0.88)	0.03
Dominant genotype				
TT	51	92	1.00 (Reference)	
TA+AA	137	118	0.38 (0.27-0.87)	0.02
Recessive genotype				
TT+TA	117	151	1.00 (Reference)	
AA	71	59	0.51(0.39-1.22)	0.09
Allele frequency				
T	168	243	1.00 (Reference)	
A	208	177	0.42 (0.33-0.91)	0.04

¹Adjusted for sex, age, smoking status, and drinking status.

RAGE-374T/A polymorphism between breast cancer cases and controls

The genotype and allele distributions of the RAGE-374T/A polymorphism in the case group and control group were summarized in **Table 2**. The observed genotype frequencies of RAGE-374T/A polymorphism was in agreement with the Hardy-Weinberg equilibrium in the control subjects ($P > 0.05$). The AA genotype was significantly higher in breast cancer patients than in controls (37.77% vs. 28.10%, $P = 0.002$). Furthermore, the A allele frequency was significantly higher in the case group than in the control group (55.32% vs. 42.14%, $P < 0.001$).

Association of RAGE-374T/A polymorphism with breast cancer risk

We performed the multivariate logistic regression to determine the independent risk factors for breast cancer. With the TT genotype as reference, the adjusted OR for AA homozygous carriers reached to 0.36 (95% CI: 0.17-0.88; $P = 0.03$) after adjustment for age, smoking status, alcohol drink, and menopausal status, while the adjusted OR for the TA genotype was 0.45 (95% CI: 0.31-1.19; $P = 0.11$). Under the dominant model of inheritance, the TA+AA genotype was associated with significantly decreased risk for breast cancer (adjusted OR = 0.38, 95% CI = 0.27-0.87; $P = 0.02$, shown in **Table 3**). The A allele carriage also presented a lower risk for breast cancer (adjusted OR = 0.42; 95% CI, 0.33-0.91; $P = 0.04$).

Discussion

Breast carcinogenesis involves a complex combination of genetic, environmental as well as lifestyle factors. Inherited susceptibility makes an important contribution to breast cancer development and the risk is around two times more in first degree relatives of women with the disease [10]. Rare mutations in several high-penetrance genes like breast cancer genes (BRCA1, BRCA2) account for less than 25% of the familial breast cancer risk, and less than 5% of the overall risk [11]. Therefore, common variants present in other low penetrance genes may be more imperative and contribute to breast cancer along with lifestyle and environmental factors [12]. However, all of the common low risk variants described so far collectively account for 10% of the familial risk of breast cancer, leaving ample room for uncovering additional variants that confer risk of this disease and account for the genetic basis of the remaining major breast cancer fraction. Single nucleotide polymorphisms are the most common type of germline variations present in at least 1% of a population [13, 14]. The effect of an individual SNP is usually small, but combinations of relevant SNPs across the genome may additively contribute to higher risk in a polygenic model [15]. Though supposed to be functionally insignificant, current evidence emphasizes their predominantly unexplored functional relevance [16].

The gene for RAGE is found on chromosome 6p21.3 in the MHC locus class II/III junction and is composed of a 1.7-kb 5' flanking region and 11 exons [17]. RAGE is a 45-kDa cell surface receptor comprising three Ig domains including one V-type and two C-types followed by a single transmembrane region and a short C-terminal cytoplasmic tail. Two N-glycosylation sites located in the V-type domain are responsible for most (but not all) extracellular ligand binding [18]. The cytoplasmic tail affects intracellular signaling possibly by binding to diaphanous-1, which finally induces the cellular response [19]. RAGE is a multiligand receptor for an expanding array of ligands including advanced glycation end-products (AGEs), high mobility group box protein 1 (HMGB1), several

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members of the S100 family like S100A8/A9, and amyloid β -peptides [4, 20]. RAGE-ligand binding initiates cell signal transduction pathways such as NF- κ B, MAPKs, Rac/Cdc42, p38, p21ras, and Janus kinase (JAK)/signal transducers and activator of transcriptions (STATs) [21].

To date, several genetic variants have been identified in RAGE gene, including 82G>S (rs2070600), -429C>T (rs1800625), -374T>A (rs1800624) and 1704G>T (rs184003). A lot of studies documented positive associations between the genetic variants of RAGE and a variety of cancers. Previously, researchers have investigated the RAGE-374T/A polymorphism and breast cancer risk, however, their results were not consistent [7-9].

In the present study, we found that the AA genotype was significantly higher in breast cancer patients than in controls, furthermore, the A allele frequency was significantly higher in the case group than in the control group. We then performed the multivariate logistic regression to determine the independent risk factors for breast cancer. With the TT genotype as reference, the AA homozygous carriers was associated with significantly decreased risk for breast cancer after adjustment for age, smoking status, alcohol drink, and menopausal status. Under the dominant model of inheritance, the TA+AA genotype was associated with significantly decreased risk for breast cancer. Furthermore, the A allele carriage also presented a lower risk for breast cancer. In conclusion, our findings suggest that the polymorphic variants of RAGE-374T/A may have an influence on breast cancer risk among Chinese women.

Disclosure of conflict of interest

None.

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