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Original Article

IL-8 -251A/T and +781C/T polymorphisms were associated with risk of breast cancer in a Chinese population

Jubiao Zhang[1], Xiuhua Han[2], Shengrong Sun[1]

[1]*Department of Breast and Thyroid Surgery, Renmin Hospital of Wuhan University, Wuhan, Hubei Province, China;* [2]*Department of Breast Neoplasms Surgery, Inner Mongolia People's Hospital, Hohhot, The Inner Mongolia Autonomous Region, China*

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Abstract: Breast cancer is the leading cause of death of women in worldwide. The real mechanism of breast cancer is still unclear. IL-8 is a member of the chemokine superfamily, which plays an important role in regulating both inflammatory and immune processes. We performed a hospital-based case-control study to estimate the association between IL-8 -251A/T, -353A/T and +781C/T polymorphisms and risk of breast cancer in a Chinese population, and interaction between IL-8 polymorphism and environmental factors. During January 2014 and July 2016, a total of 442 patients with breast cancer and 447 normal control subjects were enrolled into our study. The IL-8 -251A/T, -353A/T and +781C/T polymorphisms were analyzed by the polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP). The TT genotype of IL-8 -251A/T was associated with higher risk of breast cancer as compared with the AA genotype (adjusted OR=1.96, 95% CI=1.26-3.05). Individuals carrying TT genotype of IL-8 -251A/T was correlated with an elevated risk of breast cancer in recessive model, when compared with the AA+AT genotype (adjusted OR=1.91, 95% CI=1.26-2.90). For the IL-8 +781C/T, the TT genotype showed lower association with risk of breast cancer in comparison to the CC genotype (adjusted OR=0.49, 95% CI=0.26-0.91); the TT genotype was also correlated with a decreased risk of breast cancer in recessive model (adjusted OR=0.46, 95% CI=0.25-0.84), as compared with the CC+CT genotype. In conclusion, our study suggests that IL-8 -251A/T and +781C/T could potentially be a biomarker for susceptibility to breast cancer risk.

Keywords: IL-8, polymorphism, breast cancer

Introduction

In female, breast is particularly prominent as the hallmark of pubertal development. Breast cancer begins in any part of breast, caused by abnormal cells growth and division. It is one of the oldest known forms of malignancies. Unfortunately globally, it remains a major public health issue in China as well as world. Breast cancer is the leading cause of death of women in worldwide due to it spread to other organ [1, 2]. Development of human breast cancers is a multistep process, arising from genetic alterations that drive the transformation of normal mammary epithelial cells into highly malignant derivatives [3]. The real mechanism of breast cancer is still unclear. Epidemical studies have revealed that many environmental factors play an important role in the risk of breast cancer, such as age, history of benign breast diseases, family history of cancer, lack of exercises, obesity, alcohol drinking, hormone uses and reproductive history [4-7]. However, not all individuals would develop breast cancer even when they exposure to the similar potential environmental risk factors, which indicated that hereditary factors are involved in the risk of breast cancer. Increasing studies have revealed that genetic factors, such as single nucleotide polymorphisms (SNPs), have played a critical role in the risk of breast cancer [8-10].

The *IL-8* gene is located on chromosome 4q13-q21, and comprises four exons, three introns, and a proximal promoter region [10]. IL-8 is a member of the chemokine superfamily, which

Table 1. Primers for genotyping IL-8 -251A/T, -353A/T and +781C/T

Polymorphic sites	Primers	Restriction enzyme	Lengths of products
IL-8 -251A/T	F: 5'-ATTGGCTGGCTTATCTTCA-3'	*Mun*I	TT: 272 bp; AA: 170 bp, 102 bp; AT: 272 bp, 170 bp, 102 bp
	R: 5'-CAAATACGGAGTATGACGAAAG-3'		
IL-8 -353A/T	5'-GAATTCAGTAACCCAGGCAT-3'	*Bel*I	TT: 360 bp; AA: 280 bp, 80 bp; AT: 360 bp, 280 bp, 80 bp
	5'-AAGCTTGTGTGCTCTGCTGTCTCT-3'		
IL-8 +781C/T	5'-GTGGTATCACAGAGGATTATGC-3'	*Ase*I	CC: 162 bp; TT: 118 bp, 44 bp; CT: 162 bp, 118 bp, 44 bp
	5'-CAGTCATAACTGACAACATTGATC-3'		

plays an important role in regulating both inflammatory and immune processes [11-13]. The IL-8 is produced by neutrophil, and it is a kind of neutrophil chemotactic factor and active factor. The IL-8 could induce new blood vessels and promote cell movement and mitosis, and it could promote the development, progression and metastasis of cancer [11, 14]. Previous studies have indicated that IL-8 -251A/T and +781C/T polymorphisms were associated with the transcriptional level of this protein, which may be correlated with the development of diseases [15]. Currently, few studies reported the association between IL-8 -251A/T polymorphism and risk of breast cancer, but no study investigated the association of IL-8 -353A/T and +781C/T with the development of this cancer. Therefore, we performed a hospital-based case-control study to estimate the association between IL-8 -251A/T, -353A/T and +781C/T polymorphisms and risk of breast cancer in a Chinese population, and interaction between IL-8 polymorphism and environmental factors.

Materials and methods

Subjects

A case-control study design was taken in this study. During January 2014 and July 2016, a total of 442 patients with breast cancer and 447 normal control subjects were enrolled into our study. All the patients with breast cancer were collected from the outpatient clinics and inpatient in the Renmin Hospital of Wuhan University and Inner Mongolia People's Hospital. All the patients were confirmed without any other malignant tumors, metastatic tumors, recurrent tumors and malnutrition. In addition, patients who receive any form of anti-cancer treatment prior to enrollment were also excluded from this study.

Controls who were recruited from the hospital's outpatient clinics and health examination centers of the Renmin Hospital of Wuhan University and Inner Mongolia People's Hospital. All the control subjects are free of any malignant tumors and metabolic diseases. Controls were matched to patients with regard to age.

All cases were divided into two subgroups according to breast status: a) patients with early cancer stage (including stages I and II) and b) patients with advanced cancer stage (including stages III and IV), according to the American Joint Committee for Cancer Staging and End-Results reporting in 1992 [16]. Demographic information of all the participants were collected from medical records, and included sex, age, family history of cancer, smoking and drinking habits.

The subjects were sub-divided into non-smokers and smokers; smokers were defined as those who smoked at least one cigarette per day for a period of six months. Furthermore, individuals were also categorized as non-drinkers and drinkers; drinkers were defined as those who drank at least 50 mL white wine or a bottle of beer at least once a week for six consecutive months. Written informed consents were obtained from all subjects prior to enrollment, and the study protocol was approved by the ethics committee of in the Renmin Hospital of Wuhan University and Inner Mongolia People's Hospital.

DNA extraction

Peripheral blood was collected from all the subjects in 0.5M EDTA tubes. Genomic DNA was isolated from whole blood using the standard phenol-chloroform extraction method [17]. The DNA was stored at -80°C till further study.

Table 2. Demographic and lifestyle variables of patients with breast cancer and controls

Variables	Patients N=442	%	Controls N=447	%	t or χ^2 value	*P* value
Age, years	49.52±11.00		49.83±11.12		-0.41	0.68
Socioeconomic status						
Lower	118	26.70	111	24.83		
Middle	183	41.40	208	46.53		
Upper	141	31.90	128	28.64	2.41	0.30
Physical activity						
Never	265	59.95	296	66.22		
Seldom	95	21.49	66	14.77		
Often	82	18.55	85	19.02	6.96	0.03
Menopausal status						
Premenopausal	284	64.25	310	69.35		
Postmenopausal	158	35.75	137	30.65	2.61	0.11
Smoking						
No	436	98.64	437	97.76		
Yes	6	1.36	10	2.24	0.97	0.32
Passive smoking from husband						
No	183	41.40	228	51.01		
Yes	259	58.60	219	48.99	8.25	0.004
Drinking						
No	371	83.94	386	86.35		
Yes	76	17.19	56	12.53	3.30	0.07
Body mass index (BMI), kg/m^2	22.81±2.97		23.33±3.12		-2.54	0.01
Nulliparous						
No	422	95.48	419	93.74		
Yes	20	4.52	28	6.26	1.32	0.25
Age at first live birth, year	25.85±3.19		26.60±3.26		-3.46	0.001
Breastfeeding						
No	64	14.48	84	18.79		
Yes	378	85.52	363	81.21	2.98	0.08
Months of breastfeeding, months	6.31±3.64		6.21±3.83		0.41	0.11
Benign breast disease						
No	357	80.77	262	58.61		
Yes	85	19.23	185	41.39	51.59	<0.001
First-degree relative with cancer						
No	390	88.24	417	93.29		
Yes	52	11.76	30	6.71	6.78	0.01

IL-8 -251A/T, -353A/T and +781C/T polymorphisms

The IL-8 -251A/T, -353A/T and +781C/T polymorphisms were analyzed by the polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP). The primers used for amplification of the IL-8 -251A/T, -353A/T and +781C/T gene polymorphisms were shown in **Table 1**.

Genomic DNA of IL-8 -251A/T, -353A/T and +781C/T was amplified (Applied Biosystems, Veriti, Singapore) using the following PCR conditions: for IL-8 -251A/T, an initial denaturation at 94°C for 4 min; 35 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 35 s and extension at 72°C for 45 s, and final extension at 72°C for 5 min; For IL-8 -353A/T, 94°C for 4 min, 35 cycles at 94°C for 30 s, 59°C for 45 s and 72°C for 45 s, and final extension at 72°C for 5 min; For IL-8 +781C/T, 94°C for 4 min, 35 cycles at 94°C for 45 s, 60°C for 35 s and 72°C for 45 s, and final extension at 72°C for 5 min. The 50 μL PCR reaction mixture contained 5 μL

Table 3. Genotype distributions of IL-8 -251A/T, -353A/T and +781C/T

Genotypes	Patients N=442	%	Controls N=447	%	χ^2 value	*P* value	χ^2 for HWE in controls	*P* value
IL-8 -251A/T								
TT	190	42.99	213	47.65				
AT	174	39.37	191	42.73				
AA	78	17.65	43	9.62	12.20	0.002	0.001	0.98
IL-8 -353A/T								
AA	358	81.00	337	75.39				
AT	67	15.16	82	18.34				
TT	17	3.85	28	6.26	4.81	0.090	39.52	<0.001
IL-8 +781C/T								
CC	199	45.02	205	45.86				
CT	225	50.90	202	45.19				
TT	18	4.07	40	8.95	9.65	0.008	0.96	0.33

Table 4. Association between IL-8 -251A/T, -353A/T and +781C/T genetic polymorphisms and risk of breast cancer

Genotypes	Patients	%	Controls	%	Crude OR (95% CI)	*P* value	Adjusted OR (95% CI)[1]	*P* value
IL-8 -251A/T								
Co-dominant								
AA	190	42.99	213	47.65	1.0 (Ref.)		1.0 (Ref.)	
AT	174	39.37	191	42.73	1.02 (0.77-1.36)	0.88	1.06 (0.78-1.42)	0.72
TT	78	17.65	43	9.62	2.03 (1.34-3.10)	0.001	1.96 (1.26-3.05)	0.003
Dominant								
AT+TT vs. AA	252	57.01	234	52.35	1.21 (0.93-1.57)	0.16	1.23 (0.93-1.62)	0.15
Recessive								
TT vs. AA+AT	364	82.35	404	90.38	2.01 (1.35-3.00)	0.001	1.91 (1.26-2.90)	0.002
IL-8 -353A/T								
Co-dominant								
AA	358	81.00	337	75.39	1.0 (Ref.)		1.0 (Ref.)	
AT	67	15.16	82	18.34	0.77 (0.54-1.10)	0.15	0.84 (0.58-1.23)	0.37
TT	17	3.85	28	6.26	0.57 (0.31-1.06)	0.08	0.54 (0.28-1.04)	0.06
Dominant								
AT+TT vs. AA	84	19.00	110	24.61	0.72 (0.52-0.99)	0.04	0.76 (0.54-1.07)	0.12
Recessive								
TT vs. AA+AT	425	96.15	419	93.74	0.60 (0.32-1.11)	0.10	0.56 (0.29-1.07)	0.08
IL-8 +781C/T								
Co-dominant								
CC	199	45.02	205	45.86	1.0 (Ref.)		1.0 (Ref.)	
CT	225	50.90	202	45.19	1.15 (0.87-1.51)	0.32	1.13 (0.85-1.50)	0.42
TT	18	4.07	40	8.95	0.46 (0.26-0.84)	0.01	0.49 (0.26-0.91)	0.02
Dominant								
CT+TT vs. CC	243	54.98	242	54.14	1.03 (0.79-1.35)	0.80	1.03 (0.78-1.35)	0.86
Recessive								
TT vs. CC+CT	424	95.93	407	91.05	0.43 (0.24-0.77)	0.004	0.46 (0.25-0.84)	0.01

[1]Adjusted for age, physical activity, BMI, age at first live birth and first-degree relative with cancer.

10X PCR buffer solution, 4 μL dNTP (2.5 mM), 2 μL forward and reverse primers (10 mM), 2.5 U TaqDNA polymerase, 2 μL DNA template, and hydrogen peroxide. Amplification success of samples was monitored on 2% agarose gel by Gel electrophoresis.

Statistical analysis

The categorical variables were showed as frequencies and percentages of total number. Pearson's chi-squared or Fisher's exact tests were adopted to analyze the inter-group differences. Whether the genotype frequencies of IL-8 -251A/T, -353A/T and +781C/T were departure from Hardy-Weinberg equilibrium (HWE) was analyzed by chi-square test. The association between IL-8 -251A/T, -353A/T and +781C/T and risk of breast cancer was analyzed using the method of multiple logistic regression analysis. Results were expressed using odds ratios (ORs) and 95% confidence intervals (CIs). The wild-type genotype of IL-8 -251A/T, -353A/T and +781C/T was considered as reference group. Three genotype models were used in the analysis, including co-dominant, dominant and recessive models. Chi-square or student t test analysis was taken to analyze the interaction between IL-8 -251A/T, -353A/T and +781C/T polymorphisms and environmental factors in the risk of breast cancer. All the analysis was adopted using SPSS version17.0 for Windows (SPSS, Inc., Chicago, IL, USA), and *P* value less than 0.05 was considered as significant difference.

Results

The demographic and lifestyle characteristics of patients and controls were shown in **Table 2**. On the basis of Chi-square test or student t test, we observed that patients are more likely to have no or less physical activity (χ^2=6.96, P=0.03), passive smoking from husband (χ^2=8.25, P=0.004), lower BMI (t=-2.54, P=0.01), younger age of age at first live birth (t=-3.46, P=0.001), a history of benign breast disease (χ^2=51.59, P<0.001) and a history of first-degree relative with cancer (χ^2=6.78, P=0.01).

According to Chi-square test, the TT, AT and AA frequencies of IL-8 -251A/T presented significant difference between patients and controls (χ^2=12.20, P=0.002), and the CC, CT and TT distributions of IL-8 +781C/T showed significant difference between the two investigated groups (χ^2=9.65, P=0.008) (**Table 3**). However, no significant difference in the AA, AT and TT genotypes of IL-8 -353A/T between the two study groups (χ^2=9.65, P=0.008). The genotype frequencies of IL-8 -251A/T and IL-8 +781C/T were not departure from HWE, while the IL-8 -353A/T were not.

We observed that the TT genotype of IL-8 -251A/T was associated with higher risk of breast cancer as compared with the AA genotype (adjusted OR=1.96, 95% CI=1.26-3.05) (**Table 4**). Individuals carrying TT genotype of IL-8 -251A/T was correlated with an elevated risk of breast cancer in recessive model, when compared with the AA+AT genotype (adjusted OR=1.91, 95% CI=1.26-2.90). For the IL-8 +781C/T, the TT genotype showed lower association with risk of breast cancer in comparison to the CC genotype (adjusted OR=0.49, 95% CI=0.26-0.91); the TT genotype was also correlated with a decreased risk of breast cancer in recessive model (adjusted OR=0.46, 95% CI=0.25-0.84), as compared with the CC+CT genotype.

We further analyzed the gene-environmental interaction by Chi-square or t tests (**Table 5**). However, we did not find significant association between IL-8 -251A/T and +781C/T genetic polymorphism and age, physical activity, BMI, age at first live birth and first-degree relative with cancer.

Discussion

It is widely accepted that breast cancer is a multifactorial disease, and generally pathogenesis of diseases can be promoted by a single dominant mutation leading to expression of susceptibility genes. It is of importance to capture targeted genetic culprits responsible for the functional changes of susceptibility gene. Single nucleotide polymorphisms (SNPs) are DNA sequence variants present in at least 1% of a population, and are caused by substitutions of individual nucleotides. Such mutations include the alteration of a single base by transversion, insertion, or deletion, and are thought to be involved in changing the protein's function and increasing disease's susceptibility in humans [18-20]. The common SNPs at position -251A/T and +781C/T of the IL-8 promoter region were associated with IL-8 production or

Table 5. Interaction between IL-8 -251A/T and +781C/T genetic polymorphisms and environmental factors

	IL-8 -251A/T				t or χ^2 value	*P* value	IL-8 +781C/T				t or χ^2 value	*P* value
	AA+AT N=768	%	TT N=121	%			CC+CT N=831	%	TT N=58	%		
Physical activity												
Never	484	63.02	77	63.64			522	62.82	39	67.24		
Seldom	138	17.97	23	19.01			153	18.41	8	13.79		
Often	146	19.01	21	17.36	0.22	0.89	156	18.77	11	18.97	0.81	0.67
Passive smoking												
No	414	53.91	64	52.89			449	54.03	29	50.00		
Yes	354	46.09	57	47.11	0.04	0.85	382	45.97	29	50.00	0.35	0.55
BMI												
<24	435	56.64	66	54.55			463	55.72	38	65.52		
≥24	333	43.36	55	45.45	0.19	0.67	368	44.28	20	34.48	2.12	0.15
Benign breast disease												
No	530	69.01	89	73.55			584	70.28	35	60.34		
Yes	238	30.99	32	26.45	1.02	0.31	247	29.72	23	39.66	2.53	0.11
First-degree relative with cancer												
No	699	91.02	108	89.26			755	90.85	52	89.66		
Yes	69	8.98	13	10.74	0.39	0.53	76	9.15	6	10.34	0.09	0.76
Age at first birth, year	26.31±3.22		25.68±3.37		1.99	0.05	26.21±3.28		26.49±2.74		-0.65	0.51

protein expression both in vivo and in vitro [21, 22]. Therefore, the SNPs in IL-8 may influence the risk of disease by alteration of protein expression. Currently, we evaluated the association of IL-8 -251A/T, -353A/T and +781C/T genetic polymorphisms with the risk of breast cancer in a Chinese population, and we found that IL-8 -251A/T and +781C/T were associated with risk of this cancer in co-dominant and recessive models.

The A allele of IL-8 -251A/T was associated with higher protein expression of IL-8 than the T allele. One study reported that the AA and AT genotype of IL-8 -251A/T was correlated with higher protein expression and neutrophil chemotactic index than the TT genotype [23]. High protein expression of IL-8 -251A/T was associated with stronger inflammatory response, and long-term inflammatory response was associated with development of many diseases [23, 24]. Currently, many studies have indicated that IL-8 -251A/T polymorphism was associated with risk of many cancers, such as glioma, gastric cancer, prostate cancer and osteosarcoma [25-29]. Currently, we observed that TT genotype was associated with risk of breast cancer as compared with the AA genotype or AA+AT genotype. Three previous studies have investigated the association between IL-8 -251A/T polymorphism and risk of breast cancer [26, 30, 31]. A previous study indicated that T allele of IL-8 -251A/T was associated with higher risk of breast cancer as compared with the A allele [32]. Huang et al. performed a pooled meta-analysis with six studies, and indicated that interleukin-8 -251A/T polymorphism is associated with the susceptibility to breast cancer in different genetic models [30]. Our results are in line with previous studies. However, some studies reported in consistent results. Kim et al. did not find significant association between IL-8 -251A/T polymorphism and risk of breast cancer [33]. Therefore, further studies with large sample size should be taken to confirm our results.

Previous studies have indicated that malignant breast cancer exhibited high expression of IL-8 in breast cancer cells in vivo and vitro studies [34]. Some studies have shown that IL-8 $A^{251}T^{781}$ haplotype could regulate the protein expression of IL-8 through transcriptional level [15, 35, 36]. Current studies have shown that IL-8 +781C/T are associated with kinds of cancers, such as glioma, osteosarcoma, ovarian cancer, hepatocellular carcinoma and oral cancer [29, 37-40]. However, no studies reported the association between IL-8 +781C/T polymorphisms and risk of breast cancer. We firstly reported those carrying the TT genotype of IL-8 -251A/T exhibited a higher risk of breast cancer, when

compared with those carrying the C allele. Further studies are greatly required to confirm the results of our findings.

Three limitations should be considered in our study. First, the study subjects were selected from the two hospitals, which may induce selection bias. However, our results were based on unadjusted estimates, and accurate analysis may be achieved with the adjustment of potential confounds, such as age, physical activity, BMI, etc. Second, our analysis might overlook the possibility of gene-gene or SNP-SNP interactions, or linkage disequilibrium between polymorphisms. Further studies should be conducted to obtain the relationship between IL-8 polymorphisms and breast cancer risk.

In conclusion, our study suggests that IL-8 -251A/T and +781C/T polymorphisms are associated with risk of breast cancer. Our data also indicate that IL-8 -251A/T and +781C/T could potentially be a biomarker for susceptibility to breast cancer risk. Additional large-scale studies should be conducted to evaluate the correlation between IL-8 polymorphisms and breast cancer in different ethnicities.

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Disclosure of conflict of interest

None.

Address correspondence to: Shengrong Sun, Department of Breast and Thyroid Surgery, Renmin Hospital of Wuhan University, Wuhan 430060, Hubei Province, China. Tel: +86-13707198696; Fax: +86-27-88042292; E-mail: sun137@sina.com; sunzz_11@163.com

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