

Association of IL-18 genetic polymorphisms and haplotypes with breast cancer risk in a Chinese population.

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Abstract

Objective: We performed a case-control study to evaluate the role of IL-18-607C/A (rs1946518) and -137G/C (rs187238) polymorphisms in the development of breast cancer. **Methods:** A total of 305 breast cancer patients and 305 healthy controls were recruited between May 2015 and May 2016. The genotyping of IL-18 -607C/A and -137G/C polymorphisms was carried out by an iPLEX GOLD SNP genotyping analysis using the Sequenom MassARRAY® System. **Results:** We observed that the CC genotype of IL-18 -607C/A was associated with breast cancer risk (OR=1.80, 95% CI=1.06-3.07), when compared with the CC+CA genotype. The CC genotype of IL-18 -137G/C had a 2.90 fold risk of breast cancer as compared to the CC genotype, and the CC genotype also showed an increased risk of breast cancer compared with the GG+GC genotype (OR=2.94, 95% CI=1.41-6.11). A completely linkage disequilibrium was found between IL-18 rs1946518 and rs187238 ($D'=1.00$, $r^2=0.19$). The A-C (OR=1.52, 95% CI=1.11-2.08) and C-G (OR=0.79, 95% CI=0.62-0.99) haplotypes were associated with breast cancer risk. **Conclusion:** We found that IL-18 -607C/A and -137G/C polymorphisms and haplotypes contribute to increase the breast cancer risk.

Keywords: IL-18, Polymorphism, Haplotype, Breast cancer.

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Introduction

Breast cancer is the most common cancer among women worldwide and is the second leading cause of cancer-related death in women, after lung cancer, in developed countries [1]. This cancer is among the top five most common cancers in Iran and ranks first among cancers diagnosed in women [2]. Breast cancer has a diverse etiology and several risk factors contribute to its development [3,4], such as a family history of cancer, lack of exercises, obesity, alcohol drinking, early menarche, late menopause, age at the birth of first child, number of months of breastfeeding and hormone uses and reproductive history [5-7]. However, the prevalence of breast cancer showed differences across different populations if they exposed similar lifestyles and environmental factors, suggesting that the hereditary factors may contribute to the development of this disease. Previous studies have reported that many genetic factors are involved in the risk of breast cancer, such as tumor necrosis factor receptor 2, ABCB1, insulin-like growth factor-I [8-10].

Chronic systemic inflammation has emerged as an important factor in the pathogenesis of chronic diseases such as diabetes, spontaneous abortion and of certain types of cancer [11-13].

There is convincing evidence describing the influence of low-grade inflammation and cytokines in breast carcinogenesis [14,15]. IL-18 is an 18-kDa cytokine, which belongs to the interleukin-1 (IL-1) superfamily [16]. Previous studies have shown that IL-18 modulates the immune system for attacking cancer cells through inhibition of breast cancer cell proliferation and potential invasion [14]. The single nucleotide polymorphic position of IL-18 -607C/A and -372C/G have confirmed impact on IL-18 activity and expression in tissues [17,18]. Currently, many studies have explored the association between IL-18 -607C/A and -137G/C and risk of several kinds of cancers, such as non-small cell lung cancer, acute myeloid leukemia, hepatocellular carcinoma and gastric cancer [19-22]. However, only several studies investigated the association of IL-18 -607C/A with risk of breast cancer [23-26], but no correlation between IL-18 -137G/C polymorphism and risk of breast cancer in Chinese population. In the present study, we performed a case-control study to evaluate the role of IL-18 -607C/A (rs1946518) and -137G/C (rs187238) polymorphisms in the development of breast cancer.

Materials and Methods

Subjects

This case-control population is composed of 610 Chinese unrelated Han adults who were enrolled from the Affiliated Hospital of Xinxiang Medical University between May 2015 and May 2016. A total of 305 breast cancer patients were collected prior to receiving any anti-cancer treatment, and all these patients were confirmed to be breast cancer by pathologic examination. The exclusion criteria for breast cancer patients were those with a history of other malignant tumors, recurrent or metastasis breast cancer.

Meanwhile, one healthy control subject was selected and matched with one patient with age (± 5 years). A total of 305 healthy controls were recruited from physical examination center and outpatient clinics of the Affiliated Hospital of Xinxiang Medical University. All the controls were confirmed to be free of cancers by health examination. Those who had a history of malignant tumor, serious liver and kidney diseases, and autoimmune diseases. The mean ages of patients and controls were 57.46 ± 9.74 and 58.29 ± 8.81 years, respectively; the mean BMI values were 23.38 ± 3.06 and 22.03 ± 3.19 , respectively.

The demographic and lifestyle characteristics of respondents were collected from a face-to-face questionnaires or medical records, including age, body mass index (BMI), age of menarche, menopausal status, age at first live birth, nulliparous, breastfeeding, months of breastfeeding, tobacco smoking, alcohol drinking, history of hormone uses, family history of cancer in the first relatives, and history of benign breast diseases. The BMI was calculated as body weight (kg) divided by square of the body height. All investigated subjects volunteered for the study and signed informed consents, and the research proposal was approved by the ethics committee of the Affiliated Hospital of Xinxiang Medical University.

Genotyping assays

A total of five ml peripheral blood samples were collected from each respondent, and kept in tubes with 5% EDTA. The genomic DNA was extracted using TIANamp Blood DNA Kit (Tiangen, Beijing, China) according to the instruction. The genotyping of IL-18 -607C/A and -137G/C polymorphisms

was carried out by an iPLEX GOLD SNP genotyping analysis using the Sequenom MassARRAY® System (Sequenom, San Diego, USA). The primers used for amplification of IL-18 -607C/A and -137G/C were designed by Sequenom Assay Design 3.1 software. Then the SAP and iPLEX reactions were carried out to analyze the PCR amplification products. The PCR products are then desalted and crystallized, and then analyzed by SpectroCHIP and MALDI-TOF MS reaction. The mass spectra peak and genotypes of IL-18 -607C/A and -137G/C was analyzed by Typer 4.0 software.

Statistical analysis

Chi-square (χ^2) test was used to compare the categorical variables between the two study groups, such as lifestyle characteristics and genotypes of IL-18 -607C/A and -137G/C. Student t test was taken to compare the continued variables. The χ^2 test with one degree of freedom was used to estimate whether the IL-18 -607C/A and -137G/C genotype distributions conformed to the Hardy-Weinberg equilibrium (HWE) in controls. The correlation between IL-18 -607C/A and -137G/C polymorphisms and breast cancer risk was analyzed by conditional multivariate logistic regression, and the results were expressed by odds ratio (OR) and 95% confident intervals (95% CI) and adjusted for potential confounding factors. The linkage disequilibrium and haplotype analyses of three SNPs were estimated by SHEsis software. All the statistical analyses were analyzed by IBM SPSS Statistics for Windows, Version 21.0. (IBM Corp, Armonk, NY, USA). Two sided P-value <0.05 was regarded as statistically significance.

Results

When compared the baseline characteristics of controls, we found that patients with breast cancer were more likely to have higher BMI, earlier age of menarche, less months of breastfeeding, more history of hormone uses and history of benign breast diseases (Table 1). The genotype distributions of IL-18 -607C/A and -137G/C were shown in Table 2. The GG, GC and CC genotypes of IL-18 -137G/C were significantly different between patients with breast cancer and controls ($\chi^2=11.57$, $P=0.003$), while the CC, CA and AA genotypes of IL-18 -607C/A did not show significant difference ($\chi^2=2.65$, $P=0.27$).

Table 1. Baseline characteristics of patients with breast cancer and controls.

Variables	Patients N=305	%	Controls N=305	%	χ^2 or t values	P values
Age, years	57.46 ± 9.74		58.29 ± 8.81		-1.11	0.27
BMI, kg/m ²	23.38 ± 3.06		22.03 ± 3.19		5.32	<0.001
<24	174	57.05	220	72.13		
≥ 24	131	42.95	85	27.87	15.17	<0.001
Age of menarche, years	12.49 ± 2.26		13.41 ± 2.65		-4.61	<0.001

Nulliparous						
No	290	95.08	294	96.39		0.42
Yes	15	4.92	11	3.61	0.64	
Menopausal status						
Premenopausal	129	42.30	145	47.54		0.19
Postmenopausal	176	57.70	160	52.46	1.7	
Breastfeeding						
Never	53	17.38	43	14.10		0.27
Ever	252	82.62	262	85.90	1.24	
Months of breastfeeding, months	5.28 ± 2.70		6.36 ± 2.94		-4.62	<0.001
Alcohol drinking						
Never	245	80.33	250	81.97		0.61
Ever	60	19.67	55	18.03	0.27	
Tobacco smoking						
Never	280	91.80	285	93.44		0.44
Ever	25	8.20	20	6.56	0.6	
History of hormone uses						
Never	242	79.34	262	85.90		0.03
Ever	63	20.66	43	14.10	4.57	
Family history of cancer in the first-degree relatives						
No	269	88.20	283	92.79		0.06
Yes	36	11.80	22	7.21	3.73	
History of benign breast diseases						
No	202	66.23	249	81.64		<0.001
Yes	103	33.77	56	18.36	18.79	

Table 2. The genotype distribution of IL-18 -607C/A and -137G/C genetic polymorphisms in the study subjects.

Genotypes	Patients	%	Controls	%	χ^2	P value	HWE χ^2 controls	for in	P value
-607C/A									
CC	92	30.16	100	32.79					
CA	133	43.61	142	46.56	2.65	0.27	0.92		0.24
AA	80	26.23	63	20.66					
-137G/C									
GG	134	43.93	151	49.51					
GC	125	40.98	134	43.93	11.57	0.003	1.83		0.18
CC	46	15.08	20	6.56					

Using conditional logistic regression analysis, we observed that the CC genotype of IL-18 -607C/A was associated with breast

cancer risk (OR=1.80, 95%CI=1.06-3.07), when compared with the CC+CA genotype (Table 3). The CC genotype of IL-18 -137G/C had a 2.90 fold risk of breast cancer as compared to the CC genotype, and the CC genotype also showed an increased risk of breast cancer compared with the GG+GC genotype (OR=2.94, 95%CI=1.41-6.11). A completely linkage disequilibrium was found between IL-18 rs1946518 and rs187238 ($D'=1.00$, $r^2=0.19$). The A-C haplotype was correlated with an increased risk of breast cancer (OR=1.52, 95%CI=1.11-2.08), while the C-G haplotype showed a reduced risk (OR=0.79, 95%CI=0.62-0.99) (Table 4).

Table 3. Association between IL-18 genetic polymorphisms and risk of breast cancer.

Genotypes	Patients	Controls	β	S.E.	Adjusted OR ¹	P value
-607C/A						
CC	92	100			1.0 (Ref.)	

CA	133	142	-0.04	0.26	0.96 (0.58-1.60)	0.89
AA	80	63	0.58	0.31	1.79 (0.97-3.28)	0.06
Dominant						
CC	92	100			1.0 (Ref.)	
CA+AA	213	205	0.18	0.23	1.19 (0.75-1.89)	0.45
Recessive						
CC+CA	225	242			1.0 (Ref.)	
AA	80	63	0.60	0.27	1.80 (1.06-3.07)	0.03
-137G/C						
GG	134	151			1.0 (Ref.)	
GC	125	134	-0.04	0.24	0.96 (0.60-1.54)	0.87
CC	46	20	1.06	0.39	2.90 (1.35-6.21)	0.01
Dominant						
GG	134	151			1.0 (Ref.)	
GC+CC	171	154	0.19	0.22	1.21 (0.78-1.88)	0.38
Recessive						
GG+GC	259	285			1.0 (Ref.)	
CC	46	20	1.08	0.37	2.94 (1.41-6.11)	<0.001

¹Adjusted for BMI, age of menarche, months of breastfeeding, history of hormone uses and history of benign breast diseases.

Table 4. Haplotype analysis of IL-18 rs1946518- rs187238 with breast cancer risk.

Haplotype	Patients	%	Controls	%	OR (95% CI)	P value
A-C	112	18.36	79	12.95	1.52(1.11-2.08)	0.008
A-G	181	29.67	189	30.98	0.94(0.73-1.20)	0.60
C-C	105	17.21	95	15.57	1.12(0.83-1.52)	0.46
C-G	212	34.75	247	40.49	0.79(0.62-0.99)	0.04

Total controls=610, total patients=610, Global $\chi^2=9.11$, P value=0.03.

Discussion

In the current study, we investigated the association of IL-18 -607C/A AA genotype and -137G/C CC genotype were associated with risk of breast cancer, and a completely linkage disequilibrium was observed between rs1946518 and rs187238. Cytokines are reported to be a protein family of regulatory factors derived from tumors and their environmental components that are involve in the onset, growth, invasion and metastasis of breast cancer [27]. Srabović et al. revealed that IL-18 expression was significantly elevated in breast cancer tumor tissue in comparison to its expression in surrounding unchanged tissue of the same patients [28]. Jung indicated that IL-18 was associated with inflammation and was up-regulated in breast cancer tissues in mice [29]. Expression of IL-18 equally showed in inflammatory and non-inflammatory locally advanced breast cancer, and was correlated with complete

response to neoadjuvant chemotherapy [29]. Therefore, expression of IL-18 was associated with the development and outcome of breast cancer. IL-18 is located on chromosome 11q22.2-q22.3, and single nucleotide polymorphic position of -607C/A (rs1946518) and -137G/C (rs187238) have impact on IL-18 activity and expression in tissues [17,18]. Currently, many studies indicated that IL-18 polymorphisms were associated with many kinds of cancers [19,20].

Similarly, several studies reported the association between IL-18 polymorphisms and risk of breast cancer in several kinds of populations [26]. However, some studies reported no association between them. Back LK et al. reported that IL-18 -607A/C and -137G/C polymorphisms contributed to increase the breast cancer risk in co-dominant and recessive genetic models [26]. Khalili-Azad et al. indicated that the CC genotype of IL-18 -137G/C was significantly associated with metastasis of breast cancer patients, but not associated with onset of breast cancer [24]. A recent meta-analysis reported that IL-18 -607C/A polymorphism is associated with increased susceptibility to breast cancer in Asian and Mixed population [23]. Our study reported the similar results with previous ones. In addition, we observed that the IL-18 rs1946518- rs187238 A-G and C-G haplotypes were associated with breast cancer risk. However, some studies reported inconsistent results. Taheri et al. conducted a study with 72 breast cancer patients and 93 cancer free women, and found that IL-18 -607A/C polymorphism was not associated with breast cancer in a sample of Iranian population [26].

Our study firstly reports that the AA genotype of IL-18 -607C/A and CC genotype of IL-18 -137G/C are associated with the development of breast cancer, and a completely linkage disequilibrium was observed between the two SNPs and A-C and C-G haplotypes also play an important role in the risk of this disease. Our findings should be confirmed in further studies with large sample size and more ethnic groups.

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