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Original Article

Genetic polymorphism of interleukin-16 rs11556218 T/G influences susceptibility to breast cancer in a Chinese population

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Abstract: Background: Interleukin-16 (IL-16) plays a fundamental role in inflammatory diseases, as well as in the development and progression of tumors. However, there are no data about the role of IL-16 polymorphism in development of breast cancer. Patients and methods: A hospital-based case-control study was conducted among 230 patients with breast cancer and 230 healthy controls to investigate the possible association between the IL-16 rs11556218 T/G and rs4072111 C/T polymorphisms respectively, and the risk of breast cancer. Results: Significant differences of genotype distribution were observed between breast cancer cases and controls at the IL-16 rs11556218 T/G genotypes. Compared with the IL-16 rs11556218 T/G homozygote TT, the heterozygous TG genotype was associated with significantly increased risk for breast cancer (OR = 2.09, 95% CI = (1.25-3.82), P = 0.023); the GG genotype was associated with increased risk for breast cancer (OR= 1.84, 95% CI = 1.46-3.99, P = 0.019).TG and GG combined variants were associated with increased risk for breast cancer compared with the TT genotype (OR = 1.92, 95% CI = 1.48-4.89, P = 0.020). Moreover, the genotype GG of IL-16 rs11556218 T/G carried a higher risk of breast cancer metastasis and later stages, compared with the TT genotype. However, the genotype and allele frequencies of IL-16 rs4072111 C/T polymorphisms in breast cancer patients were not significantly different from controls. Conclusion: Our results showed that the IL-16 rs11556218 T/G genotype was associated with increased risk for development and metastasis of breast cancer in Chinese Han population.

Keywords: IL-16, Breast cancer, single-nucleotide polymorphism, susceptibility

Introduction

Breast cancer is the most prevalent type of spontaneous tumor in human, the second most common type of non-skin cancer (after lung cancer) and ranks fifth most common cause of cancer death (458,000, 6.1%) [1]. The progression of breast cancer is a multiple-step process that involves multiple genetic alterations including the activation of oncogenes and the inactivation of tumor suppressor genes, which ultimately lead to the malignant tumor of inner lining of milk ducts or the lobules that supply the ducts with milk [2]. The risk of breast cancer is determined by both genetic factors and lifestyle factors. Some etiological aspects have been established for breast cancer, such as ionizing radiation exposure, alcohol consumption, high-fat based diets, oral contraceptives and hormone therapy use [3, 4]. Besides the environmental factors listed above, genetic variations also play an important role in increasing an individual's risk of developing breast cancer [5, 6].

Although the exact etiology of breast cancer remains unclear, studies have shown that it involves environmental and genetic factors. Molecular epidemiology studies suggested that single nucleotide polymorphisms (SNPs) in specific genes and pathways may play an important role in the pathogenesis of breast cancer.

Interleukin-16 (IL-16) is a multifunctional cytokine that was initially identified as lymphocyte

Table 1. Distribution of selected variables between the breast cancer cases andcontrols

Characteristics	Cases (%) N=230	Controls (%) N=230	*P* value*
Mean Age (years)	45.5 (±13.3)	46.7 (±12.4)	0.246
≤60	167 (72.6)	158 (68.7)	
>60	63 (27.4)	72 (31.3)	
Age at menarche (years)			0.153
≤14	129 (56.1)	148 (64.3)	
>14	101 (43.9)	82 (35.7)	
BMI, kg/m^2	23.5 (±4.3)	24.2 (±3.3)	0.315
≤25	142 (61.7)	134 (58.3)	
>25	88 (38.3)	96 (41.7)	
Menstrual history			0.158
Premenopause	164 (71.3)	158 (68.7)	
Menopause	66 (28.7)	72 (31.3)	
History of cancer			0.303
Positive	69 (30.0)	82 (35.7)	
Negative	161 (70.0)	148 (64.3)	
Stage			
Localized (I + II)	167 (72.6)		
Advanced (III + IV)	63 (27.4)		

Student's t-test for age and BMI distributions between cases and controls.

chemoattractant factor (LCF) in 1982 [7]. The *IL-16* gene is located on chromosome 15q26.3, and is initially translated into a precursor protein consisting of 631 amino acids, which is cleaved by caspase-3 to form the active C-terminal domain containing 121 amino acids [8-10]. By binding to the CD4 molecule, IL-16 can activate CD4+ T cells, monocytes, macrophages, eosinophils, and dendritic cells, and promote the secretion of inflammatory cytokines [11].

Previous studies have revealed that SNPs of the IL-16 gene were associated with the susceptibility to colorectal cancer and gastric cancer patients [12]. Furthermore, the high levels of IL-16 have been demonstrated in several malignant cancers both in vitro and in vivo [13-15]. Recently, a genome-wide association study revealed that IL-16 may be used as a candidate susceptibility gene in prostate cancer [16]. However, there is no any report on investigating IL-16 polymorphism of breast cancer patients. The aim of our study was to investigate the possible association between rs11556218 T/G and rs4072111 C/T polymorphisms of IL-16 gene and risk of breast cancer in Chinese Han population.

Material and methods

Study population

This study included 230 breast cancer patients and 230 non-cancer controls. All subjects were genetically unrelated Han ethnic group living in the same region of southwest China. Patients with breast cancer were recruited from The First Affiliated Hospital of Xinjiang Medical University, and were unrelated Chinese individuals residing in China between July 2005 and March 2008. All of them were histologically/pathologically confirmed by two experienced pathologists. The control group comprised 230 healthy volunteers for the general health checkup in our hospital during the same period. All the healthy controls had been under the health screening, and their clinical characteristics were matched to the sex and age distribution with the breast cancer cases, as outlined in **Table 1**. After obtaining written informed consent, 5 mL of peripheral blood was collected for DNA extraction. Each participant was interviewed using a standard questionnaire by a trained nurse, to collect medical histories, demographic characteristics. The present study was performed with strict protocol under the Ethics Committee of The First Affiliated Hospital of Xinjiang Medical University. All the specimens we recruited were of Chinese Han ethnicity and were filtered based on their clinical characteristics. Before the assay, we obtained a written informed consent from each participant in our study.

DNA extraction and genotyping

The polymorphisms in the promoters of the IL-16 genes analyzed in this study are shown in **Table 2**. The polymerase chain reaction (PCR) combined with the restriction fragment length polymorphism (RFLP) was used to determine the IL-16 genotypes. Genomic DNA used for the assay was extracted from peripheral blood sa-

Table 2. Details of PCR Primer sequences and RFLPs conditions in our study

Polymorphism	Primer sequence	PCR product size (bp)	PCR Conditions
rs11556218 T/G	F: GCTCAGGTTCACAGAGTGTTTCCATA	171	35 cycles: 95°C 40 s, 54°C 40 s, 72°C 60 s
	R: TGTGACAATCACAGCTTGCCTG		
rs4072111 C/T	F: CACTGTGATCCCGGTCCAGTC	164	35 cycles: 94°C 180 s, 62°C 30 s, 72°C 30 s
	R: TTCAGGTACAAACCCAGCCAG		

Table 3. Association between two SNPs (rs11556218 T/G and rs4072111 C/T) of IL-16gene and breast cancer susceptibility

Polymorphisms	Cases (N = 230) (%)	Controls (N = 230) (%)	OR (95% CI)	*P*-value*
rs11556218 T/G				
TT	118 (51.3)	162 (70.4)	1	
TG	67 (29.1)	50 (21.7)	2.09 (1.25-3.82)	**0.023***
GG	45 (19.6)	18 (7.8)	1.84 (1.46-3.99)	**0.019***
TG+GG	112 (48.7)	68 (29.5)	1.92 (1.48-4.89)	**0.020***
T	303 (65.9)	374 (81.3)	1	
G	157 (34.1)	86 (18.7)	1.86 (1.35-4.21)	**0.021***
rs4072111 C/T				
CC	106 (45.3)	110 (47.4)	1	
CT	95 (47.4)	98 (46.3)	1.51 (0.83-4.63)	0.251
TT	29 (7.3)	22 (6.3)	1.76 (0.89-5.36)	0.272
CT+TT	104 (54.7)	100 (52.6)	1.69 (0.84-4.81)	0.149
C	262 (68.9)	268 (70.5)	1	
T	118 (31.1)	112 (29.5)	1.48 (1.19-.6.41)	0.162

OR, odds ratio; CI, confidence interval. *Bold numbers indicate that the *P*-value is<0.05.

mples (96.5% of total samples) or exfoliated buccal cells (3.5% of total samples) as previously described [11]. For quality control, genotyping was repeated randomly in at least 5% of the samples, and two of the authors independently reviewed all results. PCR reactions were carried out in a total volume 10 μl containing 20 ng of genomic DNA, 0.25 mM of each Dntp (Ecogen, Biologia Molecular S.L.), 0.2 units of Taq polymerase (Biotools, Inc.) and 2.5 pmol of each primer in a 1× PCR buffer (Sigma-Aldrich Co.). The details of the primers and PCR conditions used for the amplification of IL-16 are shown in **Table 2**.

Statistical analysis

During the analysis, student t-test and chi-square (χ^2) test were performed to analysis the differences in the distribution of various considered characteristics as well as the differences of genotype frequencies between the breast cancer patients and the healthy controls, as appropriate. Similarly, the Hardy-Weinberg equilibrium (HWE) of each subject was examined by implying a two-sided chi-square (χ^2) test which was performed by comparison of observed and expected genotype frequencies. The IL-16 rs11556218 T/G and rs4072111 C/T polymorphisms genotypes related breast cancer risk was assessed by odds ratio (OR) and their corresponding respective confidence intervals 95% (CIs) value of the logistic regression, for both combined and respective genotype. We managed all the statistical analysis with the SPSS software version 19.0. A two-sided *P* value less than 0.05 was considered to be statistically significant for all the analyses.

Results

Population characteristics

This study included 230 breast cancer patients and 230 healthy controls, their age, age at menarche, BMI, menstrual history, history of cancer and tumor Stage were summarized in **Table 1**. The mean age (± SD) for case and control groups was 45.5 (13.3) and 46.7 (12.4) years, respectively. No significant difference was detected in the age at menarche, BMI, menstrual history, history of cancer between two groups (P>0.05). Regarding the clinical stage, 72.6% of patients were in stage I and II, whereas 27.4% of patients presented III and IV stage. The control population was consistent with the Hardy-Weinberg Equilibrium (HWE) for the polymorphisms in IL-16 -251 A/T and +781 C/T.

Table 4. Correlations between genotypes of two SNP (rs11556218 T/G and rs4072111 C/T) of IL-16 gene and clinicopathological features of patients with breast cancer

Genotypes		rs11556218 T/G				rs4072111 C/T			
Variable	n	TT	TG	GG	*P* value	CC	CT	TT	*P* value
Mean Age (years)		118	67	45		106	95	29	
≤60	167	88	46	33	0.506	85	62	20	0. 427
>60	63	30	21	12		21	33	9	
Age at menarche (years)									
≤14	129	81	22	26	0.315	38	44	9	0. 408
>14	101	37	45	19		48	46	5	
BMI, kg/m^2									
≤25	142	81	47	14	0.508	74	66	2	0.362
>25	88	37	20	31		32	29	27	
Menstrual history									
Premenopause	164	94	46	24	0.158	86	68	10	0.342
Menopause	66	24	21	21		20	27	19	
History of cancer									
Positive	69	20	23	25	0.149	25	32	11	0.132
Negative	161	79	40	3		61	58	33	
Stage									
Localized (I + II)	167	100	45	22	*0.029**	73	71	23	0.225
Advanced (III + IV)	63	18	22	23		33	24	6	

*Student's t-test and the chi-square (χ^2) test.

Distributions of IL-16 (rs11556218 T/G and rs4072111 C/T) genotypes and risk of breast cancer

The genotype and allele frequencies of the IL-16 (rs11556218 T/G and rs4072111 C/T) polymorphisms for all the studied variations are shown in **Table 3**. All genotype frequencies of the control group conformed to the Hardy-Weinberg equilibrium.

There were significant differences in the genotype and allele frequencies of IL-16 rs11556218 T/G genotypes between breast cancer cases and controls. Compared with the IL-16 rs11556218 homozygote TT, the heterozygous TG genotype was associated with significantly increased risk for breast cancer (OR = 2.09, 95% CI = (1.25-3.82), P = 0.023); the GG genotype was associated with increased risk for breast cancer (OR= 1.84, 95% CI = 1.46-3.99, P = 0.019). TG and GG combined variants were associated with increased risk for breast cancer compared with the TT genotype (OR = 1.92, 95% CI = 1.48-4.89, P = 0.020). However, the genotype and allele frequencies of IL-16 rs4072111 C/T polymorphisms in breast cancer patients were not significantly different from controls (P>0.05) as shown in **Table 3**.

Distributions of IL-16 (rs11556218 T/G and rs4072111 C/T) genotypes and clinicopathological characteristics

The relationships between the IL-16 (rs11556218 T/G and rs4072111 C/T) genotypes polymorphisms and clinicopathological parameters were calculated. The results are given in **Table 4**. For IL-16 rs11556218 T/G, the genotype GG frequency in tumor later stages (III + IV) patients was greater compared to patients with early stages (I + II), and the difference in frequency distribution between genotypes reached significance (P = 0.021). No significant difference was observed with respect to age, age at menarche, BMI, menstrual history, history of cancer and the IL-16 rs11556218 T/G genotypes. For IL-16 rs4072111 C/T, there are no any obvious differences in the relations between age, age at menarche, BMI, menstrual history, history of cancer and stage respectively, and IL-16 rs4072111 C/T genotypes.

Discussion

In current hospital based case-control study, we assessed the association between the polymorphisms of two SNPs of IL-16 (rs11556218 T/G and rs4072111 C/T) and risk of breast cancer in Chinese Han population and found the significant association between IL-16 rs11556218 T/G polymorphisms and risk of breast cancer. The genotype and allele distribution of IL-16 rs11556218 T/G genotypes were significantly different between case and control groups, indicating that IL-16 rs11556218 T/G might be related to breast cancer development.

Moreover, our results showed the genotype GG frequency of IL-16 rs11556218 T/G in tumor

metastasis patients was greater compared to patients without tumor metastasis. These results indicated that the genotype GG frequency of IL-16 rs11556218 T/G carried a higher risk of breast cancer metastasis, compared with the TT genotype. To the best of our knowledge, our study is the first report to describe the possible role of two polymorphisms of IL-16 (rs11556218 T/G and rs4072111 C/T) as a risk factor for breast cancer and found that IL-16 rs11556218 T/G genotype variations do influence susceptibility to breast cancer development and metastasis in the Chinese Han population.

Although the role of IL-16 as an important mediator in inflammatory diseases has been identified [17], very limited data are available regarding the association between IL-16 and tumor growth and progression. As a multifunctional cytokine, IL-16 is an important mediator in inflammatory diseases as well as tumor growth and progression. Blaschke et al. [18] reported that IL-16 messenger RNA expression increased with the stage of cutaneous T cell lymphoma diagnosed. A few studies have shown that higher serum levels of IL-16 can be associated with advanced stages of cancer [19] and a worse patient outcome depending on the type of tumor [20].

In spite of interesting findings on the association of IL-16 polymorphisms with breast cancer risk, there were several limitations that need to be addressed regarding the present study. We did not collect lifestyle data for individual participants, e.g. on local environmental factors, diet, or level of physical activity, which potentially could interact with genetic variations in influencing overall risk of developing breast cancer. Besides, the relative small sample size might hide some weak gene-disease association and gene-environment interactions. Studies need to be performed in larger study groups to confirm our preliminary results.

In conclusion, our study provided the evidence of association between the polymorphisms of IL-16 (rs11556218 T/G and rs4072111 C/T) and the risk of breast cancer and found the IL-16 rs11556218 T/G genotype was associated with increased risk for development and metastasis of breast cancer in Chinese Han population. Because this is the first report concerning the IL-16 polymorphism and the risk of breast cancer in the literature, studies with larger sample size and further investigations into the mechanism are warranted to clarify and validate the role of IL-16 polymorphisms in breast cancer carcinogenesis.

Disclosure of conflict of interest

None.

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