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Original Article

Association between IL-16 rs4778889 and rs4072111 polymorphisms and ovarian cancer risk in Chinese Han population: a case-control study

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Abstract: Background: Interleukin-16 (IL16) as a multifunctional cytokine, plays a key role in inflammatory and autoimmune diseases as well as tumour growth and progression. However, there are no data about the role of IL-16 polymorphism in development of ovarian cancer. Patients and methods: A hospital-based case-control study was conducted among 220 patients with ovarian cancer and 220 healthy controls to investigate the possible association between the IL-16 rs4778889 T/C and rs4072111 C/T polymorphisms respectively, and the risk of ovarian cancer. Results: Significant differences of genotype distribution were observed between ovarian cancer cases and controls at the IL-16 rs4778889 T/C genotypes. Compared with the IL-16 rs4778889 T/C homozygote TT, the heterozygous TC genotype was associated with significantly increased risk for ovarian cancer (OR=2.31, 95% CI=(1.38-3.91), P=0.026); the CC genotype was associated with increased risk for ovarian cancer (OR=1.89, 95% CI=1.48-3.80, P=0.015). TC and CC combined variants were associated with increased risk for ovarian cancer compared with the TT genotype (OR=1.95, 95% CI=1.42-4.85, P=0.023). Moreover, the genotype GG of IL-16 rs4778889 T/C carried a higher risk of ovarian cancer metastasis and later stages, compared with the TT genotype. However, the genotype and allele frequencies of IL-16 rs4072111 C/T polymorphisms in ovarian cancer patients were not significantly different from controls. Conclusion: This study showed that the IL-16 rs4778889 T/C genotype was associated with increased risk for development and metastasis of ovarian cancer in Chinese Han population.

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

Introduction

Ovarian cancer is the most lethal cancer of gynecologic cancers even though it causes fewer deaths than breast cancer and precursor lesions of the uterine cervix [1]. New cases of ovarian cancer are estimated to be 21,980 in the United States in 2014 and deaths due to ovarian cancer totaled 14,270, which represent 5% of deaths due to female malignancies [2]. The risk of ovarian cancer is determined by both genetic factors and lifestyle factors. Some etiological aspects have been established for ovarian 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 ovarian cancer [5, 6].

Although the exact etiology of ovarian 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 ovarian cancer.

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

Table 1. Distribution of selected variables between ovarian cancer cases and controls

Characteristics	Cases (%) N=220	Controls (%) N=220	*P* value*
Mean Age (years)	59.5 (±13.3)	60.7 (±12.4)	0.158
≤60	157 (71.4)	148 (67.3)	
>60	63 (28.6)	72 (32.7)	
Age at menarche (years)			0.294
≤12	139 (63.2)	138 (62.7)	
>12	81 (36.8)	82 (37.2)	
BMI, kg/m^2	24.5 (±5.6)	25.2 (±4.6)	0.293
≤25	132 (60)	124 (56.4)	
>25	88 (40)	96 (43.6)	
Pathological type			
Serous-papillary	79 (35.9)		
Mucinous	56 (25.5)		
Endometrioid	73 (33.2)		
Undifferentiated	12 (5.4)		
Tumor grade			
G1	28 (12.7)		
G2	42 (19.1)		
G3	150 (68.2)		
FIGO stage			
Localized (I + II)	84 (38.2)		
Advanced (III + IV)	136 (61.8)		

Student's t-test for age and BMI distributions between cases and controls. *p<0.05.

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 was significantly 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 ovarian cancer patients. The aim of our study was to investigate the possible association between rs4778889 T/C and rs4072111 C/T polymorphisms of IL-16 gene and risk of ovarian cancer in Chinese Han population.

Material and methods

Study population

This study included 220 ovarian cancer patients and 220 non-cancer controls. All subjects were genetically unrelated Han ethnic group living in the same region of southwest China. Patients with ovarian cancer were recruited from Zhejiang Provincial Hospital of Chinese Medicine, and were unrelated Chinese individuals residing in China between July 2008 and March 2013. All of them were histologically/pathologically confirmed by two experienced pathologists. The control group comprised 230 healthy volunteers for the general health check-up 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 ovarian 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 Zhejiang Provincial Hospital of Chinese Medicine. 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 samples (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

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

Polymorphism	Primer sequence	PCR product size (bp)	PCR Conditions
rs4778889 T/C	F: CTCCACACTCAAAGCCTTTTGTTCCTATG R: ATACACGCTGGTTCCTTCTGT	280	35 cycles: 95°C 40 s, 54°C 40 s, 72°C 60 s
rs4072111 C/T	F: CACTGTGATCCCGGTCCAGTC R: TTCAGGTACAAACCCAGCCAG	164	35 cycles: 94°C 180 s, 62°C 30 s, 72°C 30 s

Table 3. Association between two SNPs (rs4778889 T/C and rs4072111 C/T) of IL-16 gene and ovarian cancer susceptibility

Polymorphisms	Cases (N=220) (%)	Controls (N=220) (%)	OR (95% CI)	*P*-value*
rs4778889 T/C				
TT	113 (51.4)	162 (73.6)	1	
TC	62 (28.2)	45 (20.5)	2.31 (1.38-3.91)	***0.026****
CC	45 (20.4)	13 (5.9)	1.89 (1.48-3.80)	***0.015****
TC+CC	107 (48.6)	58 (26.4)	1.95 (1.42-4.85)	***0.023****
T	288 (65.9)	369 (81.3)	1	
C	152 (34.1)	71 (18.7)	1.86 (1.35-4.21)	**0.021***
rs4072111 C/T				
CC	101 (45.3)	110 (47.4)	1	
CT	90 (47.4)	93 (46.3)	1.74 (0.89-4.63)	0.131
TT	29 (7.3)	17 (6.3)	1.89 (0.96-5.36)	0.122
CT+TT	119 (54.7)	110 (52.6)	1.79 (0.90-4.81)	0.259
C	262 (68.9)	268 (70.5)	1	
T	118 (31.1)	112 (29.5)	1.48 (1.19-.6.41)	0.142

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

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 ovarian 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 rs4778889 T/C and rs4072111 C/T polymorphisms genotypes related ovarian 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 220 ovarian cancer patients and 220 healthy controls, their age, age at menarche, BMI, pathological type, tumor grade and FIGO stage were summarized in **Table 1**. The mean age (±SD) for case and control groups was 59.5 (13.3) and 60.7 (12.4) years, respectively. No significant difference was detected in the age at menarche, BMI between two groups (P>0.05). Regarding the FIGO stage, 38.2% of patients were in stages I and II, whereas 61.8% of patients presented III and IV stages. The control population was consistent with the Hardy-Weinberg Equilibrium (HWE) for the polymorphisms in IL-16 rs4778889 T/C and rs4072111 C/T.

Distributions of IL-16 (rs4778889 T/C and rs4072111 C/T) genotypes and risk of ovarian cancer

The genotype and allele frequencies of the IL-16 (rs4778889 T/C and rs4072111 C/T) polymorphisms for all the studied variations are shown in **Table 3**. All genotype frequencies of

Table 4. Correlations between genotypes of IL-16 rs4778889 T/C and rs4072111 C/T genetypes and clinicopathological features of patients with ovarian cancer

Genotypes		rs4778889 T/C				rs4072111 C/T			
Variable	n	TT	TC	CC	*P* value	CC	CT	TT	*P* value
Mean Age (years)		113	62	45		101	90	29	
≤60	157	88	46	33	0.436	85	62	20	0. 362
>60	63	30	21	12		21	33	9	
Age at menarche (years)									
≤12	139	81	22	26	0.402	38	44	9	0. 239
>12	81	37	45	19		48	46	5	
BMI, kg/m^2									
≤25	132	81	47	14	0.316	74	66	2	0.121
>25	88	37	20	31		32	29	27	
Tumor grade									
G1	28	8	9	11	0.349	11	12	5	0.291
G2	42	21	14	7		21	18	13	
G3	150	84	39	27		69	60	11	
FIGO stage					*0.029**				0.225
Localized (I + II)	84	58	19	7		22	47	15	
Advanced (III +IV)	136	55	43	38		79	43	14	

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

the control group conformed to the Hardy-Weinberg equilibrium.

There were significant differences in the genotype and allele frequencies of IL-16 rs4778889 T/C genotypes between ovarian cancer cases and controls. Compared with the IL-16 rs-4778889 homozygote TT, the heterozygous TC genotype was associated with significantly increased risk for ovarian cancer (OR=2.31, 95% CI=1.38-3.91, P=0.026); the CC genotype was associated with increased risk for ovarian cancer (OR=1.89, 95% CI=1.48-3.80, P=0.015). TC and CC combined variants were associated with increased risk for ovarian cancer compared with the TT genotype (OR=1.95, 95% CI=1.42-4.85, P=0.023). However, the genotype and allele frequencies of IL-16 rs4072111 C/T polymorphisms in ovarian cancer patients were not significantly different from controls (P>0.05) as shown in **Table 3**.

Distributions of IL-16 (rs4778889 T/C and rs4072111 C/T) genotypes and clinicopathological characteristics

The relationships between the IL-16 (rs47788-89 T/C and rs4072111 C/T) genotypes polymorphisms and clinicopathological parameters were calculated. The results are given in **Table 4**. For IL-16 rs4778889 T/C, the genotype CC 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=0.029). No significant difference was observed with respect to age, age at menarche, BMI, tumor grade and the IL-16 rs-4778889 T/C genotypes. For IL-16 rs4072111 C/T, there are no any obvious differences in the relations between age, age at menarche, BMI, BMI, tumor grade and FIGO 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 (rs4778889 T/C and rs4072111 C/T) and risk of ovarian cancer in Chinese Han population and found the significant association between IL-16 rs4778889 T/C polymorphisms and risk of ovarian cancer. The genotype and allele distribution of IL-16 rs4778889 T/C genotypes were significantly different between case and control groups, indicating that IL-16 rs4778889 T/C might be related to ovarian cancer development. Moreover, the results showed the genotype CC frequency of IL-16 rs4778889 T/C in tumor metastasis patients was greater compared to patients without tumor metastasis. These results indicated that the genotype CC frequency of IL-16 rs4778889 T/C carried a higher risk of ovarian 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 (rs4778889 T/C and rs40721-11 C/T) as a risk factor for ovarian cancer and found that IL-16 rs4778889 T/C genotype variations do influence susceptibility to ovarian

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 ovarian 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 ovarian 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 (rs4778889 T/C and rs4072111 C/T) and the risk of ovarian cancer and found the IL-16 rs4778889 T/C genotype was associated with increased risk for development and metastasis of ovarian cancer in Chinese Han population. Because this is the first report concerning the IL-16 polymorphism and the risk of ovarian 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 ovarian cancer carcinogenesis.

Disclosure of conflict of interest

None.

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