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Association analysis of IL-17A and IL-17F polymorphisms in Chinese women with cervical cancer

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ABSTRACT. We selected six tagged single nucleotide polymorphisms (SNPs) in the interleukin 17A (IL-17A) and IL-17F genes, and evaluated the relationship between the six common SNPs and environmental factors in cervical cancer patients. Polymerase chain reaction-restriction fragment length polymorphism was used to detect the IL-17A (rs2275913, rs3748067, and rs3819025) and IL-17F (rs763780, rs9382084, and rs1266828) SNPs. The associations between IL-17A and IL-17F gene polymorphisms and risk of cervical cancer were estimated by conditional logistic regression. Compared with the control subjects, the cervical cancer patients had a lower age at first live birth, a habit of smoking, a family history of cancer, and a greater incidence of human papillomavirus-16 or 18 infections. The logistic regression analysis showed that the variant AA genotype of rs2275913 was associated with a significantly higher risk of cervical cancer than the wild-type GG genotype (OR = 1.99, 95%CI = 1.12-3.50). However, no evidence of the association was observed between rs3748067, rs3819025, rs763780, rs9382084, and rs1266828 polymorphisms and the risk of cervical cancer. We suggest that rs2275913 may play a role

in the etiology of cervical cancer. These findings could be helpful in identifying individuals at increased risk of developing cervical cancer.

Key words: Interleukin-17A; IL-17A; Interleukin-17F; IL-17F; Single nucleotide polymorphism; Cervical cancer

INTRODUCTION

Cervical cancer is the fourth most common cancer in women, and the seventh overall, with an estimated 528,000 new cases in 2012. It is estimated that a large majority (around 85%) of the global burden due to cervical cancer occurs in less developed regions, where it accounts for almost 12% of all female cancers (IARC, 2012). It is well known that human papillomavirus (HPV) infection is the main cause of cervical cancer, and long-term HPV infection plays an important role in the development of cervical carcinogenesis (Schlecht et al., 2001; Woodman et al., 2001), and genetic variations that can influence the host primary immune response play a critical role in high-risk HPV infection (Quan et al., 2012; Shi et al., 2013).

Previous studies have shown that interleukin genes are involved in immune response, and polymorphisms in these genes are important determinants for the pathogenesis of cervical cancer (Hildesheim and Wang, 2002). The interleukin 17 (IL-17) family comprises proinflammatory cytokines, of which there are six similar members: IL-17A, IL-17B, IL-17C, IL-17D, IL-17E, and IL-17F. Of the six members, IL-17A and IL-17F lie adjacent to each other on human chromosome 6, and both cytokines are produced by T helper 17 (Th17) cells in response to IL-23 (Bettelli et al., 2006).

To date, only one study has investigated the association between two SNPs in IL-17A and IL-17F and susceptibility to cervical cancer (Quan et al., 2012). In this study, we conducted a case-control investigation to explore the possible association between IL-17A and IL-17F polymorphisms and development of cervical cancer. We selected six tagged single nucleotide polymorphisms (SNPs) in the IL-17A and IL-17F genes, and evaluated the relationship between the six common SNPs and environmental factors in cervical cancer patients.

MATERIAL AND METHODS

Subjects

This study included 216 newly diagnosed patients with cervical cancer. From May 2011 to May 2014, patients were histologically confirmed as having cervical cancer at the Jinan Central Hospital of Shandong University. Four hundred and thirty-two control subjects were randomly selected from individuals who were participating in a routine cancer-screening program for the early detection of cervical cancer during the same period. All the control subjects were found to lack cervical lesions by cytology test. Two controls were matched to each case by age at enrollment (within ±5 years).

All patients and controls signed written informed consent before participating, and the protocol of this study was approved by the institutional Ethnics Committee of Jinan Central Hospital of Shandong University.

Data collection

Demographic and lifestyle characteristics were collected using a questionnaire that we designed. Each subject was investigated by a trained interviewer to obtain demographic and lifestyle data, such as age, age at menarche, age at menopause, age at first live birth, cigarette smoking habits, alcohol consumption, menopausal status, and family history of cancer. Clinical data, including type 16 and 18 HPV infection and stage, were collected from medical records.

Blood samples and genotyping

Each subject was asked to provide a 5-mL venous blood sample for DNA preparation. Ethylenediaminetetraacetic acid (0.5 mg/mL) was used as an anticoagulant, and the blood samples were stored at -20°C until required. Genomic DNA was extracted from peripheral blood using a TIANamp Blood DNA Kit (Tiangen Biotech, Beijing, China) according to the manufacturer instructions. Polymerase chain reaction-restriction fragment length polymorphism was used to detect the six SNPs. The primers for IL-17A (rs2275913, rs3748067, and rs3819025) and IL-17F (rs763780, rs9382084, and rs1266828) were designed using the Sequenom Assay Design 3.1 software (Sequenom, San Diego, CA, USA). The cycling program for the polymerase chain reaction was as follows: preliminary denaturation at 95°C for 10 min, followed by 35 cycles of denaturation at 95°C for 30 s, 62°C for 30 s, and 72°C for 30 s, with a final extension at 72°C for 10 min. We also randomly selected 5% of the cases and control subjects to repeat the genotyping of the six SNPs and confirm the previous results.

Statistical analysis

Differences in the distributions of demographic, lifestyle, and clinical characteristics and the genotypes of IL-17A (rs2275913, rs3748067, and rs3819025) and IL-17F (rs763780, rs9382084, and rs1266828) between cases and controls were analyzed by the Student *t*-test and χ^2-test. The Hardy-Weinberg equilibriums of IL-17A and IL-17F genotype frequencies in the control subjects were compared by goodness-of-fit χ^2-test. The associations between IL-17A and IL-17F gene polymorphisms and risk of cervical cancer were estimated by conditional logistic regression, and the results are expressed as ORs and their corresponding 95%CIs. Gene-environmental interaction was evaluated by conditional logistic regression. All P-values were two sided, and a P-value was regarded as statistically significant when it was less than 0.05. All statistical analyses were conducted using the STATA version 9.0 statistical software.

RESULTS

The demographic and clinical characteristics of the 216 cervical cancer patients and the 432 cancer-free controls are shown in Table 1. There was no significant difference between the two groups in terms of age distribution and alcohol consumption. Compared with the control subjects, the cervical cancer cases had a habit of smoking, a family history of cancer, and a greater incidence of HPV-16 or -18 infections. Of the 216 patients, 178 (82.41%) patients were at stage I-II, and 38 (17.59%) patients were at stage III-IV.

The genotype distributions of IL-17A rs2275913, rs3748067, and rs3819025, and IL-17F rs763780, rs9382084, and rs1266828 in cases and controls are shown in Table 2. The genotype distributions for the six SNPs in IL-17A and IL-17F polymorphisms in the controls were in line with the Hardy-Weinberg equilibrium. The logistic regression analysis showed that the variant AA genotype of rs2275913 was associated with a significantly increased risk of cervical cancer compared with the wild-type GG genotype (OR = 1.99, 95%CI = 1.12-3.50). However, no evidence of the association was observed between rs3748067, rs3819025, rs763780, rs9382084, and rs1266828 polymorphisms and risk of cervical cancer.

Table 1. Demographic and clinical characteristics of cervical cancer patients and controls.

Variables	Cases	%	Controls	%	χ^2-test	P value
Age (years)						
<55	101	46.76	206	47.69		
≥55	115	53.24	226	52.31	0.39	0.53
Smoking status						
Ever	22	10.19	24	5.56		
Never	194	89.81	408	94.44	4.68	0.03
Alcohol consumption						
Ever	36	16.67	93	21.53		
Never	180	83.33	339	78.47	0.52	0.471
Family history of cancer						
No	178	82.41	386	89.35		
Yes	38	17.59	46	10.65	6.16	0.01
HPV-16 or 18 infection						
Negative	46	21.30	335	77.55		
Positive	170	78.70	97	22.45	188.07	<0.001
Stage						
I-II	178	82.41				
III-IV	38	17.59				

HPV = human papillomavirus.

Table 2. Interleukin 17A and 17F polymorphisms and cervical cancer risk.

Variable	Cases	%	Controls	%	HWE	OR (95%CI)[1]	P value
rs2275913							
GG	91	42.2	222	51.5		1.0 (Ref.)	-
GA	94	43.6	171	39.6		1.34 (0.93-1.93)	0.1
AA	31	14.2	38	8.9	0.54	1.99 (1.12-3.50)	0.01
rs3748067							
CC	83	38.3	179	41.4		1.0 (Ref.)	-
CT	95	44.2	188	43.5		1.09 (0.75-1.59)	0.64
TT	38	17.5	65	15.1	0.18	1.26 (0.76-2.08)	0.34
rs3819025							
AA	99	45.7	205	47.4		1.0 (Ref.)	-
AG	91	42.3	173	40.1		1.08 (0.76-1.57)	0.63
GG	26	12.0	54	12.5	0.07	0.99 (0.56-1.73)	0.99
rs763780							
TT	74	34.2	162	37.6		1.0 (Ref.)	-
TC	108	50.2	213	49.3		1.11 (0.76-1.62)	0.57
CC	34	15.6	57	13.1	0.32	1.31 (0.76-2.23)	0.3
rs9382084							
GG	76	35.4	165	38.3		1.0 (Ref.)	-
GT	95	44.2	195	45.2		1.06 (0.72-1.55)	0.76
TT	44	20.4	71	16.5	0.3	1.35 (0.82-2.19)	0.21
rs1266828							0.21
AA	80	37.2	174	40.2		1.0 (Ref.)	-
AG	96	44.3	184	42.5		1.13 (0.78-1.66)	0.49
GG	40	18.5	75	17.3	0.06	1.16 (0.71-1.89)	0.53

HWE = Hardy-Weinberg equilibrium; OR = odds ratio; 95%CI = 95% confidence interval. [1]Adjusted for age, age at first live birth, smoking habits, family history of cancer, and human papillomavirus-16 or -18 infection.

DISCUSSION

Characterization and identification of genes involved in the genetic predisposition or progression of cancer are critical for both clinical practice and basic medicine research. The proinflammatory cytokines IL-17A and IL-17F, expressed by Th17 cells, play a role in coordinating local tissue inflammation. Numerous data have shown that IL-17A and IL-17F polymorphisms are associated with cancer risk, but only one study has investigated the association between IL-17A and IL-17F polymorphisms and cervical cancer. Therefore, we investigated the influence of polymorphisms in these two cytokines on the risk of cervical cancer.

The rs2275913 polymorphism occurs in the IL-17A gene promoter region. It has been reported that 197A allele-positive peripheral blood mononuclear cells (genotypes AG/AA) secrete more IL-17 than 197A allele-negative cells (genotype GG) (Espinoza et al., 2011). Several studies have reported that IL-17A rs2275913 variation significantly increased risk of several cancers, such as gastric, breast, colorectal, and cervical cancers (Quan et al., 2012; Omrane et al., 2014; Qinghai et al., 2014; Wang et al., 2012, 2014; Zhang et al., 2014). Wang et al. (2014) conducted a case-control study in China to investigate the effect of three common IL-17A and IL-17F SNPs on susceptibility to gastric cancer, and reported that polymorphism in rs2275913 was significantly associated with increased risk of gastric cancer. Two other studies have shown that rs2275913 polymorphism increases the risk of gastric cancer, and is associated with *Helicobacter pylori* infection, tobacco smoking, and gastric cancer subsites (Qinghai et al., 2014; Zhang et al., 2014). Wang et al. (2012) conducted a case-control study in Chinese Han women, which suggested that the AA genotype of rs2275913 is associated with the risk of breast cancer. Omrane et al. (2014) investigated the association between colorectal cancer and polymorphisms of rs2275913, and found that the GG genotype of rs2275913 is involved in susceptibility to colorectal cancer and is correlated with tumor location, tumor differentiation, and TNM stage. Only one study on a Chinese population has investigated polymorphisms in IL-17A and IL-17F in cervical cancer patients; the researchers found that IL-17 rs2275913 gene polymorphism is associated with susceptibility (Quan et al., 2012). We found that the AA genotype of rs2275913 was associated with a significantly increased risk of cervical cancer compared with the wild-type GG genotype, and that rs2275913 plays an important role in the pathogenesis of tumors.

Several limitations should be considered in our study. First, cases and controls were selected from one hospital, which may not be representative of other populations. However, the controls were a random sample from a pool of individuals who came to receive a health check-up, which may well represent the general population. Second, owing to the rarity of cervical cancer, the sample size of cervical cancer patients was relatively small. The small sample size could limit the statistical power to find an association between groups. Third, the risk of cervical cancer could be modified by many other genetic factors. Therefore, further studies with more subjects are needed to confirm the association between IL-17A and IL-17F polymorphisms and the risk of cervical cancer.

As a result of this case-control study, we suggest that rs2275913 may play a role in the etiology of cervical cancer. These findings could be helpful in identifying individuals at increased risk of developing cervical cancer, but further large sample studies are needed to confirm these associations.

Conflicts of interest

The authors declare no conflict of interest.

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