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Association analysis of rs2275913G>A and rs763780T>C interleukin 17 polymorphisms in Chinese women with cervical cancer

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ABSTRACT. We conducted a case-control study with a relatively large sample size, and investigated the association between rs2275913G>A and rs763780T>C and the risk of cervical cancer. Three hundred and six newly diagnosed patients with histologically confirmed cervical cancer and 354 cancer-free control subjects were recruited from the Forestry General Hospital between May 2011 and May 2014. The gene polymorphisms rs2275913G>A and rs763780T>C were identified using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. By unconditional logistic regression analysis, our study found that the AA genotype and the A allele of rs2275913 were associated with a higher risk of cervical cancer compared with the wild-type genotype, and the ORs (95%CIs) were 2.84 (1.57-5.23) and 1.55 (1.22-1.97), respectively. Compared with the G allele, the A allele of rs699947 was associated with a significantly increased risk of cervical cancer in subjects above 20 years and who were positive for human papillomavirus 16 (HPV-16) or HPV-18 infection. Patients with the A allele of rs2275913 had increased risk of cervical cancer, regardless of the number of births they had experienced

or their smoking habits. We suggest that rs2275913 may play a role in the etiology of cervical cancer, although further large-sample studies are needed to confirm these observations.

Key words: rs2275913G>A; rs763780T>C; Single nucleotide polymorphism; Cervical cancer; Interleukin 17; IL-17

INTRODUCTION

Cervical cancer is the fourth most common cancer among women and the seventh most common of all cancers globally; it is estimated that there were 528,000 new cases and 266,000 deaths in 2012 (International Agency for Research on Cancer, 2012). About 85% of the global burden occurs in the less developed regions, where it accounts for almost 12% of all female cancers (International Agency for Research on Cancer, 2012). Cervical cancer is caused by multiple factors, including smoking, number of sexual partners, age of first intercourse, and socioeconomic status, and its carcinogenesis is a complex and multistep process (Bonneau et al., 2014; Ekechi et al., 2014).

Interleukin-17 is an important pro-inflammatory cytokine and is a multifunctional protein that plays an important role in the genesis and maintenance of the inflammatory response (Tilahun et al., 2014; van Baarsen et al., 2014). Several previous studies have found that overexpression of IL-17A and IL-17F is associated with increased risk of various cancers (Dai et al., 2014; Kaabachi et al., 2014; Karczewski et al., 2014; Niu et al., 2014; Yu et al., 2014). rs2275913G>A and rs763780T>C are two important single nucleotide polymorphisms (SNPs) in IL-17A and IL-17F. Previous studies have reported that rs2275913G>A and rs763780T>C polymorphisms are associated with the risk of cancer (Niu et al., 2014; Zhang et al., 2014).

Only one previous study has reported an association between rs2275913G>A and rs763780T>C and cervical cancer risk (Quan et al., 2012). Therefore, we conducted this case-control study with a relatively large sample size, and investigated the association between rs2275913G>A and rs763780T>C and the risk of cervical cancer.

MATERIAL AND METHODS

Subjects

The study comprised 306 newly diagnosed patients with histologically confirmed cervical cancer and 354 cancer-free control subjects recruited from the Forestry General Hospital between May 2011 and May 2014. Patients who had previously suffered from cancer, or had previously undergone radiotherapy or chemotherapy, were excluded. Of the 398 cancer-free potential control subjects recruited from patients seeking care for hysteromyoma, ovarian cysts, and ectopic pregnancies, 354 agreed to participate in this study: a participation rate of 88.94%. Written informed consent was obtained from all cervical cancer patients who participated. The protocol of our study was approved by the Ethics Committee of the Forestry General Hospital.

All the patients were investigated using a questionnaire that we designed regarding demographic and reproductive information. The demographic and reproductive information included age, age at menarche, number of live births, cigarette smoking habits, alcohol consumption, and family history of cancer. Clinical information, including HPV16 and HPV-18 infection, and tumor

stage, was collected from the cervical patients' medical records.

Blood samples and genotyping

After an interview, a 5-mL venous blood sample was collected from each subject and stored at -20°C until required for DNA analysis. Genomic DNA was extracted from peripheral blood using a TIANamp Blood DNA Kit (TIANGEN Biotech Co., Ltd., Beijing, China) and DNA was dissolved in water according to the manufacturer instructions. The gene polymorphisms rs2275913G>A and rs763780T>C were identified using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay (Applied Biosystems, Foster City, CA, USA). The primers for rs2275913G>A and rs763780T>C were designed using Sequenom Assay Design 3.1 software (Sequenom, San Diego, CA, USA). We used the following regimen for the PCR: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 62°C for 60 s, extension at 72°C for 60 s, and final extension at 72°C for 10 min. For quality control, 10% of the patients were randomly selected to repeat the genotyping procedure with different researchers. The reproducibility was 100%.

Statistical analysis

All analyses were conducted using STATA version 9.0 statistical software. Categorical variables are expressed as frequencies and percentages. The χ^2 test was used to compare the difference in the distributions of demographic, lifestyle, and clinical characteristics as well as genetic frequencies between cervical cases and controls. A goodness-of-fit χ^2-test was taken to calculate the Hardy-Weinberg equilibriums (HWEs) of rs2275913G>A and rs763780T>C genotype frequencies in the controls. Unconditional logistic regression analysis (with ORs and their corresponding 95%CIs) was used to analyze the associations between rs2275913G>A and rs763780T>C gene polymorphisms and the risk of cervical cancer. Unconditional logistic regression analysis was also conducted to assess the gene-environment interaction. Two-tailed P values of < 0.05 were considered statistically significant.

RESULTS

A total of 306 patients with cervical cancer were invited to participate in our study. The demographic and clinical characteristics of the cervical cancer patients and controls are presented in Table 1. The analysis revealed that cervical cancer patients were more likely to be older, to have experienced more live births, to have had a smoking habit, and to have had a HPV-16 or HPV-18 infection ($P < 0.05$).

The allele and genotype distributions of rs2275913G>A and rs763780T>C in cases and controls are presented in Table 2.

The genotype distributions of rs2275913G>A and rs763780T>C of the controls were in line with HWE, and the P values for HWE were 0.34 and 0.32, respectively. By unconditional logistic regression analysis, our study found that the AA genotype and the A allele of rs2275913 were associated with a higher risk of cervical cancer compared with the wild-type genotype, and the ORs (95%CI) were 2.84 (1.57-5.23) and 1.55 (1.22-1.97), respectively. However, we did not find significant correlation between rs763780 polymorphism and the risk of cervical

cancer.

Stratification analyses of age, number of live births, cigarette consumption, and HPV-16 or HPV-18 infection with the rs2275913 polymorphism are shown in Table 3. Compared with the G allele, the A allele of rs699947 was associated with a significantly increased risk of cervical cancer for those above 20 years and those who had experienced HPV-16 or HPV-18 infection. Patients with the A allele of rs2275913 had increased risk of cervical cancer, regardless of the number of births they had experienced or their smoking habits.

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

Variables	Cases (N = 306)	% (N = 354)	Controls	%	χ^2-test	P value
Age, year						
<55	133	43.5	185	52.3		
≥55	173	56.5	169	47.7	5.09	0.02
Age at menarche						
<6	243	79.4	274	77.4		
≥16	63	20.6	80	22.6	0.39	0.53
Number of live births						
≤1	141	46.1	195	55.1		
≥2	165	53.9	159	44.9	5.33	0.02
Smoking status						
Ever	35	11.4	22	6.2		
Never	271	88.6	332	93.8	5.67	0.02
Alcohol consumption						
Ever	82	26.8	87	24.6		
Never	224	73.2	267	75.4	0.43	0.51
Menopausal status						
Premenopausal	124	40.5	135	38.1		
Postmenopausal	182	59.5	219	61.9	0.39	0.53
Family history of cancer						
No	278	90.8	324	91.5		
Yes	28	9.2	30	8.5	0.09	0.76
HPV-16 or HPV-18 infection						
Negative	56	18.3	282	79.7		
Positive	250	81.7	72	20.3	247.33	<0.001
Stage						
I-II	233	76.1				
III-IV	73	23.9				

Table 2. Association between rs2275913G>A and rs763780T>C polymorphisms and cervical cancer risk.

Variable	Cases	%	Controls	%	HWE	OR (95%CI)[1]	P value
rs2275913							
GG	128	41.7	186	52.5		1.0 (Ref.)	-
GA	135	44.1	146	41.3		1.34 (0.96-1.88)	0.07
AA	43	14.2	22	6.2	0.34	2.84 (1.57-5.23)	<0.001
G allele	390	63.8	518	73.1		1.0 (Ref.)	-
A allele	222	36.2	190	26.9		1.55 (1.22-1.97)	<0.001
rs763780						2.84 (1.57-5.23)	<0.001
TT	74	36.3	162	41.7		1.0 (Ref.)	-
TC	108	46.4	213	47.1		1.55 (1.22-1.97)	<0.001
CC	34	17.3	57	11.2	0.32		
T allele	256	59.5	537	65.2		1.0 (Ref.)	-
C allele	176	40.5	327	34.8		1.11 (0.76-1.62)	0.57

HWE: Hardy-Weinberg equilibrium; OR: odds ratio; 95%CI: 95% confidence interval. [1]Adjusted for age, number of live births, smoking habits, and HPV-16 or HPV-18 infection.

Table 3. Association between rs2275913 and cervical cancer risk stratified by demographic and clinical characteristics.

Variables	rs2275913				OR (95%CI)	P value
	Cases		Controls			
	G allele	A allele	G allele	A allele	A allele *vs* G allele	
Age, years						
≤20	176	269	90	101	1.36 (0.95-1.94)	0.08
>20	215	249	131	89	1.71 (1.22-2.39)	0.001
Number of live births						
≤1	173	279	109	111	1.58 (1.13-2.22)	0.006
≥2	218	239	112	79	1.56 (1.10-2.24)	0.01
Smoking status						
Ever	35	31	35	13	2.38 (1.01-5.81)	0.03
Never	366	487	176	177	1.32 (1.02-1.71)	0.03
HPV-16 or HPV-18 infection						
Negative	82	413	30	151	1.01 (0.61-1.61)	0.99
Positive	309	105	191	39	1.66 (1.09-2.58)	0.01

OR = odds ratio; 95%CI = 95% confidence interval.

DISCUSSION

In this case-control study, we investigated the relationship between rs2275913G>A and rs763780T>C polymorphisms and the risk of cervical cancer. We also explored gene-environment interactions and the development of the cancer. Our study found that the AA genotype and the A allele of rs2275913 were associated with a higher risk of cervical cancer compared with the wild-type genotype.

Previous studies have reported that rs2275913 polymorphism in the IL-17A gene promoter region is associated with the risk of several kinds of cancers, such as gastric, colorectal, breast, and cervical cancers (Wang et al., 2012; Omrane et al., 2014; Qinghai et al., 2014; Zhang et al., 2014). Wang et al. (2012) conducted a case-control study in China and showed that rs2275913 is associated with increased risk of breast cancer. One study conducted in a Chinese population reported that rs2275913G>A and rs763780T>C polymorphisms increase gastric cancer risk, and interact with *Helicobacter pylori* infection and subsites (Zhang et al., 2014). Omrane et al. (2014) conducted a case-control study to investigate the association between colorectal cancer and the rs2275913G>A polymorphism; they found that the A allele of the IL-17A gene is involved in susceptibility to colorectal cancer and is associated with clinical features such as tumor location, tumor differentiation, and TNM stage. IL-17A polymorphism may serve as a biomarker for disease location and progression. One previous study has found that IL-17 gene polymorphism rs2275913 is associated with susceptibility to cervical cancer, as well as positive peritumor intravascular cancer emboli and advanced clinical stage, in Chinese women (Quan et al., 2012). The present study found that the AA genotype of rs2275913 was associated with increased risk of cervical cancer, which is in line with previous studies.

Our study found that rs2275913 had an association with HPV infection. Inflammation plays an important role in maintaining and promoting cancer progression, and in effecting the full malignant phenotype, by such processes as tumor tissue rebuilding, angiogenesis, metastasis, and suppression of the innate anticancer immune response (Chechlinska et al., 2010). Genetic and epigenetic mutations can trigger cell transformation and maintain the autonomous proliferation of the transformed cancer cells. Therefore, rs2275913 may have an association with HPV infection

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. Further large-sample studies are needed to confirm these associations. Large-scale case-control studies are urgently needed to determine more precisely the relationship between the polymorphism and potential gene-gene and gene-environment interactions.

Conflicts of interest

The authors declare no conflict of interest.

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