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Original Article

Interleukin-31 single nucleotide polymorphisms are significantly associated with endometrial cancer in Chinese Han women

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Abstract: Objective: At present, cancer genetic markers of susceptibility (CGEMS) are a very important cancer research topic. This study aims to investigate possible correlations between the emergence of endometrial cancer (EC) and the presence of polymorphisms in two gene sites (rs4758680 and rs7977932) of interleukin-31 (IL-31) in a well-defined Chinese cohort. Methods: Polymerase Chain-Reaction (PCR) was performed to determine the genotypic composition and the allelic frequencies of IL-31 gene variants in 255 EC patients and 370 healthy controls. Results: Our results revealed a statistically significant association between the presence of allele A of rs4758680 and increased EC risk (P = 0.009). Moreover, genotypic frequencies in the codominant (P = 0.0041), dominant (P = 0.0018), and overdominant (P<0.001) genetic models of rs4758680 were associated with EC susceptibility. For rs7977932, allele G was statistically more frequent in EC patients (P = 0.043). Conclusions: Our findings suggest that polymorphisms in rs4758680 and rs7977932 of IL-31 may have a role in increased susceptibility to EC in Chinese Han women.

Keywords: Endometrial cancer, interleukin-31, single nucleotide polymorphisms

Introduction

Endometrial cancer (EC) is the sixth most common tumor in women worldwide and the leading carcinoma of the female genital tract in the developed countries. Worldwide, more than 287,100 women are newly diagnosed with this disease each year [1], with most patients being diagnosed at an early stage. Although the five-year survival rate for stage I EC patients can reach up to 81%-91% [2], metastatic EC remains therapeutically challenging. Numerous risk factors for the development of EC have been validated including obesity, unopposed estrogen therapy, and anovulation [3]. However, the molecular mechanisms of endometrium carcinogenesis remain poorly understood. In recent years, considerable progress has been made to clarify the association between inflammatory pathways and cancer development [4]. Some inflammatory mediators such as C-reactive protein, NF-κB and interleukins [4, 5] were shown to be involved in the process of angiogenesis, neoplastic transformation and immune response.

Interleukin 31 (IL-31), a pro-inflammatory cytokine of to the gp130/IL-6 cytokine family, was first reported in 2004 as a four-helix bundle cytokine [6]. This cytokine is mainly secreted by activated T helper type 2 (Th2) cells [6], CD45RO+ CLA+ (cutaneous lymphocyte-associated antigen-positive) T cells [7], mast cells [8], monocytes/macrophages and dendritic cells [9]. IL-31 signals through a heteromeric receptor complex composed of the IL-31 recep-

Table 1. Information about PCR-RELP in EC and control groups

SNP ID	Primer sequence	Major/ minor gene	Annealing temperature (°C)	Restriction enzyme	PCR product (bp)	Allele (bp)
rs4758680	F: 5'-AGGTCTGTGGGTGGAGACAG-3'	C/A	60.0°C	MboII	130	C (99+31)
	R: 5'-TTTCCCCCGAGATAAGATGA-3'					A (130)
rs7977932	F: 5'-GGTCAGTGTTGGGTTTGCAATG-3'	C/G	58.3°C	ScrFI	131	G (74+57)
	R: 5'-TTGGTGATGGCACAGCCTCATA-3'					C (131)

tor alpha (IL-31RA) and the oncostatin M receptor beta (OSMR) subunits [10].

An increased IL-31 level has been detected in serum or tissues from patients with atopic dermatitis, chronic spontaneous urticaria, allergic contact dermatitis, prurigo nodularis, primary cutaneous mastocytosis, bowel diseases, allergic asthma and rhinitis [11, 12], which suggests that IL-31 is involved in the pathogenesis of allergic diseases. Tseng et al. [13] demonstrated that IL-31 was significantly associated with coronary artery lesion formation in patients with Kawasaki disease. Moreover, there has been increasing evidence that IL-31 may play an important role in cancer-related diseases, including human malignant lymphomas of T-cell lineage, human follicular lymphoma [12], lung cancer [14], and endometrial cancer [15]. However, the effect of the different genetic polymorphisms of IL-31 on the onset of EC remains unexplored. Thus, we conducted a pilot study to clarify the association between IL-31 gene polymorphisms (rs7977932, rs4758680) and the risk of EC in the Chinese Han population.

Material and methods

Study population

All the EC women (n = 255, mean age: 51.77±9.99 years) had the same ethnic background (Chinese Han) and were recruited from the West China Second University Hospital from July 2008 to October 2015. In all patients, EC was diagnosed by histological examination of the endometrium sample after hysterectomy or diagnostic curettage. The International Federation of Gynecology and Obstetrics (FIGO) criteria were used to define the operation-pathology stages of EC. The patients with a history of any other malignancy or autoimmune diseases were excluded from this study. The control subjects were age-matched healthy women (n = 370, mean age: 50.67±11.45 years) as assessed by a routine medical checkup during the same period. The present study was ratified by a medical ethics committee, in accordance with the principles of the Declaration of Helsinki. All included subjects were informed regarding this research project, and their written informed consent was obtained. Peripheral venous blood samples from each of the EC women and control subjects were obtained and stored at -80°C.

SNP genotyping

We extracted genomic DNA from 10 ml blood samples using a whole blood DNA isolation kit (BioTeke, Peking, China) according to the manufacturer's protocol. The purified DNA was stored at -20°C until use. Genotyping of rs4758680 (alleles A and C) and rs7977932 (alleles C and G) SNPs sites the IL-31 gene was carried out by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. The software Primer 3 (http://bioinfo.ut.ee/primer3-0.4.0/primer3/) [16] was used to design the PCR primers which sequences are listed in **Table 1**. PCR amplification was carried out in the total volume of 10 μl containing: 5 μl 2× Power Taq PCR Master Mix (BioTeke, Peking, China), 0.5 ul DNA template, 0.1 μl of each primer and 4.3 ul sterilized water. The PCR cycle conditions consisted of an initial denaturation step at 94.0°C for 3 minutes followed by 35 cycles as follows: denaturing for 30 seconds at 94.0°C, primer annealing for 30 seconds at 60°C for rs4758680 and 58.3°C for rs7977932, primer extension for 30 seconds at 72°C. A final elongation step was carried out at 72°C for 10 minutes. The PCR products were digested with the restriction enzymes MboII (30 minutes, 37°C) and ScrFI (overnight, 37°C) for rs4758680 and rs7977932, respectively. Both enzymes were obtained from New England Biolabs, Peking, China. The bands were separated by electrophoresis using 6% polyacrylamide gels and stained with silver nitrate for visualization. About 10% of the PCR-amplified products were randomly selected for DNA sequencing analyses in order to confirm the genotype, and the two detection methods showed the results had a reproducibility of 100%.

Table 2. Descriptive characteristics of the EC patients and the control

Characteristics		Number of case (%)	Number of controls (%)	*P* value
Sample size		255	370	
Age mean ± SD (range) (year)		51.77±9.99 (25-81)	50.67±11.45 (19-72)	0.215
BMI mean ± SD (kg/m^2)		24.23±3.43	23.78±3.64	0.120
Menopausal status	Premenopausal	124 (48.6%)	169 (45.7%)	0.467
	Postmenopausal	131 (51.4%)	201 (54.3%)	
Family history of cancer	Yes	21 (8.2%)	20 (5.4%)	0.160
	No	234 (91.8%)	350 (94.6%)	
History of pregnancy	Yes	242 (94.9%)	352 (95.1%)	0.895
	No	13 (5.1%)	18 (4.9%)	
Abnormal uterine bleeding	Yes	247 (96.9%)		
	No	8 (3.1%)		
FIGO grade	G1	90 (35.3%)		
	G2	94 (36.9%)		
	G3	71 (27.8%)		
FIGO stage	I	191 (74.9%)		
	II	21 (8.2%)		
	III	29 (11.4%)		
	IV	12 (4.7%)		
	Unknow[a]	2 (0.8%)		
Histology	Endometrioid adenocarcinoma	214 (83.9%)		
	Non-endometrioid adenocarcinoma[b]	41 (16.1%)		

Abbreviations: SD, standard deviation; BMI, body mass index; FIGO, International Federation of Gynecology and Obstetrics. [a]No surgery; [b]Serous adenocarcinoma: 10; clear cell adenocarcinoma: 5; neuroendocrine carcinoma: 2; mixed: 24 (including adenosquamous carcinoma 12, carcinosarcoma 2 and other 10).

Statistical analysis

The chi-square test was employed to test for Hardy-Weinberg equilibrium, as in to compare the expected genotype frequencies with observed genotype frequencies. The distribution of family cancer history, menopausal status, pregnancy history between EC patients and controls, as well as allelic frequencies of the IL-31 polymorphic sites (rs4758680 and rs7977932) were also analyzed by a chi-square test. The odds ratios (ORs) with the respective confidence intervals (95% CI) were obtained. Continuous variables (Age and Body Mass Index) are presented as mean ± standard deviation and were analyzed by a Student's t test. All statistical analyses were by SNPstats online program and SPSS statistical software (version 13.0, Chicago, USA). A *p* value <0.05 was considered statistically significant.

Results

Clinical characteristics and Hardy-Weinberg equilibrium test

The general characteristics of the EC and control subjects were shown in **Table 2**. No statistically significant differences in mean age, mean BMI, menopausal status, family history of cancer and history of pregnancy were identified between the EC patients and the control groups (P>0.05). From all the 255 eligible EC patients, 253 (99.2%) patients underwent surgical treatment, 191 (74.9%) were diagnosed with FIGO's stage I, and endometrioid adenocarcinoma was the most common pathological type (214, 83.9%). In the present study, the genotypic frequencies of rs4758680 and rs7977932 polymorphic sites in the EC patients and control subjects conformed to the Hardy-Weinberg equilibrium expectations (HWE, with P>0.05 for all).

The distribution of IL-31 genotypes and allele frequencies

As shown in **Table 3**, the frequency of allele A of the rs4758680 was significantly higher in EC patients (21.8%) than in controls (15.9%) (P = 0.009, OR (95% CI) = 0.68 (0.51-0.91)). In the codominant model, the genotype frequencies of the CC, CA, and AA for rs4758680 were 59.6%, 37.2%, and 3.1% in the EC group and 71.6%, 24.9%, and 3.5% in the control group. A significant statistical difference was observed

Table 3. Genotype and allele distributions of IL-31 SNPs in patients with EC and controls

		Genotype	Cases			Controls n = 370 (%)	Logistic regression					
							Cases vs. Controls		FIGO I vs. Controls		EA vs. Control	
			Total n = 255 (%)	FIGO I n = 191 (%)	EA n = 214 (%)		OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
rs4758680												
Genetic model	Codominant	C/C	152 (59.6%)	115 (60.2%)	127 (59.4%)	265 (71.6%)	1.00	0.0041	1.00	0.015	1.00	0.0029
		C/A	95 (37.2%)	70 (36.6%)	82 (38.3%)	92 (24.9%)	0.56 (0.39-0.79)		0.57 (0.39-0.83)		0.54 (0.37-0.77)	
		A/A	8 (3.1%)	6 (3.1%)	5 (2.3%)	13 (3.5%)	0.93 (0.38-2.30)		0.94 (0.35-2.54)		1.25 (0.43-3.57)	
	Dominant	C/C	152 (59.6%)	115 (60.2%)	127 (59.4%)	265 (71.6%)	1.00	0.0018	1.00	0.0065	1.00	0.003
		C/A-A/A	103 (40.4%)	76 (39.8%)	87 (40.6%)	105 (28.4%)	0.58 (0.42-0.82)		0.60 (0.42-0.87)		0.58 (0.41-0.82)	
	Recessive	C/C-C/A	247 (96.9%)	185 (96.9%)	209 (97.7%)	357 (96.5%)	1.00	0.8	1.00	0.82	1.00	0.42
		A/A	8 (3.1%)	6 (3.1%)	5 (2.3%)	13 (3.5%)	1.12 (0.46-2.75)		1.12 (0.42-3.00)		1.52 (0.54-4.33)	
	Overdominant	C/C-A/A	160 (62.8%)	121 (63.4%)	132 (61.7%)	278 (75.1%)	1.00	<0.001	1.00	0.0039	1.00	<0.001
		C/A	95 (37.2%)	70 (36.6%)	82 (38.3%)	92 (24.9%)	0.56 (0.39-0.79)		0.57 (0.39-0.83)		0.53 (0.37-0.77)	
	Log-additive	-	-	-	-	-	0.68 (0.51-0.91)	0.0093	0.70 (0.51-0.95)	0.024	0.69 (0.51-0.94)	0.018
Allele		C	399 (78.2%)	300 (78.5%)	336 (78.5%)	622 (84.1%)	1.00	0.009	1.00	0.022	1.00	0.017
		A	111 (21.8%)	82 (21.5%)	92 (21.5%)	118 (15.9%)	0.68 (0.51-0.91)		0.69 (0.51-0.95)		0.69 (0.51-0.94)	
rs7977932												
Genetic model	Codominant	C/C	191 (74.9%)	141 (73.8%)	164 (76.6%)	300 (81.1%)	1.00	0.13	1.00	0.069	1.00	0.21
		C/G	58 (22.8%)	44 (23%)	44 (20.6%)	66 (17.8%)	0.72 (0.49-1.08)		0.71 (0.46-1.08)		0.82 (0.54-1.26)	
		G/G	6 (2.4%)	6 (3.1%)	6 (2.8%)	4 (1.1%)	0.42 (0.12-1.52)		0.31 (0.09-1.13)		0.36 (0.10-1.31)	
	Dominant	C/C	191 (74.9%)	141 (73.8%)	164 (76.6%)	300 (81.1%)	1.00	0.066	1.00	0.05	1.00	0.2
		C/G-G/G	64 (25.1%)	50 (26.2%)	50 (23.4%)	70 (18.9%)	0.70 (0.47-1.02)		0.66 (0.43-1.00)		0.77 (0.51-1.15)	
	Recessive	C/C-G/G	249 (97.7%)	185 (96.9%)	208 (97.2%)	366 (98.9%)	1.00	0.22	1.00	0.091	1.00	0.13
		G/G	6 (2.4%)	6 (3.1%)	6 (2.8%)	4 (1.1%)	0.45 (0.13-1.62)		0.34 (0.09-1.21)		0.38 (0.11-1.36)	
	Overdominant	C/C-G/G	197 (77.2%)	147 (77%)	170 (79.4%)	304 (82.2%)	1.00	0.13	1.00	0.15	1.00	0.42
		C/G	58 (22.8%)	44 (23%)	44 (20.6%)	66 (17.8%)	0.74 (0.50-1.10)		0.73 (0.47-1.11)		0.84 (0.55-1.28)	
	Log-additive	-	-	-	-	-	0.70 (0.50-0.99)	0.047	0.66 (0.46-0.95)	0.026	0.75 (0.52-1.07)	0.12
Allele		C	440 (86.3%)	326 (85.3%)	372 (86.9%)	666 (90.0%)	1.00	0.043	1.00	0.021	1.00	0.122
		G	70 (13.7%)	56 (14.7%)	56 (13.1%)	74 (10.0%)	0.70 (0.49-0.99)		0.65 (0.45-0.94)		0.74 (0.51-1.07)	

Abbreviations: FIGO, International Federation of Gynecology and Obstetrics; OR, odds ratio; CI, confidence interval; EA, endometrioid adenocarcinoma.

Table 4. Analysis of clinical characteristics and polymorphism of locus rs4758680

Clinical characteristics		Genotype			Genetic model								Allele	
		CC	CA	AA	Codominant (CC vs. CA vs. AA)		Dominant (CC vs. CA/AA)		Recessive CC/CA vs. AA)		Overdominant (CC/AA vs. CA)		C vs. A	
					OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Pathological type	EA	127	82	5	CA: 0.81 (0.39-1.66)	0.28	0.93 (0.47-1.85)	0.85	3.30 (0.76-14.39)	0.14	0.75 (0.37-1.53)	0.42	1.10 (0.63-1.93)	0.74
	Non-EA	25	13	3	AA: 3.05 (0.68-13.58)									
FIGO grade	G1	54	34	2	CA: 0.99 (0.58-1.69)	0.82	1.03 (0.61-1.73)	0.92	1.66 (0.33-8.40)	0.52	0.97 (0.57-1.64)	0.9	1.06 (0.68-1.65)	0.79
	G2-G3	98	61	6	AA: 1.65 (0.32-8.47)									
FIGO stage	I	115	70	6	CA: 1.10 (0.60-1.99)	0.96	1.09 (0.61-1.96)	0.77	1.03 (0.20-5.23)	0.97	1.09 (0.61-1.97)	0.77	1.07 (0.66-1.74)	0.79
	II-IV	36	24	2	AA: 1.06 (0.21-5.51)									
BMI	<28	124	80	5	CA: 0.31 (0.11-0.84)	0.021	0.29 (0.11-0.79)	0.008	0.00 (0.00-NA)	0.24	0.32 (0.12-0.88)	0.015	0.33 (0.13-0.85)	0.017
	≥28	25	5	0	AA: 0.00 (0.00-NA)									
Menopausal status	Premenopausal	74	45	5	CA: 1.05 (0.63-1.76)	0.71	1.01 (0.61-1.66)	0.98	0.56 (0.13-2.38)	0.42	1.08 (0.65-1.80)	0.76	0.95 (0.63-1.45)	0.83
	Postmenopausal	78	50	3	AA: 0.57 (0.13-2.47)									
Myometrial invasion	<1/2	119	69	6	CA: 1.35 (0.74-2.46)	0.62	1.34 (0.74-2.41)	0.33	1.10 (0.22-5.60)	0.91	1.33 (0.74-2.41)	0.35	1.24 (0.76-2.01)	0.39
	≥1/2	32	25	2	AA: 1.24 (0.24-6.44)									
Cervical invasion	Negative	124	81	6	CA: 0.74 (0.36-1.51)	0.58	0.79 (0.40-1.58)	0.5	1.71 (0.33-8.77)	0.54	1.71 (0.33-8.77)	0.54	0.90 (0.50-1.63)	0.72
	Positive	27	13	2	AA: 1.53 (0.29-8.00)									
Parametrial invasion	Negative	139	85	7	CA: 1.23 (0.50-3.03)	0.85	1.26 (0.52-3.03)	0.61	1.52 (0.18-12.98)	0.71	1.19 (0.49-2.90)	0.7	1.22 (0.60-2.51)	0.58
	Positive	12	9	1	AA: 1.65 (0.19-14.59)									
Lymph node status	Negative	135	84	7	CA: 1.00 (0.44-2.32)	0.99	1.02 (0.45-2.30)	0.96	1.20 (0.14-10.17)	0.87	0.99 (0.44-2.27)	0.99	1.03 (0.52-2.04)	0.93
	Positive	16	10	1	AA: 1.21 (0.14-10.44)									
Peritumor intravascular cancer emboli	Negative	128	82	8	CA: 0.81 (0.38-1.73)	0.26	0.74 (0.35-1.57)	0.43	0.00 (0.00-NA)	0.12	0.87 (0.41-1.83)	0.7	0.71 (0.37-1.38)	0.32
	Positive	23	12	0	AA: 0.00 (0.00-NA)									

Abbreviations: FIGO, International Federation of Gynecology and Obstetrics; BMI, body mass index; OR, odds ratio; CI, confidence interval; EA, endometrioid adenocarcinoma.

Table 5. Analysis of clinical characteristics and polymorphism of locus rs7977932

Clinical characteristics		Genotype			Genetic model								Allele	
		CC	CG	GG	Codominant (CC vs. CG vs. GG)		Dominant (CC vs. CG/GG)		Recessive (CC/CG vs. GG)		Overdominant (CC/GG vs. CG)		C vs. G	
					OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Pathological type	EA	164	44	6	CG: 1.93 (0.93-4.00)	0.076	1.70 (0.83-3.49)	0.16	0.00 (0.00-NA)	0.14	2.00 (0.97-4.14)	0.067	1.37 (0.72-2.59)	0.34
	Non-EA	27	14	0	GG: 0.00 (0.00-NA)									
FIGO grade	G1	68	19	3	CG: 1.13 (0.61-2.12)	0.7	1.06 (0.58-1.91)	0.86	0.54 (0.11-2.72)	0.46	1.16 (0.62-2.15)	0.64	0.98 (0.58-1.66)	0.94
	G2-G3	123	39	3	GG: 0.55 (0.11-2.81)									
FIGO stage	I	141	44	6	CG: 0.93 (0.47-1.85)	0.18	0.82 (0.42-1.62)	0.57	0.00 (0.00-NA)	0.064	0.97 (0.49-1.93)	0.94	0.74 (0.40-1.38)	0.35
	II-IV	48	14	0	GG: 0.00 (0.00-NA)									
BMI	<28	159	45	5	CG: 1.35 (0.56-3.24)	0.77	1.36 (0.59-3.17)	0.48	1.41 (0.16-12.47)	0.77	1.33 (0.55-3.18)	0.53	1.32 (0.63-2.76)	0.458
	≥28	21	8	1	GG: 1.51 (0.17-13.60)									
Menopausal status	Premenopausal	87	32	5	CG: 0.68 (0.38-1.23)	0.088	0.61 (0.34-1.08)	0.089	0.18 (0.02-1.59)	0.073	0.71 (0.40-1.28)	0.26	0.59 (0.35-0.98)	0.04
	Postmenopausal	104	26	1	GG: 0.17 (0.02-1.46)									
Myometrial invasion	<1/2	146	43	5	CG: 1.18 (0.60-2.34)	0.82	1.13 (0.58-2.19)	0.71	0.65 (0.07-5.69)	0.69	1.20 (0.61-2.36)	0.61	1.06 (0.59-1.92)	0.84
	≥1/2	43	15	1	GG: 0.68 (0.08-5.97)									
Cervical invasion	Negative	159	46	6	CG: 1.38 (0.66-2.91)	0.23	1.22 (0.58-2.56)	0.6	0.00 (0.00-NA)	0.14	1.43 (0.68-3.02)	0.35	1.05 (0.54-2.05)	0.9
	Positive	30	12	0	GG: 0.00 (0.00-NA)									
Parametrial invasion	Negative	171	54	6	CG: 0.70 (0.23-2.17)	0.47	0.63 (0.21-1.95)	0.41	0.00 (0.00-NA)	0.29	0.73 (0.24-2.24)	0.57	0.60 (0.21-1.73)	0.34
	Positive	18	4	0	GG: 0.00 (0.00-NA)									
Lymph node status	Negative	168	52	6	CG: 0.92 (0.35-2.41)	0.5	0.83 (0.32-2.15)	0.69	0.00 (0.00-NA)	0.24	0.96 (0.37-2.49)	0.93	0.76 (0.31-1.84)	0.54
	Positive	21	6	0	GG: 0.00 (0.00-NA)									
Peritumor intravascular cancer emboli	Negative	167	46	5	CG: 1.98 (0.91-4.30)	0.24	1.93 (0.91-4.11)	0.093	1.25 (0.14-11.05)	0.84	1.95 (0.90-4.21)	0.098	1.70 (0.89-3.25)	0.11
	Positive	22	12	1	GG: 1.52 (0.17-13.60)									

Abbreviations: FIGO, International Federation of Gynecology and Obstetrics; BMI, body mass index; OR, odds ratio; CI, confidence interval; EA, endometrioid adenocarcinoma.

among the genotypic frequencies (P = 0.0041) between the EC patient and the control groups. In the dominant model, the CC genotype for rs4758680 was associated with a decreased risk of cancer as compared to the CA-AA genotypes (P = 0.0018, OR (95% CI) = 0.58 (0.42-0.82)). Compared with the CC-AA genotypes, subjects with heterozygous genotype (CA) were found to be over-represented in patients with EC (P<0.001, OR (95% CI) = 0.56 (0.39-0.79)). Similar results for rs4758680 were observed between FIGO's early stage I EC patients and controls, as well as patients with endometrioid adenocarcinoma and controls. In terms of rs7977932, allele G was significantly more frequent in EC patients (13.7%) vs. controls (10.0%) (P = 0.043, OR (95% CI) = 0.70 (0.49-0.99)), especially in FIGO I stage patients (14.7%) (P = 0.021, OR (95% CI) = 0.6 (0.45-0.94)). However, no differences were observed in genotypic frequency of rs7977932 polymorphisms between EC and control subjects when Chi-Square test was used (P>0.05).

Association between IL-31 gene polymorphisms and clinical features

As listed in **Tables 4** and **5**, a stratification analysis was performed among the EC patients according to histological types, FIGO stages and grades, BMI, menopausal status, myometrial invasion, cervical invasion, parametrial invasion, lymph node status and peritumor intravascular cancer emboli. For rs4758680, allele C frequency significantly increased in obese EC patients, with obesity defined as BMI>28 [17] (BMI<28 (78.5%) vs. BMI≥28 (91.7%), P = 0.017, OR (95% CI) = 0.33 (0.13-0.85)). Moreover, we observed an increased distribution of CC genotype in EC patients with BMI≥28 both in the codominant genetic model (P = 0.021, OR (95% CI) = 0.31 (0.11-0.84)) and in the dominant genetic model (P = 0.0075, OR (95% CI) = 0.29 (0.11-0.79)). For rs7977932, the frequency of allele G was higher in premenopausal (16.9%) than in postmenopausal (10.7%) women of the EC group (P = 0.04, OR (95% CI) = 0.59 (0.35-0.98)). However, we did not find evidence for any other significant association between gene polymorphisms and the remaining clinical features.

Discussion

The IL-31 gene is located on chromosome 12q24.31 and includes 3 exons spanning 2.2 kb of genomic DNA. Recently, IL-31 gene polymorphisms have been associated with several diseases. Schulz et al. [18] found 15 SNPs within IL31 by sequencing the complete gene and identified 3 most common haplotypes that were present in 93.4% of the 78 unrelated German individuals. Furthermore, it was the first to report that a particular IL-31 gene haplotype is strongly associated with non-atopic eczema. However, Hong et al. [19] analyzed two IL-31 SNPs, rs10847385 and rs7974857, and found that the distribution of the IL-31 gene polymorphisms was not statistically different between extrinsic atopic dermatitis (AD) patients and controls. Their study also suggested that the IL-31 rs10847385 polymorphism might influence the IL-31 blood level, IgE level and disease severity in extrinsic AD patients. Furthermore, a large sample study was performed by Lan et al. [20] including 1132 Taiwanese females (nursing staff) showing that allele G of rs7977932 site of IL-31 gene was associated with increased risk of development of atopic eczema. Similar findings were reported by Sokołowska-Wojdyło M et al. [21] in Polish patients with atopic dermatitis.

Besides allergic skin disorders, IL31 may be involved in the pathogenesis of the connective tissue disease and other allergic diseases. The results by Huang HT et al. [22] indicated that the CG or GG genotypes of IL-31 rs7977932 had higher IL-31 levels than CC genotype in Chinese population with systemic lupus erythematosus. Furthermore, Yu JI et al. [23] showed that the g.1066G>A, g.586C>A and g.1449C>G polymorphisms of IL-31 were associated with the level of rheumatoid factors and anti-cyclic citrullinated peptide in female rheumatoid arthritis patients. In patients with asthma, the rs7312610 and rs7974857 of the IL-31 were significantly associated with the total serum IgE levels [24]. In the recently published studies, the relationship between the distinct polymorphic variants of the IL-31 gene and mastocytosis [25] and dilated cardiomyopathy [26] were also investigated.

In recent years, many studies indicated that IL-31 might be closely related to the pathogenesis of tumors. Shiri Davidi et al. reported that IL-31 anti-tumor cell activity is mediated by an inhibitory effect on angiogenesis and metastasis, demonstrating its potential use as a novel drug for the treatment of cancer [27]. Mycosis fungoides (MF) and Sézary syndrome

(SS) belong to the group of primary cutaneous T-cell lymphomas (CTCL) which are characterized by an infiltration of the skin with neoplastic skin-homing T-cells. Möbs M et al. [28] observed increased IL-31 levels in MF and SS patients with severe pruritus. Evidence provided by Cedeno-Laurent F et al. [29] suggested that an inhibitor of the IL-31 pathway may relieve itch by down-regulating the levels of IL-31-expressing T cells in CTCL. Malek M et al. [30] reported that compared with the early stages of CTCL patients, the distribution of allele A IVS2+12A/G locus was more frequent in the advanced stages, indicating it might be a promoting factor for tumor progression. Furthermore, Ferretti E et al. [12] studied the relations between the IL-31/IL-31 receptor complex and human follicular lymphoma (FL), a prototypic germinal center (GC)-derived B-cell malignancy, and discovered that the IL-31/IL-31RA axis plays a paracrine/autocrine role in the promotion of FL growth by microvesicle shedding. Besides human lymphomas, a negative correlation between concentrations of IL-31 before and after chemotherapy in non-small cell lung cancer (NSCLC) patients, was reported by Naumnik W et al. [14]. In 2016, Zeng X et al. [15] firstly reported that serum levels of IL-31 were significantly increased in EC patients compared to healthy women, with concentrations closely following FIGO cancer stages, myometrial invasion, status of lymph node and distant metastases. Using 113.1 pg/mL as a cut-off value, IL-31 showed a better diagnostic sensitivity (92.68%) and specificity (92.68%) than CEA, CA-125 and CA-199.

Although the study of cancer genetic markers of susceptibility (CGEMS) is one of the main foci in current cancer research, the correlation between genetic polymorphisms of IL-31 and EC remains unclear. Given the wealth of evidence described earlier, we conducted a study to assess the influence of IL-31 genetic polymorphisms on EC. In the present study, we assumed that both rs4758680 and rs7977932 polymorphisms might contribute to the pathogenesis of EC. Since both rs4758680 and rs7977932 are located in introns of IL-31 gene, therefore they may have influence on the regulation of gene express. Our data showed that the risk of developing EC decreased in a population with increased numbers of the rs4758680 allele C. Furthermore, the genotypic frequency of rs4758680 CC genotype was linked with lower EC risk in the codominant and dominant models. For rs7977932, the frequency of allele C significantly decreased in EC women as compared with healthy women. Therefore, allele C could be regarded as a protective factor for EC, whereas allele G might be considered as the disease-causing factor.

Stage I of EC was defined as tumor confined to the corpus uteri, neither invading the cervical stroma nor extending beyond the uterus, as according to the FIGO system. In our study, 74.9% EC patients were diagnosed at FIGO stage I similarly to a previous study (73%) [2]. We analyzed two SNPs in the IL-31 in 191 FIGO I patients and 370 controls (**Table 3**), suggesting both rs4758680 and rs7977932 polymorphisms might play an important role in the onset of EC. According to worldwide statistics, type I endometrioid adenocarcinoma accounts for ~80% of all endometrial neoplasms [31], which compares to the proportion of 83.9% analyzed in our study. Our data showed that the frequency of allele C in rs4758680 was significantly decreased in the endometrioid adenocarcinoma patients, indicating a special correlation between IL-31 and endometrioid adenocarcinoma. However, no significant difference for genotypic frequency in rs7977932 between the two groups was observed. Obesity is the most common risk factor for EC. Shaw E et al. [32] reported that obesity was associated with a 2.6-fold increase in EC risk compared to normal-weight women. General obesity was defined as BMI ~28 kg/m^2 in Chinese populations [17]. In the present study, stratified results revealed that rs4758680 polymorphisms were significantly associated with BMI of the patients. Further research with larger sample size and diverse ethnic populations is needed to fully understand the roles of the IL-31 polymorphisms and the clinicopathological parameters of EC.

In summary, polymorphisms found in rs4758680 and rs7977932 sites of the IL-31 gene may serve as novel genetic markers of susceptibility to EC in the Chinese Han women. Our data identified that genotypic frequencies as obtained from the codominant, dominant, and overdominant genetic models for rs4758680 were associated with EC risk. The clinical characteristics such as menopausal status and BMI of EC patients may be also affected by the two SNPs polymorphisms. Our results provide supporting

evidence for a relationship between inflammatory responses and the pathogenesis of EC. However, the function and the underlying signal transduction mechanisms of rs4758680 and rs7977932 SNPs of IL-31 in EC development needs to be clarified.

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Disclosure of conflict of interest

None.

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