

# Association of *IL-17* and *IL-23* Gene Variants with Plasma Levels and Risk of Vulvovaginal Candidiasis in a Chinese Han Population

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**Background:** Vulvovaginal candidiasis (VVC) is a common vaginal inflammatory disease in females. The interleukin (IL)-23/IL-17 axis was involved in vaginal inflammation. Nevertheless, the relationship between gene polymorphisms in the IL-23/IL-17 axis and VVC risk is still unexplored.

**Methods:** We enrolled 217 VCC cases and 326 controls in this study. The genotyping of all polymorphisms was implemented by PCR-RFLP methods.

**Results:** Data indicated that *IL-17F* gene rs763780, *IL-17A* gene rs2275913, and *IL-23R* rs11209026 polymorphisms were linked with an elevated risk of VVC in Chinese ethnicity. Subgroup analyses uncovered that *IL-23R* rs11209026, *IL-17A* rs10484879 and *IL-17F* rs763780 polymorphisms increased the risk of VVC among smokers or individuals with BMI  $\geq 25$  kg/m<sup>2</sup>. Additionally, *IL-17F* rs763780 polymorphism was shown to increase the risk of recurrent VVC (RVVC). Furthermore, IL-23 and IL-17 serum levels were higher among VVC cases than controls. We also observed that IL-23 and IL-17 gene polymorphisms were related to their serum levels. Receiver operating characteristics (ROC) curve analysis found that IL-17 and IL-23 serum levels were associated with the relapse of VVC.

**Conclusion:** In conclusion, this study indicates that polymorphisms in the IL-23/IL-17 axis increase the risk of VVC.

**Keywords:** IL-17, IL-23, case-control study, vulvovaginal candidiasis, polymorphism

## Introduction

Vulvovaginal candidiasis (VVC) is a common vaginal infection caused mainly by the opportunistic *Candida albicans*, which is second only to bacterial vaginosis.<sup>1</sup> VVC is the most prevalent human candidal infection, and more than 75% childbearing women are affected by overgrowth of opportunistic *Candida* species at least once in their life.<sup>2</sup> In addition, recurrent VVC (RVVC) affects approximately 8% of the women globally, which is defined as 4 or more episodes of this disorder per year.<sup>3</sup> Vaginal itching, pain, burning and redness are the primary disease symptoms of VVC.<sup>4</sup> Up to date, the underlying pathogenic mechanisms of VVC are still poorly understood. Predisposing factors including genetic factors, the use of antibacterial agents, hormones, sexual activity, age, and some pathologies is reported to contribute to the development of VVC.<sup>5</sup> Thus, different variants of relevant genes may exert effects on the vaginal mucosal defense mechanisms against *Candida* species, thereby affecting susceptibility to VVC.<sup>6,7</sup>

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VVC, a primary disease associated with insufficient clearance and persistent fungal infections, has been regarded as the outcome of inadequate host defenses against colonization.<sup>3</sup> The vaginal immune response includes innate immunity and acquired immune response. The vaginal mucosa consists of different lymphoid tissues and lymphocytes, which have important immune functions and exert anti-infective effects.<sup>8</sup> *Candida* components are mainly processed by phagocytic cells; and T helper (Th) cells are differentiated in cellular subsets based on antigen specificity, which leads to pathogen clearance ultimately.<sup>9</sup> Th17 cells, a subtype of T cells, secrete interleukin-17 (IL-17) and IL-22.<sup>10</sup> Studies have indicated that pro-inflammatory cytokines including IL-17A and IL-17F stimulated the chronic vaginal inflammation, which produced by activated Th17 lymphocytes.<sup>3</sup> Peters et al indicated that Th17/IL-17 axis signaling was indispensable for the immunopathogenesis of VVC.<sup>11</sup> An animal study suggested that IL-17 played a key role in the immune reaction of vaginal candidiasis.<sup>12</sup> Th17 phenotype is stabilized by IL-23, belonging to the IL-12 cytokine family, which could stimulate IL-17 production.<sup>13</sup> IL-23/IL-17 axis was reported to alter the patterns and the potential role of Th1 cells in some autoimmune and infectious diseases such as VVC.<sup>14</sup>

The *IL23 receptor (IL23R)* gene, containing at least 11 exons, which is located on chromosome 1p31. *IL-17A* and *IL-17F* genes are shown to locate on chromosome 6p12. The link of *IL-17* and *IL-23* gene variants with VVC risk was not investigated before. We assumed that *IL-17* and *IL-23* gene variants may associate with the pathogenesis of VVC. Thus, we conducted this study to assess the link between variants of *IL-17* and *IL-23* gene variants and VVC risk in Chinese Han individuals. Additionally, we evaluated the predicting effects of the serum levels of IL-17 and IL-23 on the relapse of VVC.

## Patients and Methods

### Subjects

This study enrolled 217 women who were diagnosed with VVC according to their clinical examination and symptoms, and the diagnosis was confirmed by culture of the vaginal discharge collected from the posterior fornix. Healthy controls were 326 women with no history of vaginal *Candida* infection, and the culture of vaginal pathogens is currently negative. Controls who had vaginal diseases, gynecological diseases, or infectious diseases were excluded. To the population, annual VVC attack frequency was questioned. Forty-six women who had four or more symptomatic VVC attacks

were diagnosed with RVVC. Family history of VVC was regarded as at least one first-degree relatives or at least two second-degree relatives having VVC. All patients and control subjects were enrolled from the Fourth Affiliated Hospital of Jiangsu University (Zhenjiang, China).

Written informed consent of all participants was got from all the participants. This study was completely approved by the Ethics committees of Fourth Affiliated Hospital of Jiangsu University and in accordance with the standards of Declaration of Helsinki.

### Genotyping

The DNA samples of peripheral blood leukocytes were extracted from the study participants using a DNA Purification Kit (Tiangen Biotech). Genotyping was done by PCR-RFLP methods. The primers were as follows: 5'-CTTTTCTGGCAGGGTCATTTTG-3' (rs11209026F), 5'-CAG AAGACCTACATGTTACT-3' (rs2275913F) and forward: 5'-GTGTAGGAACTTGGGCTGCATCAAT-3' (rs763780F). IL-17 and IL-23 serum levels were assessed by use of human IL-17 and IL-23 ELISA kits (Sino Biological, Beijing, China).

### Statistical Analysis

All relevant statistical analyses were performed on SPSS 22.0 for Windows (SPSS Inc., Chicago, USA). Student's *t*-test and Chi-square ( $\chi^2$ ) test were used for analyzing continuous variables and categorical variables, respectively.  $\chi^2$ -test was utilized to evaluate Hardy–Weinberg equilibrium (HWE). Using logistic regression analysis, the comparison of allele and genotype distributions in two groups was estimated. ORs and 95% CIs were presented. Subgroup analyses and cross-over analysis were analyzed. Regression models were adjusted for age, smoking, and drinking. Using receiver operating characteristics (ROC) curves, we assessed the area under ROC curves (AUC), specificity and sensitivity of the serum levels of IL-17 and IL-23 for predicting the relapse of VVC. All data were regarded as statistically significant with a *P* value lower than 0.05.

## Results

### Estimation of the Sample Size

We used the APP Quanto to estimate the sample sizes of this study before conduction. We set the following conditions:  $\alpha = 0.05$ ,  $\beta = 0.1$ , power value = 0.8, and the estimated OR=2.0. The allele frequency of rs763780 polymorphism was 0.19, and the estimated sample sizes of

case and control groups were 140 and 140, respectively; For rs2275913 polymorphism, the allele frequency was 0.29, and the sample sizes of case and control groups were 133 and 133, respectively; For rs11209026 polymorphism, the allele frequency was 0.09, and the sample sizes of case and control groups were 197 and 197, respectively. In this study, we enrolled 217 VCC cases and 326 controls, indicating that the sample size of this study had enough power value (>80%) to evaluate the association between those polymorphisms and VCC risk.

## Characteristics of the Study Population

We summarize the clinical features of all individuals in Table 1. The numbers of children and miscarriages were higher in VCC cases than those of controls ( $P < 0.05$ ). As for age, smoking, BMI, and drinking, no significant differences between the VCC cases and controls were obtained. Among all VCC cases, 46 (21.2%) were RVVC, and 171 (78.8%) were VVC, respectively.

## Association Between *IL-17* and *IL-23* Gene Polymorphisms and VVC Risk

The genotype and allele distributions for *IL-17* and *IL-23* gene polymorphisms are shown in Table 2. We found that *IL-23R* rs11209026 polymorphism was connected to the

risk of VVC. AA+GA or GA genotype or A allele increased the risk of VVC (GA vs AA: adjusted OR, 1.58; 95% CI, 1.02–2.43;  $P = 0.043$ ; A vs G: OR, 1.50; 95% CI, 1.02–2.19;  $P = 0.038$ ). In addition, *IL-17F* rs763780 and *IL-17A* rs10484879 polymorphisms were also found to increase the risk of VVC. These results were still positive in previous genetic models even after adjusting for age, smoking and drinking. Next, we assessed the subgroup analyses of BMI, age, drinking and smoking. A significantly increased risk of VVC was shown in VVC patients with BMI  $\geq 25$  kg/m<sup>2</sup> or smoking for *IL-17F* rs763780, *IL-23R* rs11209026, and *IL-17A* rs10484879 polymorphisms (Table 3). Additionally, we evaluated the associations between *IL-23R* and *IL-17* gene variants and types of VVC, and found that rs763780 polymorphism of the *IL-17F* gene increased the risk of RVVC (Supplemental Table 1). No significant relationship was indicated in the analyses of *IL-17A* rs10484879, and *IL-23R* rs11209026 polymorphisms and status of VVC.

## Cross-Over Analysis

Due to the potential interaction between genetic factors and environmental factors, we next analyzed the combined effects of the *IL-23R* and *IL-17* gene variants and either alcohol consumption or smoking on VVC risk. For *IL-23R* gene rs11209026 polymorphism, data indicated that smokers carrying the GA genotype increased the risk of VVC when compared with non-smokers who carried GG genotype (OR = 2.32, 95% CI = 1.17–4.57;  $P = 0.014$ ) (Table 4). Regarding *IL-17A* gene rs2275913 polymorphism, comparing with non-smokers carrying GG genotype, smokers carrying the AA genotype elevated the risk of VVC (OR = 2.82, 95% CI = 1.09–7.29;  $P = 0.028$ ). As for *IL-17F* gene rs763780 polymorphism, smokers with the CC genotype also showed an increased susceptibility to VVC when comparing with non-smokers carrying TT genotype. However, no positive association was observed between VVC risk and drinking. The data suggested significant interactions between *IL-23* and *IL-17* gene variants and smoking.

## Association of *IL-23* and *IL-17* Serum Levels with the Relapse of VVC

Next, we measured the serum levels of *IL-17* and *IL-23*. Data revealed that serum levels of *IL-17* and *IL-23* among VVC patients were markedly higher than controls (Supplemental Figure 1 and Supplemental Figure 2). In

**Table 1** Patient Demographics and Risk Factors in Vulvovaginal Candidiasis

Characteristics	Case (N=217)	Control (N=326)	P
Age	33.03±7.82	32.08±8.16	0.180
Smoking			0.558
Yes	75 (34.6%)	105 (32.2%)	
No	142 (65.4%)	221 (67.8%)	
Drinking			0.811
Yes	68 (31.3%)	99 (30.4%)	
No	149 (68.7%)	227 (69.6%)	
BMI	23.61±2.71	23.51±3.05	0.704
Number of children	1.47±1.13	1.00±0.85	<b>0.000</b>
Number of miscarriages	1.05±0.80	0.16±0.37	<b>0.000</b>
Family history of VVC	28 (12.9%)	13 (4.0%)	<b>0.000</b>
Disease types			
RVVC	46 (21.2%)		
VVC	171 (78.8%)		

**Note:** Bold values are statistically significant ( $P < 0.05$ ).

**Abbreviations:** RVVC, recurrent vulvovaginal candidiasis; VVC, vulvovaginal candidiasis.

**Table 2** Genotype Frequencies of *IL-23R* and *IL-17* Gene Polymorphisms in Cases and Controls

Models	Genotype	Case (n, %) <sup>a</sup>	Control (n, %) <sup>a</sup>	OR (95% CI)	P-value	*OR (95% CI)	*P-value
<b>rs11209026</b>							
Co-dominant	GG	163 (75.1%)	269 (82.5%)	1.00 (reference)			
Heterozygote	GA	50 (23.0%)	53 (16.3%)	<b>1.56 (1.01–2.40)</b>	<b>0.044</b>	<b>1.58 (1.02–2.43)</b>	<b>0.043</b>
Homozygote	AA	4 (1.8%)	4 (1.2%)	1.65 (0.41–6.69)	0.733	1.63 (0.40–6.65)	0.721
Dominant	GG	163 (75.1%)	269 (82.5%)	1.00 (reference)			
	AA+GA	54 (24.9%)	57 (17.5%)	<b>1.56 (1.03–2.38)</b>	<b>0.036</b>	<b>1.53 (1.02–2.35)</b>	<b>0.032</b>
Recessive	GA+GG	213 (98.1%)	322 (98.8%)	1.00 (reference)			
	AA	4 (1.8%)	4 (1.2%)	1.51 (0.37–6.11)	0.826	1.54 (0.33–6.13)	0.831
Allele	G	376 (86.6%)	591 (90.6%)	1.00 (reference)			
	A	58 (13.3%)	61 (9.3%)	<b>1.50 (1.02–2.19)</b>	<b>0.038</b>		
<b>rs2275913</b>							
Co-dominant	GG	85 (36.9%)	161 (49.4%)	1.00 (reference)			
Heterozygote	GA	102 (47.0%)	136 (41.7%)	1.42 (0.98–2.05)	0.061	1.41 (0.97–2.02)	0.059
Homozygote	AA	30 (13.8%)	29 (8.9%)	<b>1.96 (1.10–3.48)</b>	<b>0.020</b>	<b>1.94 (1.12–3.46)</b>	<b>0.017</b>
Dominant	GG	85 (39.1%)	161 (49.4%)	1.00 (reference)			
	AA+GA	132 (60.8%)	165 (50.6%)	<b>1.52 (1.07–2.15)</b>	<b>0.019</b>	<b>1.51 (1.05–2.14)</b>	<b>0.018</b>
Recessive	GA+GG	187 (86.2%)	297 (91.1%)	1.00 (reference)			
	AA	30 (13.8%)	29 (8.9%)	1.64 (0.96–2.83)	0.071	1.63 (0.94–2.81)	0.067
Allele	G	272 (62.7%)	458 (70.2%)	1.00 (reference)			
	A	162 (37.3%)	194 (29.7%)	<b>1.41 (1.09–1.82)</b>	<b>0.009</b>		
<b>rs763780</b>							
Co-dominant	TT	120 (55.3%)	213 (65.3%)	1.00 (reference)	–		
Heterozygote	TC	83 (38.2%)	101 (31.0%)	<b>1.46 (1.01–2.10)</b>	<b>0.043</b>	<b>1.44 (1.02–2.08)</b>	<b>0.041</b>
Homozygote	CC	14 (6.4%)	12 (3.7%)	2.07 (0.93–4.62)	0.071	2.05 (0.92–4.60)	0.069
Dominant	TT	120 (55.3%)	213 (65.3%)	1.00 (reference)	–		
	CC+TC	97 (44.7%)	113 (34.7%)	<b>1.52 (1.07–2.17)</b>	<b>0.019</b>	<b>1.51 (1.05–2.15)</b>	<b>0.017</b>
Recessive	TT+TC	203 (93.5%)	314 (96.3%)	1.00 (reference)	–		
	CC	14 (6.5%)	12 (3.7%)	1.81 (0.82–3.98)	0.139	1.80 (0.81–3.95)	0.136
Allele	T	323 (74.4%)	527 (80.8%)	1.00 (reference)	–		
	C	111 (25.6%)	125 (19.2%)	<b>1.45 (1.08–1.94)</b>	<b>0.012</b>		

**Notes:** <sup>a</sup>Rs11209026, rs2275913 and rs763780 polymorphisms were successfully genotyped for all individuals; Bold values are statistically significant ( $P < 0.05$ ); \*Adjust for age, smoking and drinking.

addition, we assessed whether *IL-23* and *IL-17* gene polymorphisms were linked with their serum levels. We found that GA genotype carriers of *IL-23R* gene rs11209026 polymorphism showed higher *IL-23* serum levels compared with GG genotype carriers among VVC patients ([Supplemental Figure 1](#)). Besides, this study revealed that rs2275913 and rs763780 polymorphisms were related to *IL-17* serum levels in VVC patients ([Supplemental Figure 2](#)).

Last, a ROC curve was utilized to evaluate the predictive values of *IL-23* and *IL-17* serum levels in detecting RVVC in this Chinese Han population ([Table 5](#)). The cutoff values of *IL-23* and *IL-17* were 28.67 with a sensitivity of 73.91% and a specificity of 56.73%, and 5.98 with a sensitivity of 69.57% and a specificity of

71.35%, respectively. The AUC values of *IL-17* and *IL-23* serum levels for predicting RVVC were 0.755 (95% CI: 0.69–0.81), and 0.653 (95% CI: 0.58–0.71, [Figure 1](#)), respectively. The Youden index of *IL-17* and *IL-23* was 0.409 and 0.306, respectively.

## Discussion

In this study, we found that *IL-17A* gene rs2275913, *IL-17F* gene rs763780, and *IL-23R* rs11209026 polymorphisms increased the risk of VVC in Chinese Han population. Subgroup analyses showed *IL-17A* rs10484879, *IL-23R* rs11209026, and *IL-17F* rs763780 polymorphisms increased the risk for VVC among smokers or individuals with BMI  $\geq 25$  kg/m<sup>2</sup>. In addition, rs763780 polymorphism of the

**Table 3** Stratified Analyses Between *IL-23R* and *IL-17* Gene Polymorphisms and the Risk of Vulvovaginal Candidiasis

Variables	Genotypes (Case/Control)			Heterozygous Model	Homozygous Model	Recessive Model	Dominant Model
	Wild	Heterozygote	Homozygous				
rs11209026	GG	GA	AA	GA vs GG	AA vs GG	AA vs GG+GA	AA+GA vs GG
BMI (kg/m <sup>2</sup> )							
<25	115/187	35/43	3/2	1.32 (0.80–2.19); 0.274	2.44 (0.40–14.82); 0.592	2.30 (0.38–13.93); 0.637	1.37 (0.84–2.42); 0.204
≥25	48/82	15/10	1/2	<b>2.56 (1.07–6.15); 0.031</b>	0.85 (0.08–9.67); 1.000	0.73 (0.07–8.23); 1.000	1.95 (0.86–4.40); 0.105
Smoking							
Yes	50/87	23/16	2/2	<b>2.50 (1.21–5.17); 0.012</b>	1.74 (0.24–12.74); 0.979	1.41 (0.19–10.25); 1.000	<b>2.42 (1.20–4.86); 0.012</b>
No	113/182	27/37	2/2	1.18 (0.68–2.04); 0.564	1.61 (0.22–11.60); 1.000	1.56 (0.22–11.23); 1.000	1.44 (0.85–2.45); 0.176
Drinking							
Yes	46/77	21/20	1/2	1.76 (0.86–3.59); 0.119	0.84 (0.07–9.49); 1.000	0.73 (0.06–8.15); 1.000	1.67 (0.84–3.35); 0.144
No	117/192	29/33	3/2	1.44 (0.83–2.50); 0.190	2.46 (0.41–14.95); 0.585	2.31 (0.38–14.00); 0.633	1.50 (0.88–2.55); 0.133
Age (years)							
<30	60/116	18/23	3/2	1.51 (0.76–3.02); 0.238	2.90 (0.47–17.83); 0.470	2.67 (0.44–16.34); 0.269	1.62 (0.84–3.14); 0.147
≥30	103/153	32/30	1/2	1.58 (0.91–2.77); 0.104	0.74 (0.07–8.30); 1.000	0.68 (0.06–7.55); 1.000	1.53 (0.89–2.65); 0.125
rs2275913	GG	GA	AA	GA vs GG	AA vs GG	AA vs GG+GA	AA+GA vs GG
BMI (kg/m <sup>2</sup> )							
<25	57/101	75/110	21/21	1.21 (0.78–1.87); 0.397	1.77 (0.89–3.52); 0.100	1.60 (0.84–3.04); 0.150	1.30 (0.86–1.97); 0.220
≥25	28/60	27/26	9/8	<b>2.23 (1.10–4.49); 0.024</b>	2.41 (0.84–6.91); 0.095	1.76 (0.64–4.83); 0.269	<b>2.27 (1.19–4.34); 0.013</b>
Smoking							
Yes	28/54	35/43	12/8	1.57 (0.83–2.97); 0.165	<b>2.89 (1.06–7.90); 0.034</b>	2.31 (0.89–5.97); 0.078	1.78 (0.97–3.25); 0.061
No	57/107	67/93	18/21	1.35 (0.86–2.12); 0.187	1.61 (0.79–3.26); 0.185	1.38 (0.71–2.70); 0.341	1.40 (0.91–2.15); 0.122
Drinking							
Yes	24/47	36/45	8/7	1.57 (0.81–3.03); 0.181	2.24 (0.73–6.91); 0.155	1.75 (0.60–5.09); 0.297	1.66 (0.88–3.13); 0.118
No	61/114	66/91	22/22	1.36 (0.87–2.11); 0.179	1.87 (0.96–3.64); 0.064	1.61 (0.86–3.03); 0.134	1.46 (0.96–2.21); 0.078
Age (years)							
<30	31/69	35/56	15/16	1.39 (0.77–2.53); 0.279	2.09 (0.92–4.75); 0.076	1.78 (0.83–3.82); 0.138	1.55 (0.89–2.70); 0.124
≥30	54/92	67/80	15/13	1.43 (0.89–2.28); 0.135	1.97 (0.87–4.44); 0.100	1.64 (0.75–3.57); 0.209	1.50 (0.96–2.35); 0.075
rs763780	TT	TC	CC	TC vs TT	CC vs TT	CC vs TT+TC	CC+TC vs TT
BMI (kg/m <sup>2</sup> )							
<25	89/147	53/76	11/9	1.15 (0.74–1.79); 0.527	2.02 (0.81–5.06); 0.128	1.92 (0.78–4.75); 0.152	1.24 (0.82–1.89); 0.306
≥25	31/66	30/25	3/3	<b>2.56 (1.29–5.05); 0.006</b>	2.13 (0.41–11.16); 0.642	1.49 (0.29–7.64); 0.953	<b>2.51 (1.30–4.86); 0.006</b>
Smoking							
Yes	37/71	28/28	10/6	1.92 (0.99–3.70); 0.051	<b>3.20 (1.08–9.49); 0.030</b>	2.54 (0.88–7.32); 0.077	<b>2.15 (1.17–3.95); 0.014</b>
No	83/142	55/73	4/6	1.29 (0.83–2.01); 0.260	1.14 (0.31–4.16); 1.000	1.04 (0.29–3.75); 1.000	1.28 (0.83–1.97); 0.266
Drinking							
Yes	39/67	23/27	6/5	1.46 (0.74–2.89); 0.273	2.06 (0.59–7.20); 0.249	1.82 (0.53–6.22); 0.334	1.56 (0.82–2.95); 0.173
No	81/146	60/74	8/7	1.46 (0.95–2.26); 0.087	2.06 (0.72–5.89); 0.170	1.78 (0.63–5.03); 0.268	1.51 (0.99–2.31); 0.054
Age (years)							
<30	51/94	24/43	6/4	1.03 (0.56–1.88); 0.927	2.77 (0.75–10.25); 0.217	2.74 (0.75–10.02); 0.213	1.18 (0.67–2.09); 0.577
≥30	69/119	59/58	8/8	<b>2.03 (1.86–4.62); 0.000</b>	2.88 (1.04–7.98); 0.067	1.38 (0.51–3.78); 0.526	<b>1.75 (1.12–2.75); 0.015</b>

**Note:** Bold values are statistically significant ( $P < 0.05$ ).

*IL-17F* gene was shown to elevate the risk of RVVC. And then, we observed that IL-17 and IL-23 serum levels among VVC patients were higher among than controls. Last, we found that increased IL-23 and IL-17 serum levels were associated with the relapse of VVC.

The IL-23/IL-17 axis was shown to mediate vaginal immunity and inflammation.<sup>12,15,16</sup> The IL-23R is mainly expressed on activated T cells, and it is indispensable for maintaining and activating the effects of Th17 cells on secreting IL-17.<sup>17,18</sup> Transcriptomic analysis of vaginal

**Table 4** Genetic (G) and Environmental (E) Factors 2 × 4 Fork Analysis

G <sup>a</sup>	E <sup>b</sup>	Case	Control	OR (95% CI); P value	Reflecting Information
rs11209026					
AA vs GG	Smoking				
+	+	2	2	1.61 (0.22,11.60); 1.000	G, E combined effect
+	-	2	2	1.61 (0.22,11.60); 1.000	G alone effect
-	+	50	87	0.93 (0.61,1.41); 0.718	E alone effect
-	-	113	182	1.00 (reference)	Common control
GA vs GG	Smoking				
+	+	23	16	<b>2.32 (1.17,4.57); 0.014</b>	G, E combined effect
+	-	27	37	1.18 (0.68,2.04); 0.564	G alone effect
-	+	50	87	0.93 (0.61,1.41); 0.718	E alone effect
-	-	113	182	1.00 (reference)	Common control
AA vs GG	Drinking				
+	+	1	2	0.82 (0.07,9.15); 1.000	G, E combined effect
+	-	3	2	2.46 (0.41,14.95); 0.585	G alone effect
-	+	46	77	0.98 (0.64,1.51); 1.000	E alone effect
-	-	117	192	1.00 (reference)	Common control
GA vs GG	Drinking				
+	+	21	20	1.72 (0.90,3.31); 0.100	G, E combined effect
+	-	29	33	1.44 (0.83,2.50); 0.190	G alone effect
-	+	46	77	0.98 (0.64,1.51); 1.000	E alone effect
-	-	117	192	1.00 (reference)	Common control
rs2275913					
AA vs GG	Smoking				
+	+	12	8	<b>2.82 (1.09,7.29); 0.028</b>	G, E combined effect
+	-	18	21	1.61 (0.79,3.26); 0.185	G alone effect
-	+	28	54	0.97 (0.56,1.70); 0.924	E alone effect
-	-	57	107	1.00 (reference)	Common control
GA vs GG	Smoking				
+	+	35	43	1.53 (0.88,2.65); 0.130	G, E combined effect
+	-	67	93	1.35 (0.86,2.12); 0.187	G alone effect
-	+	28	54	0.97 (0.56,1.70); 0.924	E alone effect
-	-	57	107	1.00 (reference)	Common control
AA vs GG	Drinking				
+	+	8	7	2.14 (0.74,6.17); 0.153	G, E combined effect
+	-	22	22	1.87 (0.96,3.64); 0.064	G alone effect
-	+	24	47	0.95 (0.53,1.71); 0.875	E alone effect
-	-	61	114	1.00 (reference)	Common control
GA vs GG	Drinking				
+	+	36	45	1.50 (0.87,2.56); 0.141	G, E combined effect
+	-	66	91	1.36 (0.87,2.11); 0.179	G alone effect
-	+	24	47	0.95 (0.53,1.71); 0.875	E alone effect
-	-	61	114	1.00 (reference)	Common control
rs763780					
CC vs TT	Smoking				
+	+	10	6	<b>2.85 (1.00,8.13); 0.042</b>	G, E combined effect
+	-	4	6	1.61 (0.58,4.47); 0.359	G alone effect
-	+	37	71	0.89 (0.55,1.44); 0.640	E alone effect
-	-	83	142	1.00 (reference)	Common control

(Continued)

**Table 4** (Continued).

G <sup>a</sup>	E <sup>b</sup>	Case	Control	OR (95% CI); P value	Reflecting Information
TC vs TT + + - -	Smoking				
	+	28	28	1.71 (0.95,3.09); 0.073	G, E combined effect
	-	55	73	1.29 (0.83,2.01); 0.260	G alone effect
		37	71	0.89 (0.55,1.44); 0.640	E alone effect
	-	83	142	1.00 (reference)	Common control
CC vs TT + + - -	Drinking				
	+	6	5	2.16 (0.64,7.31); 0.205	G, E combined effect
	-	8	7	2.06 (0.72,5.89); 0.170	G alone effect
		39	67	1.05 (0.65,1.69); 0.844	E alone effect
	-	81	146	1.00 (reference)	Common control
TC vs TT + + - -	Drinking				
	+	23	27	1.54 (0.83,2.85); 0.173	G, E combined effect
	-	60	74	1.46 (0.95,2.26); 0.087	G alone effect
		39	67	1.05 (0.65,1.69); 0.844	E alone effect
	-	81	146	1.00 (reference)	Common control

**Notes:** <sup>a</sup>G (+): *IL-23R* gene rs11209026 and *IL-17* gene rs36084323/rs7421861 polymorphisms (heterozygous or homozygous); G (-): wild type; <sup>b</sup>E(+): smoking/non-smoking; E(-): non-smoking/non-drinking. Bold values are statistically significant ( $P < 0.05$ ).

tissue showed an elevated expression of host genes including Th17 cytokine secretion in a murine model of VVC.<sup>3</sup> The *IL-17* gene polymorphisms were reported to be associated with several immunopathologies related to inflammation.<sup>19,20</sup> As for other infectious and immune diseases, a host of studies have explored the relationship between *IL-23/IL-17* pathway gene polymorphisms and corresponding disease risk. McGovern et al indicated that variants of *IL-23/IL-17* pathway genes were markedly associated with the susceptibility to Crohn's disease.<sup>21</sup> Omrane et al observed that *IL-17F* and *IL-23R* gene polymorphisms were not related to susceptibility to colorectal cancer.<sup>22</sup> However, they obtained an association between these SNPs and clinical features of colorectal cancer.<sup>22</sup> Regarding necrotizing enterocolitis in premature infants, *IL-23R* rs10889677 and *IL-17A* rs2275913 polymorphisms were not related to the susceptibility to necrotizing enterocolitis; however, *IL-17F* rs763780 polymorphism elevated the risk of necrotizing enterocolitis.<sup>23</sup> In addition, Louahchi et al from Algeria found no association between *IL-23R* and *IL-17* gene variants and rheumatoid arthritis risk.<sup>24</sup> A study by Catanoso et al revealed that *IL-23R* and

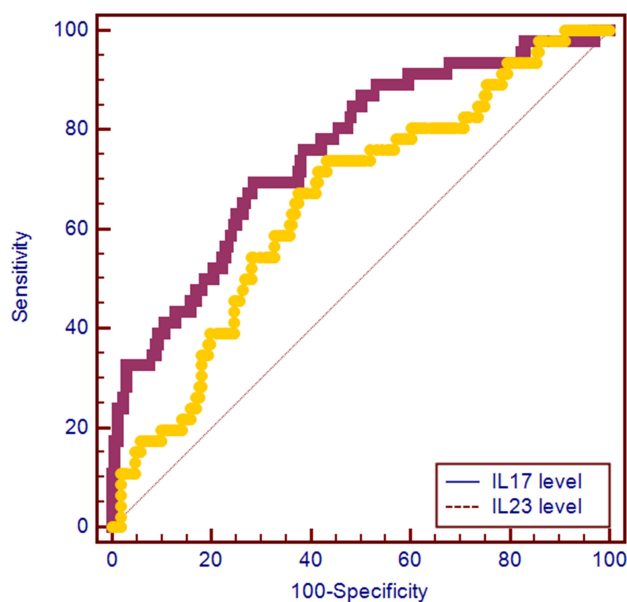
*IL-17* gene variants showed no association with psoriatic arthritis susceptibility.<sup>25</sup> A Turkish study suggested *IL-23R* and *IL-17* polymorphisms had no link with alopecia areata risk.<sup>26</sup> On the whole, *IL-23R* and *IL-17* polymorphisms were related to the risk of some specific disorders. Types of disease, various sample sizes, clinical heterogeneity, and different races may account for conflicting findings of these studies, which need further studies to validate it.

Given the crucial role of the *IL-23R* and *IL-17* genes in the development of infectious and immune diseases, we assumed that *IL-23R* and *IL-17* gene variants may be associated with the susceptibility to VVC. Firstly, we searched the SNPinfo Web Server and found the potential functional effects of these chosen variants: *IL-23R* rs11209026 and *IL-17F* rs763780 polymorphisms are nsSNPs, which could cause amino acid variation and lead to the alteration of protein after translation; *IL-17A* gene rs2275913 is a transcription factor binding site (TFBS), which helps the transcription factors regulate relevant gene expressions. Although functional effects of those variants are important, the potential link of these single nucleotide polymorphisms (SNPs) in the *IL23/IL17* axis with the risk of VVC remains

**Table 5** Predictive Values of IL-17 and IL-23 Serum Levels in Detecting Recurrent Vulvovaginal Candidiasis

	Youden Index J	Associated Criterion	Sensitivity	Specificity	AUC*	95% CI	P-value
IL-23	0.306	>28.67	73.91%	56.73%	<b>0.653</b>	<b>(0.58–0.71)</b>	<b>&lt;0.0001</b>
IL-17	0.409	>5.98	69.57%	71.35%	<b>0.755</b>	<b>(0.69–0.81)</b>	<b>&lt;0.0001</b>

**Notes:** \*AUC, area under the ROC curve; Bold values are statistically significant ( $P < 0.05$ ).



**Figure 1** ROC curve for IL-23 and IL-17 serum levels to predict the occurrence of RVVC.

unexplored. In this study, we uncovered a link between *IL-17A* gene rs2275913, *IL-23R* rs11209026, and *IL-17F* gene rs763780 polymorphisms and VVC risk. Data indicated that all these SNPs increased the risk of VVC. Subgroup analyses indicated that these SNPs elevated the risk of VVC among smokers and individuals with BMI  $\geq 25$  kg/m<sup>2</sup>, indicating that individuals with genotypes of these SNPs exposing to these factors are more prone to suffering VVC. In addition, we evaluated the association of VVC types with *IL-23R* and *IL-17* polymorphisms, and found that *IL-17F* rs763780 polymorphism elevated the risk of RVVC, inferring that this SNP was related to the relapse of VVC.

Next, we evaluated the serum levels of IL-23 and IL-17 among VVC patients and healthy controls, and found that VVC patients had higher serum levels of IL-17 and IL-23 when comparing with healthy controls. It is of note that IL-23 expression in murine vaginal candidiasis was associated with its immune status and infection.<sup>27</sup> Kolben et al revealed that levels of IL-23 were markedly lower in the VVC group,<sup>28</sup> which indicated a compromised local immune response in vaginal mucosa. Obviously, the findings uncovered by Kolben et al were inconsistent with those of this study. Potential factors including different ethnicities, distinct sample sizes, and various stage severity of VVC may explain these disaccords. Additionally, we found that *IL-17* and *IL-23* gene polymorphisms were related to the serum levels of IL-17 and IL-23. Maybe *IL-17* and *IL-23* gene polymorphisms increased the risk of

VVC via affecting their serum levels. Last, we used the ROC curve to evaluate the predictive values of IL-23 and IL-17 serum levels in detecting relapse of VVC, and found the AUC values of IL-23 and IL-17 serum levels for predicting RVVC were 0.653, and 0.755, respectively, indicating good ability of IL-23 and IL-17 to predict recurrence of VVC.

Several limitations were shown in this study. One, the sample size was small, which may present untrustworthy results. Two, we only investigated limited SNPs of *IL-17* and *IL-23* genes. Three, the interaction between genetic factors and environmental factors was not explored. Four, whether IL-23 and IL-17 affected the therapeutic effects of clinical medications should be addressed. Fifth, the possible gene-gene interactions were not investigated. We utilized the String online tool (<http://string-db.org/>) to observe these effects. Several genes including *IL-2*, *IL-6*, *IL-13*, *IL-10*, *STAT3*, *CTLA4*, and *STAT6* participated in the interaction of *IL-17* (Supplemental Figure 3). *RELA*, *TYK2*, *JAK2*, *STAT3*, *IL12B*, *STAT4*, *STAT6*, *STAT1*, and *TYK2* showed interactions with *IL-23* (Supplemental Figure 4). Last, cell and animal experiments should be performed to explore the role of IL-23 and IL-17 in the development of VVC.

In conclusion, *IL-17* and *IL-23* gene polymorphisms increase the risk of VVC. IL-17 and IL-23 serum levels are related with the relapse of VVC. Further studies are urgently warranted to verify these results.

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## Disclosure

The authors report no conflicts of interest for this work.

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