Research Square

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Correlation study between Interleukin-17 Gene Polymorphisms and risk of development of recurrent aphthous ulcer in Han Chinese Population

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Research article

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Abstract

Objective Interleukin-17 (IL-17) is a pleiotropic cytokine which plays important role in the inflammatory diseases.

Methods: Polymorphisms of IL-17A rs2275913 and IL17F rs763780 were measured in 125 RAU cases and 116 healthy controls. The polymerase chain reaction-restriction fragment length was measured. The genotype distribution and disease risk, and its' relationship with RAU severity was analyzed.

Results: RAU risk were related with polymorphism of IL-17 gene at rs2275913 site after adjusting BMI, sex, age, smoking and drinking status (AA vs. GG: odds ratio (OR), 1.624; 95% confidence interval (CI), 1.125–2.250; P = 0.030; A allele vs. G allele: OR, 1.192; P = 0.037; 95% CI, 1.012–1.404;). In addition, the rs763780 variant genotypes (TC and CC) and C allele also have higher relevance to RAU compared with subjects who bears the TT genotype (TC vs. TT, OR: 1.312; P = 0.039; 95% CI: 1.017–1.692; CC vs. TT, OR: 2.812, P = 0.006, 95% CI: 1.338-5.909; C allele Vs. T allele, OR:1.413, P=0.002, 95% CI:1.141-1.751). We also found serum IL-17 levels were greatly higher in RAU patients compared with controls (P = 0.001), and serum IL17 concentration is correlated with IL17 polymorphism.

Conclusion: Our research showed polymorphisms of IL-17 gene might related to the high-risk of RAU occurrence.

Introduction

Recurrent oral ulceration (RAU) is also known as recurrent aphthous stomatitis, with characters of recurrent episodes of oral ulcers. Reports indicated that RAU affects about 20% of populations [1,2]. The etiology of RAU is multifactorial, genetic, immune, nutritional, and microbial factors and local trauma might contribute to the occurrence. In addition, family history of RAU is confirmed to be a risk factor for RAU [3].

The etiology of RAU is not well demonstrated clearly, previous studies indicated that inflammatory factors play an key role in the development of RAU [4,5]. Borra et al revealed that cytokine genes in peripheral blood mononuclear cells, such as IL-2, TNF-α and IL-6, contribute to the development of RAU [6]. Other research indicated that IL-10 mRNA levels decreased in RAU patients, which suggests a failure of suppressing inflammatory reaction to oral mucosa in immune system [7].

So far, few studies reported the association between polymorphism of inflammatory genes and risk of RAU. The polymorphisms of IL-1β -3954 C/T (rs1143634), IL-6-174G/C (rs1800795), IL-10-1082A/G (rs1800896), and IL-10-819C/T (rs1800871) have been reported correlated with risk of RAU in a Chinese population [1]. While no association study about IL-17 polymorphisms and RAU was reported.

IL 17 play an important role in adaptive and innate immune systems. Five confirmed receptors (IL-17RA-RD and SEF) and six members (IL-17A-F) [8,9] of IL 17 have been identified so far. Among them, IL-17A and IL-17F are secreted by Th17 cells, and composed a distinct lineage of CD4 + effector cells [10]. As a pro-inflammatory cytokine, IL-17 could trigger the release of chemokines, cytokines, antimicrobial peptides, and matrix metalloproteinases (MMPs) from mesenchymal and myeloid cells [11]. On the other hand, IL-17 is expressed in synovial tissues, and could participate in the process of cartilage breakdown and synovial infiltration in RAU through inducing the release of chemokines from chondrocytes [12]. IL-17 also augments nitric oxide production via nuclear factor kappa B activation in RAU cartilage [13].

Compelling studies indicated that genetic polymorphisms of IL-17 are related to the susceptibility of a scope of inflammation-related diseases, including ulcerative colitis, gastric cancer, breast cancer, and rheumatoid arthritis [14–17]. So far, there is no relative report about association of IL-17 polymorphism with RAU in the publicly available Genome database, we hypothesize that IL-17 is a potential risk factor for RAU, and aim to explore the correlation between the polymorphisms of IL-17A and IL-17F with risk of RAU among Chinese population. The polymorphs of IL-17A rs2275913 and IL-17F rs763780 with the risk factors of RAU were evaluated in our recruited subjects. This case-control study was designed to explore the relationship of these two SNPs with the morbidity and severity of RA.

Materials And Methods

Subjects

A total of 125 patients who were diagnosed of ROU were enrolled. The diagnostic criteria were horizontal range of oral ulcer being covered with yellow pseudomembrane, surrounding hyperemia, and with central sag and obvious causalgia, ulcer having cyclicity and being self-limiting and being at differential phase. Inclusion criteria included: (1) ≥ 6-month of regularly recurrent episodes of oral aphthous ulcer; (2) at least two ulcers per month within 6 months; (3) full blood count was normal with serum B12 within 200–900 ng/l, red cell folate within 110–700 μg/l and serum ferritin within range of 20–400 μg/l. Exclusion criteria included: (1) pregnant woman; (2) history of in which oral ulcer caused by systemic disease such as Behc¸et's syndrome, coeliac disease, Crohn's disease, ulcerative colitis or AIDS; (3) combined medication with systemic steroids, immunomodulatory drugs or cytotoxics. 116 healthy control without ROU were recruited from individuals receiving routine check-up in our hospital. The subjects from control group was confirmed with no history of ROU and matched with cases group with sex and age. The gender and age

distribution in the RAU and healthy control group were shown in Table 1. Informed consent was obtained from all subjects. The protocol of study was approved by the Ethics Committee of the second hospital of Hebei Medical University.

Table 1
Demographic characteristics of the study population.

Variables	Healthy control (n = 116)	RAU (n = 125)	*P* value
Age (mean ± SD)	38.3 ± 12.6	39.5 ± 12.9	0.234
Sex			0.617
Male	56	62	
Female	60	63	
Body mass index (kg/m^2)	25.6 ± 3.2	26.0 ± 3.9	0.178
Smoking			
Yes	10	6	0.321
No	106	119	
Drinking			0.032
Yes	29	55	
No	87	70	
Number of lesions			
< 3 lesions		74	
≥ 3 lesions		51	
IL-17A concentration (x ± S, pg/mL)	2.38 ± 0.14	4.31 ± 0.27	0.0221
IL-17F concentration (x ± S, pg/mL)	104.5 ± 13.6	144.6 ± 14.5	0.0314

Sample collection

A total of 10 ml peripheral blood was collected into EDTA tube by venipuncture from each subject for DNA isolation. The biochemical parameters in the serum were also detected.

Genomic DNA isolation

DNA Blood Mini Kit (QIAGEN, Hilden, Germany) was used for DNA extracted for genomic studies. 200 µL of whole blood was used following the manufacturer's instructions. DNA concentration was measured by spectrophotometry (NanoDrop 2000, Thermo Scientific) and diluted to about 40 ng/µL.

DNA Sequencing Analysis

IL-17 copies were measured by PCR through amplification. The used primers of SNP were: rs2275913, forward 5'-ATTTCTGCCCTTCCCATTTT-3' and reverse 5'- CCAGGAGTCATCGTTGTTT-3'; rs763780, forward 5'-GCAGAGCACTGGGTAAGGAG-3' and reverse 5'-CTGCATCAATGCTCAAGGAA-3'. Sequencing was measured by a Bio-Informatic company (Life technology, Shang Hai, China).

Serum IL-17A and IL-17F levels

Collected samples were allowed to clot for 30 min at 4 °C before centrifugation at 3,000 rpm for 10 min at 4 °C. Total serum was separated, stored at -20 °C for further using. Sandwich ELISA The concentrations of serum IL-17A and F were measured following the manufacturer's instructions. The intra- coefficients of variation were set as 10%.

Statistical analysis

The demographic and clinical data were demonstrated as Mean ± SD and compared among groups with the Student's *t*-test. The genotype and allelic frequencies were measured by Hardy-Weinberg equilibrium (HWE) and compared by Chi-square test and Fisher's exact test through an online calculator (http://www.oege.org/software/hardy-weinberg.html). Odds ratio (OR) and 95% confidence interval (CI) for the additive model were evaluated to association between the SNP and the ROU risk. The dominant model, the recessive model was also evaluated by SPSS 19.0 software (IBM SPSS, USA). *P* values < 0.05 were regarded as statistically significant.

Results

Study participants

125 Chinese patients with RAU (mean age ± s.d. = 39.5 ± 12.9 years) were recruited, 62 was men and 63 was women, 6 were smokers. All subjects were assessed by an oral medicine specialist, the presence of aphthous ulcers were confirmed by them.

Blood samples were also obtained from 116 age and sex-matched healthy Chinese controls. Their RAU status confirmed by medical records and inquiries of patients, there is still possible that a small proportion subjects suffered from RAU. This might weaken the identified strength of associations. In our study, only six smokers were recruited to the patient group while 10 smokers were recruited in healthy control, which make it impossible to evaluate the effect of smoking on any gene associations.

The detailed demographic characteristics of the study population was listed in Table 1. Comparison showed there was no significant difference in mean age, sex, BMI and smoking status between the 2 groups, indicating subjective matching. However, it seems that alcohol drinking could increase the risk of RAU occurrence (P = 0.032, Table 1).

An allele of rs2275913 and C allele of rs763780 increase the risk of RAU

The distribution of each allele and genotype is shown in Table 2. Both SNPs were within the Hardy-Weinberg equilibrium. As for the genotype and allele frequencies in rs2275913 polymorphism, we observed a remarkably difference between RAU patients and healthy controls (Table 2). In addition, all subjects carrying AA genotype have significantly higher risks of RAU compared with GG genotype ($P < 0.05$). That is subjects with the A allele were more likely to get RAU compared with subjects bearing the G allele (OR, 1.1192; 95% CI, 1.012–1.404; $p = 0.037$). In addition, analysis also indicated the higher risk of AA genotype and A allele mainly existed in female sub-population (P < 0.05), but not in male population ($p > 0.05$, Table 2).

Table 2
Distributions of IL-17 SNPs genotypes in each group and analyses of associations between these polymorphisms and RAU

Genotype	Overall (N)				Female (N)				Male (N)			
	control	RAU	OR (95% CI)	*P*	control	RAU	OR (95% CI)	*P*	control	RAU	OR (95% CI)	*P*
rs2275913	116	125			60	63			56	62		
GG	46	29	1.00	1.000	26	16	1.00	1.00	20	13	1.00	1.000
GA	47	56	1.890(1.032–3.462)	0.048	23	30	2.120(0.928–4.843)	0.098	24	26	1.669(0.683–4.066)	0.273
AA	23	40	2.759(1.381–5.512)	0.006	11	17	2.511(0.941–6.701)	0.088	12	23	2.949(1.099–7.914)	0.051
Allele												
G	139	114	1.00	1.000	75	62	1.00	1.00	64	52	1.00	1.000
A	93	136	1.783(1.242–2.560)	0.002	45	64	1.720(1.035–2.861)	0.040	48	72	1.846(1.101–3.096)	0.026
rs763780												
TT	82	67	1.00	1.000	43	32	1.00	1.000	39	35	1.00	1.000
TC	31	48	1.895(1.088–3.301)	0.026	15	26	2.329(1.064–5.097)	0.051	16	22	1.532(0.696–3.373)	0.235
CC	3	10	4.080(1.079–15.425)	0.040	2	5	2.759(1.381–5.512)	0.235	1	5	5.571(0.620-50.031)	0.201
Allele												
T	195	182	1.00	1.000	101	90	1.00	1.000	94	92	1.00	1.000
C	37	68	1.969(1.257–3.083)	0.003	19	36	2.126(1.139–3.969)	0.021	18	32	1.816(0.953–3.462)	0.080

RAU: recurrent aphthous ulcer; OR: odds ratio; CI: confidence interval

We also observed similar results about rs763780 polymorphism. There was a significant difference in the genotype and allele frequencies between RAU patients and control subjects, as demonstrated in Table 2. In addition, C allele rather than T allele increases the risk of developing RAU (Table 2). Furthermore, our study indicated that C allele caused higher risk than in T allele in female subgroup (P = 0.004). As for the male

population, the CC genotype and C allele did not induce significant higher risk for RAU. Dominant and recessive model also indicated the similar results that A allele of rs2275913 and C allele of rs763780 increase the risk of RAU.

IL-17 polymorphism is related to RAU severity

RAU severity was evaluated based on number of lesions in one patient. In the current study, we classified into two sub-groups, less than three lesions and ≥ three lesions in RAU patients. The distribution of SNP among RAU severity was listed in Table 3. Results showed there is a significant difference between all the SNPs and alleles in different severity-groups, which suggests that these two IL-17 polymorphisms are risk factors for RAU severity.

Table 3
The association of different genotypes and alleles of various polymorphisms with the severity of RAU.

Genotype	N	< 3 lesions	≥ 3 lesions	*P* value
rs2275913	125	74	51	0.003
GG	29	21	8	
GA	56	38	18	
AA	40	15	25	
Allele				0.048
G	114	80	34	
A	136	78	58	
rs763780	125	74	51	< 0.001
TT	67	51	16	
TC	48	20	28	
CC	10	3	7	
Allele				< 0.001
T	182	122	60	
C	68	26	42	

Serum IL-17A/F levels are higher in RAU patients

The median serum concentration of IL-17A was 2.38 ± 0.14 pg/ mL and 4.31 ± 0.27 pg/ mL in healthy controls and RAU patients, respectively (Table 1). IL-17A serum levels in RAU patients were higher than healthy controls (P = 0.0221, Table 1). The serum levels of IL-17F in RAU patients were also higher than healthy controls (P = 0.0314, Table 1). Further, for the average serum concentration of IL-17A and IL-17F in each genotype, there was significant difference among all 3 genotypes for these two SNPs (Table 4), which indicated that A allele in rs2275913 and C allele in rs763780 possibly increased the IL17A and IL17F secretion respectively in RAU patients.

Table 4
The serum levels of IL-17A and IL-17F in the different genotypes in RAU patients

Genotype	IL-17A (x ± SD, pg/mL)	IL-17F (x ± SD, pg/mL)
rs2275913		
GG	2.92 ± 0.47	119.9 ± 21.6
GA	3.61 ± 0.33*	134.5 ± 18.5*
AA	3.95 ± 0.28**	153.4 ± 22.3*
rs763780		
TT	2.81 ± 0.34	108.3 ± 23.4
TC	3.72 ± 0.28#	142.1 ± 22.1#
CC	3.91 ± 0.29##	150.8 ± 23.8#

*, P < 0.05, **, P < 0.01, compared with GG genotype. #, P < 0.05, ##, P < 0.01, compared with TT genotype. The significance was calculated by student's t-test.

Discussion

Previous studies indicated that IL-17/IL23 pathway and TH17 cells play important roles in pathology of inflammation-related diseases [18]. Several meta-analysis demonbstrated the relationship between gene polymorphisms of IL-17A (rs2275913) and IL-17F (rs763780) and the risk of inflammatory diseases, including periodontitis, rheumatoid arthritis (RA), and inflammatory bowel disease [19]. Research also reported polymorphism of IL17A (rs2275913) is associated with the occurrence of rheumatic heart disease in south Indian population [20], and a variant of IL17F (rs763780) may participated in the development of necrotizing enterocolitis [21]. All these researches suggest that IL17 polymorphism may be associated with immune mediated diseases widely.

A previous studies indicated that increasing of IL-17 A/F are effective in the pathogenesis of minor aphthous, particularly in the ulcerative stage [22]. No study reported the relationship between rs2275913 (IL-17A SNP) and rs763780 (IL-17F SNP) with risk of RAU. Our study might be first time to investigate the involvement of IL-17 gene polymorphisms in RAU and whether these polymorphisms are correlated with serum levels of IL-17A/F. So far, there is no enough evidence to demonstrated whether the 2 SNPs affect the IL-17 secretion in the human plasma. Present results from Table 4, these two SNPs seem to influence the plasma concentration of IL-17A/F in serum. An allele in rs2275913 and C allele in rs763780 possibly increased the plasma concentration of IL17A and IL17F respectively, which provides the explanation that these 2 alleles increase the risk of RAU (Table 2). Our results suggested that the polymorphism of IL-17A rs2275913 has a significant impact on the risk of RAU. In addition, subjects carrying the rs2275913 A allele are at a higher risk of developing RAU as compared with subjects carrying the G allele. Furthermore, rs763780 C allele, was also related to an increased risk of developing RAU greatly. Further assessment of the effect of IL-17 polymorphisms on RAU risk was stratified by sex, and we also find increased risk ratio in both male and female patients' subgroups, but no statistical significance was found due to limited population, more sample size is still need to further revealed the underlying mechanisms of this association.

Higher average serum concentrations of IL17A/F have been found in RAU patients when it was compared with healthy controls. Analysis of inflammatory cells, such as Th17, some strongly positive IL-17A^{+} cells ae seen in the vascularized superficial connective tissue of human oral mucosa, and aggravate RAU. Cytokine genes such as IL-17A/F are associated with development and function of Th17 cell. Therefore, IL17 polymorphism regulated Th17 cell population is another possible mechanism that involved in RAU-associated inflammatory response.

There are some other limitations except for the small sample size population in 0ur study. The population of study was confined to Han Chinese, the findings might not apply to other population. It would of great significance if RAU patient from other ethnic population is studied. In addition, we only investigated 2 SNPs in the IL-17 gene. If more SNPs would be defined for the occurrence of RAU, it will also make our research more significance. More importantly, allele frequency differences between cases and controls after population stratification could cause spurious associations in disease studies due to systematic ancestry differences, and lead to false positive results [23]. Therefore, more detailed statistical analysis is need to make our results more accurately.

In a conclusion, our results demonstrated that functional polymorphisms of IL-17 are correlated with the risk of RAU significantly. The variant alleles rs2275913 AA and rs763780 CC might lead to the increased risk of RAU. Elevated serum IL-17 levels correlated with increased risk of RAU significantly, which taking together might facilitate defining high risk subjects to prevent the initial development of RAU.

Declarations

Ethics approval and consent to participate

The protocol of study was approved by the Ethics Committee of the second hospital of Hebei Medical University.

Consent for publication

Informed consent was obtained from each patient for their medical data using and consent for publication.

Availability of data and material

The relevant raw data will be freely available to any scientist wishing to use them for non-commercial purposes, without breaching participant confidentiality.

Competing interests

None.

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None.

Authors' contributions

Conception and design: Xiang HD and Gao Q; Acquisition of data, Xiang HD, Cheng DM, Gao H and Wang Y; Statistical analysis, Jai ZY.

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