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

Keywords: IL-17, rs763780, pro-inflammation, polymorphism, recurrent oral ulceration

Posted Date: July 29th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-41582/v2

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 aphthous ulcer (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 innate and adaptive immune functions. Five identified receptors (IL-17 receptor A to E) and six members (IL-17A-F) [8, 9] of IL 17 have been identified so far. Among them, IL-17A and IL-17F are mainly produced and secreted by T helper 17 (Th17) cells, and composed a unique lineage of $CD4^+$ effector cells [10]. Currently, IL-17 was considered as one of pro-inflammatory cytokines, and IL-17 could initiate the release of pro-inflammation cytokines and chemokines, , matrix metalloproteinases, and antimicrobial peptides from myeloid cells, mesenchymal cells and even epithelial cells [11]. Recently, IL-17 has emerged as an important driver of pathogenic inflammation, and is considered a key underlying element in the pathogenesis of autoimmune diseases such as systemic lupus erythematosus, membranous glomerulonephritis, and others [12, 13].

Compelling studies indicated that genetic polymorphisms of IL-17 are associated with the susceptibility of several immune-mediated diseases, including ulcerative colitis, systemic lupus erythematosus, membranous glomerulonephritis and rheumatoid arthritis [14-17]. So far, however, the relationship between IL-17 polymorphism and RAU has been rarely reported in the literature, as well as public available genetic database. Therefore we postulate that IL-17 is a possible susceptibility factor for RAU, and aim to explore the association between the IL-17 polymorphisms with risk of RAU in Chinese population. The polymorphisms of IL-17A rs2275913 and IL-17F rs763780 with the susceptibility 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 RAU.

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 pseudo membrane, 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 investigation was approved by the Ethics Committee of the second hospital of Hebei Medical University.

Sample collection

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

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 menu instructions. DNA concentration was measured by spectrophotometry (NanoDrop 2000, Thermo Scientific, CA, USA) and diluted to about 40 ng/μL.

DNA Sequencing Analysis

IL-17 copies were measured by PCR through amplification. The used primers of polymorphisms 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

Total serum was separated from collected non-anticoagulant blood samples by centrifugation at 3,000 rpm for 10 min at 4°C after blood clotted. The concentrations of serum IL-17A and F were measured by sandwich ELISA (Abcam, CA, USA) following the menu's instructions.

Statistics

The clinical data were described as mean ± standard deviation (SD) in each group and analyzed by Student's *t*-test. The genotype and allelic frequencies had been calculated by Hardy-Weinberg equilibrium (HWE) through an online calculator (http://www.oege.org/software/hardy-weinberg.html), and further by Chi-square test following Fisher's exact test. Odds ratio (OR) and 95% confidence interval (CI) were calculated to evaluate to association between the SNP and the ROU risk by SPSS 19.0 software (IBM SPSS, USA). Statistically significance threshold was set up as $P < 0.05$.

Results

Study participants

125 Chinese patients with RAU (mean age ± SD = 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**. Statistical analysis showed there was no significant difference in age, gender, BMI and smoking between the two groups, which indicated that enrolled subject from two groups matched in the current study. However, it seems that alcohol drinking could increase the risk of RAU occurrence (P=0.032, table 1).

A allele of rs2275913 and C allele of rs763780 raise the risk of RAU

The distributions of each genotype and allele from SNP sites are shown in **Table 2**, and their distribution complied with Hardy-Weinberg equilibrium. As for the genotype and allele distribution in rs2275913, a significant difference was observed between healthy control and RAU patients (Table 2). In addition, AA genotype carrier had remarkably increased risks of RAU occurrence compared with genotype GG ($P < 0.05$). Moreover, A allele carrier had higher risk on RAU occurrence compared with G allele carriers (OR, 1.1192; 95% CI, 1.012–1.404; $p = 0.037$). In addition, data from table 2 also indicated the AA genotype and A allele were substantially distributed in female population (P<0.05), instead of male sub-population ($p>0.05$, Table 2).

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). However, in the male sub-population, the CC genotype did not cause significant change of RAU risk ratio, as well as C allele.

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 was a significant difference between all the genotypes and alleles in different severity-groups, which suggests that these two IL-17 polymorphisms are risk factors for RAU severity.

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

The average serum IL-17A concentration 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 significantly higher compared with healthy subjects (P=0.0314, Table 1). Further, f of in each genotype, there was significant difference of serum IL-17A and IL-17F concentration among all three genotypes about these two genic

loci (**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.

Discussion

Recent researches indicated that Th17 cells and IL-17/IL23 pathway play important roles in pathology of inflammation-related diseases [13]. Several meta-analysis demonbstrated the relationship between gene polymorphisms of IL-17A (rs2275913) and IL-17F (rs763780) and the pathogenesis of inflammatory diseases, including periodontitis, SLE, golomerulonephritis, rheumatoid arthritis (RA), and inflammatory bowel disease [12]. Research also reported polymorphism of IL17A (rs2275913) is associated with osteitis after the Bacillus Calmette-Guérin vaccination [18, 19], and a variant of IL17F (rs763780) may participate in the development of immune thrombocytopenia [20] and necrotizing enterocolitis [21]. All these researches indicate that IL17 polymorphisms are possibly 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 and rs763780 with risk of RAU occurrence. Present study seems to be first research to investigate the involvement of IL-17 gene polymorphisms in RAU and their correlation with serum levels of IL-17A/F. So far, there is no enough evidence to demonstrate whether these two SNPs affect the serum content of IL-17 A and F. From the data in table 4, these two SNPs possibly affect the serum concentration of IL-17A/F. 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). Current results show that the polymorphism of IL-17A affects the risk of RAU. In addition, subjects bearing the rs2275913 A allele have higher risk of developing RAU compared with G allele carriers. Furthermore, rs763780 C allele, was also related to an increased risk of developing RAU greatly. Further evaluation of the effect of IL-17 polymorphisms on RAU occurrence ratio was separated by gender, and we also find increased risk ratio in both male and female patients' subgroups, but no statistical significance was found due to limited population, bigger population size is still need to revealed the in-depth mechanisms of this correlation.

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 artificial positive results [23]. Therefore, more detailed statistical analysis is necessary to make our conclusions more reliable.

In a summary, our results indicated that IL-17 functional polymorphisms are correlated with the occurrence risk of RAU significantly. The variant alleles rs2275913 AA and rs763780 CC might lead to the higher risk of RAU, elevated serum IL-17A/F levels, as well as increased severity. All these results inhibiting IL17A/F might be a effective strategy to prevent and treat 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.

Funding

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.

Acknowledgement

None.

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Tables

Table 1 Demographic characteristics of the study population.

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

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

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	

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.