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Association between interleukin gene polymorphisms and risk of recurrent oral ulceration

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ABSTRACT. We conducted a case-control study to investigate the association between the functional IL-1β+3954 (C/T), IL-6-174 (G/C), IL-10-1082 (G/A), and IL-10-819C/T genetic polymorphisms and risk of recurrent oral ulceration (ROU) in a Chinese population. Polymorphisms of IL-1β+3954C/T, IL-6-174G/C, IL-10-1082A/G and IL-10-819C/T were assessed by polymerase chain reaction-restriction fragment length polymorphism. The genotype distributions of the IL-1β+3954 C/T and IL-10-819C/T were in Hardy-Weinberg equilibrium in the control group. Conditional logistic regression analyses showed that subjects carrying the IL-1β+3954CC and IL-10-1082AA genotypes had a significantly increased risk of ROU, with adjusted ORs (95%CI) of 2.86 (1.37-6.33) and 1.72 (1.02-2.89), respectively. In summary, we found that IL-1β+3954C/T and IL-10-1082A/G polymorphisms are associated with an increased risk of ROU.

Key words: Recurrent oral ulceration; Interleukin gene; Polymorphism

INTRODUCTION

Recurrent oral ulceration (ROU) is known as recurrent aphthous stomatitis, which is characterized by recurrent episodes of oral ulcers in healthy individuals and affects about 20% of populations (Stanley, 1972; Porter et al., 1998). It is well known that ROU has multi-factor causes, including both genetic and environmental factors. Family history of ROU is confirmed to be a risk factor for ROU (Natah et al., 2004). It has been reported that psychological factors can also influence the pathologic process of ROU (McCartan et al., 1996; Natah et al., 2004), and a previous study reported that serotonin transporter gene polymorphism (5-HTTLPR) is associated with anxiety-related traits (McCartan et al., 1996; Natah et al., 2004; Victoria et al., 2005).

Since the etiology of ROU is not well understood, previous studies reported that inflammatory factors play an important role in the development of ROU (Sun et al., 2013; Najafi et al., 2014). A previous study reported that cytokine genes in peripheral blood mononuclear cells were associated with disease development in ROU patients, such as IL-2, TNF-α and IL-6 (Borra et al., 2004). Moreover, decreased IL-10 mRNA levels were found in ROU patients, which suggests a failure of the immune system to suppress inflammatory reaction to oral mucosa (Lewkowicz et al., 2005).

Up to now, there are no reported studies on the association between inflammatory genes and risk of ROU. Therefore, considering the immunological alterations in the pathogenesis of ROU, we conducted a case-control study to investigate the association between the functional IL-1β +3954 C/T (rs1143634), IL-6-174G/C (rs1800795), IL-10-1082A/G (rs1800896), and IL-10-819C/T (rs1800871) genetic polymorphisms and risk of ROU in a Chinese population.

MATERIAL AND METHODS

Patients

A total of 264 consecutive patients with ROU were enrolled in the study, and they were diagnosed by a dentist. The diagnostic criteria were horizontal range being covered with yellow pseudomembrane, surrounding hyperemia, with central sag and obvious causalgia, being at different ictal phase, having cyclicity and being self-limiting. For the control group, 176 subjects without ROU were recruited from the individuals getting a routine check-up in the health examination center. The subjects in the control group had no history of ROU. The control subjects were matched with the cases by sex and age.

There were 117 males and 147 females in the case and control groups, and the age of case and control groups ranged from 19 to 74 years old. Informed consent was obtained from all cases and control subjects or their relatives before being enrolling in this study. The protocol of this study was approved by the Ethics Committee of the First Affiliated Hospital of PLA General Hospital.

DNA isolation

Each case and control was asked to provide 5 mL blood for DNA sequencing after agreeing to participate in our study. The IL-1β, IL-6 and IL-10 polymorphisms were analyzed using genomic DNA purified from peripheral blood. All study participants provided 5 mL venous blood, and their blood samples were kept at -20°C until use, where 0.5 mg/mL ethyl-

enediaminetetraacetic acid was used as the anticoagulant. Genomic DNA was isolated from peripheral blood leukocytes using the TIANamp Blood DNA kit (Tiangen, Beijing, China) according to the manufacturer instructions, and genomic DNA was stored at -20°C until use.

Determination of IL-1β, IL-8 and IL-10 polymorphisms

Polymorphisms of IL-1β+3954C/T, IL-6-174G/C, IL-10-1082A/G, and IL-10-819C/T were assessed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Primers of IL-1β+3954C/T (rs1143634), IL-6-174G/C (rs1800795), IL-10-1082A/G (rs1800896), and IL-10-819C/T (rs1800871) were designed using the Sequenom Assay Design 3.1 software, and are shown in Table 1. PCR was performed in a 50-μL reaction mixture containing 25 mM $MgCl_2$, 2 mM dNTPs, 20 μM primer, and 5 U/μL Taq DNA. PCR was performed using the following conditions: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 62°C for 60 s and extension at 72°C for 60 s, and final extension at 72°C for 10 min. The PCR products were visualized by 1.0% agarose gel electrophoresis with ethidium bromide staining and UV light. For quality control, a randomly chosen group of 10% of cases and control subjects were selected, and the results of repeated samples showed 100% concordance.

Table 1. Primer sequences and restriction enzymes for each polymorphism.

Genes	Primers	Restriction enzyme
IL-1β+3954C/T	GCCTGCCCTTCTGATTTTATACC CATCGTGCACATAAGCCTCGTTA	*Taq*I
IL-6-174G/C	CAGAAGAACTCAGATGACCTG GTGGGGCTGATTGGAAACC	*Hsp*92II
IL-10-1082A/G	CCAAGACAACACTACTAAGGCTCCTTT GCTTCTTATATGCTAGTCAGGTA	*Xag*I
IL-10-819C/T	ATGGTGTACAGTAGGGTGAG TTTCCACCTCTTCAGCTGTC	*Hin*la

Statistical analysis

Continuous variables are reported as means ± standard deviation (SD), and categorical variables are reported as N (%) of study participants. The Student *t*-test was used to compare continuous variables between patients and control subjects, and χ^2-test was used to compare categorical variables between patients and control subjects. Hardy-Weinberg equilibrium among controls was compared using the χ^2-test. Unconditional logistic regression was conducted to assess the effects of the IL-1β, IL-6 and IL-10 polymorphisms on the risk of ROU, with results expressed as odds ratios (OR) and corresponding 95% confidence intervals (CIs) on risk of ROU. Homozygotes of the most frequent genotype were regarded as the reference group. All P values were two sided, and $P < 0.05$ was considered as statistically significant. All statistical analyses were conducted using SPSS® statistical package, version 11.0 (SPSS Inc., Chicago, IL, USA) for Windows®.

RESULTS

The demographic and clinical characteristics of study subjects are shown in Table 2. Of the 264 patients with ROU, 117 subjects (44.32%) were males and 147 (55.68%) were females. The mean age of patients and controls were respectively 51.6 ± 11.3 and 35.4 ± 16.8 years old. A total of 166 patients (37.12%) had 3 or more lesions of ROU, and 64 (24.24%)

had ROU on floor of the mouth, 89 (33.71%) on lips, 80 (30.30%) on oral mucosa, 25(9.47%) on tongue, and 6 (2.27%) on soft palate.

The genotype distributions of the IL-6-174G/C and IL-10-1082A/G were in Hardy-Weinberg equilibrium in the control group (Table 3). However, genotype distributions of IL-1β+3954C/T and IL-10-819C/T were not. Moreover, we found that minor allele frequencies of the five gene polymorphisms in the control group were similar to that in dbSNP.

Conditional logistic regression analyses showed that subjects carrying the IL-1β+3954CC genotype were significantly associated with increased risk of ROU, with adjusted ORs (95%CI) of 2.86 (1.37-6.33) (Table 4). Moreover, we found that IL-10-1082AA genotype can increase the risk of ROU when compared with GG genotype, and that the OR (95%CI) was 1.72 (1.02-2.89). However, we did not observe a significant association between IL-6-174G/C and IL-10-819C/T polymorphisms and risk of ROU.

Table 2. Characteristics of ROU patients and controls.

Characteristics	Cases	%	Controls	%	χ^2 or *t*	P value
Mean age (range of years)	35.4 ± 16.8		36.2 ± 16.5		0.55	0.29
Gender						
Male	117	44.32	117	44.32	0.00	1.00
Female	147	55.68	147	55.68		
Number of lesions of ROU						
<3 lesions	166	62.88				
≥3 lesion	98	37.12				
Sites of ROU						
Floor of the mouth	64	24.24				
Lips	89	33.71				
Buccal mucosa	80	30.30				
Tongue	25	9.47				
Soft palate	6	2.27				

Table 3. Genotype characteristics of the four SNPs in the cases and controls.

Gene	SNP	Alleles	MAF[1]		HWE[2] (P value) in controls
			Control group	From dbSNP	
IL-1β+3954 C/T	rs1143634	C/T	0.165	0.146	0.025
IL-6-174G/C	rs1800795	G/C	0.207	0.185	0.63
IL-10-1082A/G	rs1800896	A/G	0.329	0.303	0.01
IL-10-819C/T	rs1800871	C/T	0.409	0.409	0.08

[1]MAF: minor allele frequencies; [2]HWE: Hardy-Weinberg equilibrium.

Table 4. Association between four SNPs and risk of ROU.

SNPs		Cases (N = 264)	%	Controls (N = 264)	%	Adjusted OR (95%CI)[1]	P value
IL-1β+3954 C/T	CC	165	62.4	189	71.7	1.0 (Ref.)	-
	CT	69	26.2	62	23.6	1.27 (0.84-1.94)	0.24
	TT	30	11.4	12	4.7	2.86 (1.37-6.33)	0.002
IL-6-174G/C	TT	151	57.2	165	62.4	1.0 (Ref.)	-
	TA	95	35.9	89	33.8	1.17 (0.80-1.71)	0.41
	AA	18	6.9	10	3.8	1.97 (0.83-4.92)	0.09
IL-10-1082A/G	AA	106	40.2	128	48.5	1.0 (Ref.)	-
	AG	104	39.5	98	37.2	1.28 (0.86-1.90)	0.2
	GG	54	20.3	38	14.3	1.72 (1.02-2.89)	0.03
IL-10-819C/T	CC	89	33.6	99	37.5	1.0 (Ref.)	-
	CT	119	45.2	114	43.18	1.16 (0.78-1.74)	0.45
	TT	56	21.2	51	19.32	1.22 (0.74-2.02)	0.41

[1]Adjusted for gender and age.

DISCUSSION

ROU is a common oral disease, and the etiology of ROU is not well understood. Previous studies reported that many factors play an important role in the development of ROU, including immunological, psychological, genetic and microbiological factors (Natah et al., 2004; Jurge, 2006). Our study showed that IL-1β+3954 C/T and IL-10-1082A/G polymorphism may increase the risk of ROU.

Previous studies have shown that polymorphisms of cytokines have been associated with risk of oral mucosal disease (Bazrafshani et al., 2002; Bai et al., 2007; Xavier et al., 2007; Guimarães et al., 2007a; Kalkan et al., 2013). Kalkan et al. (2013) reported that intron 3 VNTR polymorphism in the IL-4 gene plays an important role in the development of recurrent aphthous stomatitis in the Turkish population. Bai et al. (2007) conducted a cohort study in a Chinese cohort, and reported that IL-18 polymorphism is associated with pathogenesis of oral lichen planus. Xavier et al. (2007) reported that IL-6 and TNF-α gene polymorphisms are associated with occurrence of oral lichen planus. For ROU, Guimarães et al. (2007a) reported that polymorphism of high IL-1β production was correlated with an increased risk of ROU. IL-6 gene polymorphisms can increase susceptibility to ROU (Bazrafshani et al., 2002). Our study showed that IL-1β+3954 CC and IL-10-1082AA genotypes were significantly associated with increased risk of ROU. Two studies reported similar results. Bazrafshani et al. (2003) reported that IL-10 and IL-12 gene polymorphisms can influence susceptibility to ROU. Guimarães et al. (2007b) showed that polymorphism of IL-1β+3954 C/T is correlated with an increased risk of ROU development. However, another study reported inconsistent results with ours. Sun et al. (2013) reported no association between IL-10 polymorphism and development of ROU. Discrepancies in ethnicity, sample size, control selection and study design can cause the difference between results of studies. Further studies are greatly needed to clarify the association between IL-1β and IL-10 polymorphisms and risk of ROU.

There were two limitations in our study. First, a relatively small sample size would limit the statistical power to find the difference between groups. Second, genotype distributions of IL-1β+3954C/T and IL-10-1082A/G were not in Hardy-Weinberg equilibrium in the control group, which showed that the control could not represent well the distribution of the general population. Therefore, selection bias may exist in our study. Third, other genetic polymorphisms may greatly influence the risk of ROU, besides the inflammatory cytokines. Therefore, further well-designed, large sample and multicenter studies are greatly needed to investigate the association between inflammatory cytokines and risk of ROU.

In summary, we found that IL-1β+3954C/T and IL-10-1082A/G polymorphisms are associated with an increased risk of ROU, and that no significant association exists between IL-6-174G/C and IL-10-819C/T gene polymorphisms and risk of ROU. Due to the limitations of our study, further well-designed, large sample and multicenter studies are greatly needed.

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