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Association of Interleukin-8 Gene Polymorphisms and Haplotypes with Oral Lichen Planus in a Chinese Population

Hongxia Dan,[1] Wenzhao Liu,[1,2] Yu Zhou,[1] Jiayi Wang,[1] Qianming Chen,[1] and Xin Zeng[1,3]

Abstract—Interleukin-8 (IL-8), a CXC chemokine with multiple biological functions, plays an important role in the pathogenesis of oral lichen planus (OLP). The aim of this study was to investigate the association of single nucleotide polymorphisms (SNPs) of IL-8 gene with OLP in a Chinese population. Four SNPs of the IL-8 gene at positions −845 T/C (rs2227532), −738 T/A, −251 A/T (rs4073) and +781 C/T (rs2227306) were analyzed in 109 patients with OLP and 101 normal controls using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method. The data revealed that the −251 AA genotype and −251 A allele frequency was significantly lower in the erosive OLP (eOLP) group than in the control group (P=0.012 and P=0.031, respectively). Haplotype analysis revealed that the −251 A/+781 C haplotype frequency was lower in the eOLP group than in the control group (P=0.029) while the −251 T/+781 C haplotype frequency was higher in the eOLP patients than in the healthy controls (P=0.028). The study suggests that the IL-8 polymorphisms may be associated with the severity of OLP in this Chinese cohort.

KEY WORDS: IL-8 gene; oral lichen planus; single nucleotide polymorphism; haplotype.

INTRODUCTION

Lichen planus is a chronic inflammatory disease of skin and mucosa of squamous cell origin. The oral form of lichen planus (OLP) is more common than the cutaneous type, affecting about 0.5–2% of the world population, more often females at a mean age of onset in their forties. The etiology of OLP is unknown. However, it is widely accepted that T cell-mediated immune response plays an important part in the pathogenesis [1–2]. The immune response in OLP is characterized by infiltration of T lymphocytes in lamina propria, and release of chemokines and cytokines. Variation of cytokine levels in the serum, saliva and lesion tissue of OLP patients has been reported by many researchers [3–5].

Genetic and environmental influences play important roles in the variation of cytokine levels. Genetic variations that result in structure or expression alternation of a cytokine can potentially lead to a number of chronic diseases, increased risk of infection and altered outcome of acute disorders or surgery [6]. Single nucleotide polymorphism (SNP) is one of the most common manifestations of genetic variation. Lots of studies have been performed to assess the association of SNPs with various diseases, however, only a few of them focused on the correlation of SNPs and OLP [7–8].

Interleukin-8 (IL-8) is a CXC chemokine produced by monocytes, macrophages, neutrophils, fibroblasts, keratinocytes and some other cells [9]. IL-8 has a great

[1] State Key Laboratory of Oral Diseases, West China College of Stomatology, Sichuan University, No. 14, Section 3, Renminnan Road, Chengdu, Sichuan 610041, China
[2] Hospital of Stomatology, Wenzhou Medical College, Wenzhou, China
[3] To whom correspondence should be addressed at State Key Laboratory of Oral Diseases, West China College of Stomatology, Sichuan University, No. 14, Section 3, Renminnan Road, Chengdu, Sichuan 610041, China. E-mail: zengxin22@163.com

ABBREVIATIONS: LP, lichen planus; OLP, oral lichen planus; eOLP, erosive oral lichen planus; neOLP, nonerosive oral lichen planus; SNP, single nucleotide polymorphism; IL, interleukin; RFLP, restriction fragment length polymorphism; PCR, polymerase chain reaction; EtBr, ethidium bromide.

ability of attracting neutrophils [10]. Furthermore, IL-8 also induces migration of T lymphocytes [11], facilitates tumor growth and angiogenesis [12–13], and inhibits collagen synthesis [14]. Recently, IL-8 has been reported to be a sensitive serologic marker which could monitor the progress of several immune-related diseases, such as recurrent alphthous ulcer and Behcet's disease [15–16]. Previous studies also indicated that serum and saliva level of IL-8 was elevated in OLP patients and that was related to the severity of this disease [17–18].

Previous studies have revealed that SNPs of the IL-8 gene were associated with several diseases, such as respiratory syncytial virus bronchiolitis, acute respiratory distress syndrome and gastric cancer [19–21]. Recently, relationship between IL-8 SNPs and haplotypes and some autoimmune diseases has also been reported [22–24]. As OLP is a kind of disease which correlate with auto-immune reaction [1–2], we hypothesized that SNPs and haplotypes of IL-8 gene might be associated with the susceptibility of individuals to OLP, as well as the progression and prognosis of this disease.

The IL-8 gene is located at chromosome 4q13-21 and contains numerous SNPs, as it's difficult to study all the positions, we chosed four SNPs that were commonly referred, including −845 T/C (rs2227532), −738 T/A [23], −251 A/T (rs4073) and +781 C/T (rs2227306). A case–control study was conducted to assess the association of IL-8 gene SNPs and haplotypes with OLP in ethnic Chinese.

SUBJECTS AND METHODS

Subjects

A sum of 109 Chinese patients clinically and pathologically diagnosed as OLP were enrolled for this study [25], according to the definition of OLP by the World Health Organization. Among those, 54 of them were classified as erosive subtype while the rest 55 were classified as nonerosive subtype based on the criteria of the Society for Oral Medicine, Chinese Stomatological Association [26].

At the same time, 101 healthy volunteers were recruited as the controls. The clinical characteristics of both groups are listed in Table 1.

All subjects were nonsmokers that neither have any systemic disorders (such as cardiovascular disease, diabetes mellitus, etc) nor any soft tissue lesions in the oral cavity. Furthermore, none of them received any medical treatment within 90 days prior to the specimen collection.

Table 1. Clinical Characteristic of OLP Patients and the Controls

	OLP (n=109)	Control (n=101)
Age (years)		
Mean age	43.9	43.5
Range	16–71	18–69
Gender		
Female	33	29
Male	76	72
Disease duration (months)	12	
Range (months)	1–30	
Clinical classification		
Erosive	54	
Nonerosive	55	

This research was carried out with approval of Committee for the Use of Human Subjects in Research in Sichuan University, and informed consents were obtained from both patients and controls.

Sample Collection

A blood sample was drawn from each subject, and genomic DNA was extracted by using the E.Z.N.A Blood DNA Kit (Omega, USA) according to the manufacturer's protocol. All samples were stored at −80°C until use.

Genotyping

The polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method was used to genotype the IL-8 −845 T/C, −738 T/A, −251 A/T and +781 C/T polymorphisms in patients and controls, as described elsewhere [22, 27]. All the subjects' genotypes were analyzed blinded.

The PCR was performed under identical conditions. The reaction mixture was 25 μl, containing 1.5 mM $MgCl_2$, 50 mM KCl, 200 μM of each dNTP, 0.5 μM of each primer, 1 U of Taq DNA polymerase and 50 ng genome DNA.

The PCR cycle began with an initial denaturation step at 94°C for 5 min, followed by 35 cycles of 30 s at 94°C, 1 min at the annealing temperature (61°C for −845 T/C and −738 T/A, 55°C for −251 A/T and 52 C° for +781 C/T), 1 min at 72°C, and a final extension at 72°C for 8 min.

After PCR reaction, 10 μl of the reaction mixture was digested overnight at 37°C with 10 U of each restriction enzyme, and visualized on an EtBr stained 3% agarose gel.

The primers for genotyping IL-8 −845 T/C were 5′-AACCCAGCAGCTCCAGTG-3′ and 5′-AGATAAGC

CAGCCAATCATT-3′. The PCR product (534 bp) was digested with *Vsp*I (New England Biolabs), producing fragments of 341 bp and 193 bp for allele "T", or 534 bp for allele "C".

The primers for genotyping IL-8 −738 T/A were the same as −845 T/C. The PCR product (534 bp) was digested with *Xba*I (New England Biolabs), producing fragments of 302 bp and 232 bp for allele "T", or 534 bp for allele "A".

The primers for genotyping IL-8 −251 A/T were 5′-CCATCATGATAGCATCTGA-3′ and 5′-CCACAATTT GGTGAATTATTAA-3′. The PCR product (173 bp) was digested with *Ase*I (New England Biolabs), producing fragments of 152 bp and 21 bp for allele "A", or 173 bp for allele "T".

The primers for genotyping IL-8 +781 C/T were 5′-CTCTAACTCTTTATATAGGAATT-3′ and 5′-GATTG ATTTTATCAACAGGCA-3′. The PCR product (203 bp) was digested with *Eco*RI (New England Biolabs), producing fragments of 180 bp and 23 bp for allele "C", or 203 bp for allele "T".

Statistical Analysis

All genotype frequencies of control population were tested for Hardy–Weinberg equilibrium. The differences between the practical and expected number of each genotypes were compared by using χ^2 test. Hardy–Weinberg equilibrium was assumed for the P values more than 0.05.

Statistical reconstruction of haplotypes using population genotypic data for current study was performed by using PHASE software.

The differences of genotype frequencies under additive, dominant and recessive genetic models and haplotype distribution between different groups were examined by χ^2 test or Fisher's exact test when appropriate. Odds ratio (OR) and 95% confidence intervals (CI) were calculated for significant associations. Statistically significance was assumed for the P values less than 0.05.

RESULTS

Genotype Analysis of IL-8 Gene

Two SNPs, IL-8 −845 T/C and −738 T/A were not present in our sample set, so these two SNPs were excluded from the further analysis.

All genotype frequencies of the control group conformed to the Hardy–Weinberg equilibrium (P=0.765 for −251 A/T, P=0.630 for +781 C/T; data not shown).

Genotype and allele frequencies of IL-8 gene in patients and controls were summarized in Table 2. There was no significant difference in the genotype distribution between patients with OLP and the controls at position −251 and +781 (data not shown).

The clinical manifestation of OLP can be classified into two subtypes: erosive and nonerosive for the purpose of As shown in Table 2, the frequency of the −251 A allele was significantly lower in eOLP patients than in the controls (P=0.031, OR=0.709, 95%CI=0.512–0.982), and the genotype frequencies at position −251 was significantly different between eOLP patients and the controls (P=0.012, OR=0.208, 95%CI=0.050–0.862, genotype AA compared with AT+TT).

There was no significant difference with genotype and allele frequencies between eOLP patients and controls at the position +781; no significant difference was found between the nonerosive OLP group and the erosive OLP group or the control group at the two SNPs (data not shown).

Haplotype Frequencies of IL-8 Gene

Haplotype analysis of the IL-8 gene was performed by using the SNPs at position −251 and +781. The possible four haplotype frequencies with OLP patients

Table 2. Comparison of IL-8 Gene Polymorphisms Between OLP Patients and the Controls

Polymorphism	Erosive OLP n=54(%)	Nonerosive OLP n=55(%)	Patient n=109(%)	Control n=101(%)
Position −251				
Genotypes				
AA	2 (3.7)*	11 (20.0)	13 (11.9)	18 (17.8)
AT	29 (53.7)	26 (47.3)	55 (50.5)	51 (50.5)
TT	23 (42.5)	18 (32.7)	41 (37.6)	32 (31.7)
Alleles				
A	33 (30.6)**	48 (43.6)	81 (37.2)	87 (43.1)
T	75 (69.4)	62 (56.4)	137 (62.8)	115 (56.9)
Position +781				
Genotypes				
CC	21 (38.9)	19 (34.5)	40 (36.7)	38 (37.6)
CT	30 (55.6)	29 (52.7)	59 (54.1)	46 (45.5)
TT	3 (5.6)	7 (12.7)	10 (9.2)	17 (16.8)
Alleles				
C	72 (66.7)	67 (60.9)	139 (63.7)	122 (60.3)
T	36 (33.3)	43 (39.1)	79 (36.2)	80 (39.6)

*P=0.012, OR=0.208, 95%CI=0.050–0.862 compared to the control group (AA *vs* AT+TT); **P=0.031 compared to the control group

and control subjects were listed in Table 3, and no significant difference was found between the two groups. However, the frequency of eOLP patients carrying haplotype AC was significantly lower than that of the healthy controls (P=0.029, OR=0.332, 95%CI= 0.111–0.992), whereas the haplotype TC was found to be more prevalent in eOLP group than in the control group (P=0.028, OR=1.700, 95%CI=1.054–2.742).

DISCUSSION

OLP is one of the most common diseases of the oral mucosa. Though OLP is not a fatal disease, it can interfere with the quality of the patients' life because of its chronicity and recurrence.

Elucidating the pathophysiology of OLP is one of the major objectives of specialists in oral medicine. Recent studies on cytokine gene polymorphisms and OLP had shed a new light on this. SNPs of several cytokines, including IL-18, IL-4 and IFN-γ, were associated with the development of OLP [7–8].

IL-8 is a multi-functional cytokine that plays an important role in the regulation of inflammation. Although the precise mechanisms were still unknown, it had been reported that serum and saliva level of IL-8 might be a sensitive marker of monitoring the clinical course of OLP [18].

In this study, we investigated the role of four polymorphisms located in the IL-8 loci among 109 OLP patients and 101 healthy controls in an ethnic Chinese cohort. Our results showed that there was no significant difference in the genotype and haplotype distribution between OLP group and the control group, indicating that these SNPs may have no relationship with individuals' susceptibility to OLP.

The severity of OLP was usually evaluated according to the clinical manifestations [26]. Previous investigations indicated that the erosive subtype seemed to cause more serious symptoms such as pain and bleeding compared with nonerosive subtype [28], Therefore, it was considered to be the more severe subtype. In this study, we also divided the OLP patients into erosive and nonerosive subtypes and then we found that there was a minor association of −251 A/T SNP and haplotype of −251/+781 with OLP severity.

IL-8 −251 A/T is a commonly referred site in disease-association studies of IL-8 gene. IL-8 −251 A allele and AA genotype were associated with increased risk and poor prognosis of several diseases [19–21]. Based on these data, we originally speculated that the A allele at position −251, might be associated with individuals' susceptibility to OLP, especially eOLP. Contrary to our expectation, the results of this study showed a significant decreased level of AA genotype and A allele in eOLP patients than in healthy controls, indicating that −251 A allele might be an protective factor against eOLP while T allele might be the risk factor. At the same time, the haplotype analysis also suggested that the haplotype I (−251 A/+781 C) of the IL-8 gene was associated with a significantly decreased risk of eOLP, while the haplotype III (−251 T/+781 C) was associated with a significantly increased risk of eOLP. The reason for the controversy remains obscure to us. However, there're some speculations we can make. Although it has been reported that IL-8 −251 A allele is the high producer allele [20–21], it's still controversial. Lee *et al*. have described that IL-8 −251 T allele possessed transcriptional activity twofold to fivefold stronger than the −251 A counterpart [29]. However, Hacking *et al*. found no difference in promoter activity of the two alleles by performing analysis on the −251 T/A promoter SNP [30]. It is also possible that SNPs at other positions of the IL-8 gene may also have an effect on IL-8 production. Moreover, as IL-8 expression is greatly affected by the activation of nuclear factor-kappa B (NF-κB), it can be induced by the activators of NF-κB, such as TNF-α and CXCL12 [31, 32], so increased

Table 3. Haplotype Frequencies of IL-8 Gene Polymorphisms in OLP Patients and the Controls

Haplotype	−251/+781	Erosive OLP 2n=108(%)	Nonerosive OLP 2n=110(%)	Patient 2n=218(%)	Control 2n=202(%)
I	AC	4 (4.2)*	9 (8.2)	13 (6.4)	21 (11.3)
II	AT	29 (26.4)	39 (35.4)	68 (30.7)	65 (31.3)
III	TC	68 (62.5)**	58 (52.7)	126 (57.3)	101 (49.1)
IV	TT	7 (6.9)	4 (3.6)	11 (5.5)	15 (8.3)

*P=0.029 OR=0.332, 95%CI=0.111–0.992 compared to the control group; **P=0.028 OR=1.700, 95%CI=1.054–2.742 compared to the control group

secretion of IL-8 in OLP patients might be the result of IL-8 gene expression induced by an active immune response, rather than the genetic tendency to express more of this protein. We will try to prove our speculation in the near future by further investigation on the function of the promoter region of IL-8 gene.

In conclusion, our study suggested that the −251 A/T SNP and −251 A/+781 C, −251 T/+781 C haplotype of IL-8 gene could play a role in the severity of OLP in this ethnic Chinese cohort. To the best of our knowledge, this study is the first to demonstrate the association of IL-8 gene polymorphisms and the severity of OLP. However, the sample size of this study was relatively small and only one ethnic group was included, further studies with larger sample size and multiple ethnic groups are required to illustrate the genetic effects of IL-8 gene polymorphisms on OLP. Further investigation on the IL-8 sequence variants and their biological function is also needed.

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REFERENCES

1. Sugerman, P.B., N.W. Savage, L.J. Walsh, *et al.* 2002. The pathogenesis of oral lichen planus. *Critical Reviews in Oral Biology and Medicine* 13(4):350–365.
2. Lodi, G., C. Scully, M. Carrozzo, *et al.* 2005. Current controversies in oral lichen planus: Report of an international consensus meeting. Part 1. Viral infections and etiopathogenesis. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology & Endodontics* 100(2):40–51.
3. Karagouni, E.E., E.N. Dotsika, and Sklavounou. 1994. Alteration in peripheral blood mononuclear cell function and serum cytokines in oral lichen planus. *Journal of Oral Pathology & Medicine* 23 (1):28–35.
4. Simark Mattsson, C., M. Jontel, G. Benrgenholtz, *et al.* 1998. Distribution of interferon-gamma mRNA-positive cell in oral lichen planus lesions. *Journal of Oral Pathology & Medicine* 27 (10):483–488.
5. Simark Mattsson, C., G. Benrgenholtz, M. Jontel, *et al.* 1999. Distribution of interleukin-2-4-10, tumor necrosis factor alpha and transforming growth factor-beta mRNA in oral lichen planus. *Archives of Oral Biology* 44(6):499–507.
6. Smith, A.J.P., and S.E. Humphries. 2009. Cytokine and cytokine receptor gene polymorphisms and their functionality. *Cytokine & Growth Factor Reviews* 20(1):43–59.
7. Bai, J., Y. Zhang, M. Lin, *et al.* 2007. Interleukin-18 gene polymorphisms and haplotypes in patients with oral lichen planus: A study in an ethnic Chinese cohort. *Tissue Antigens* 70(5):390–397.
8. Bai, J., M. Lin, X. Zeng, *et al.* 2008. Association of polymorphisms in the human IFN-γ and IL-4 gene with oral lichen planus: A study in an ethnic Chinese cohort. *Journal of Interferon & Cytokine Research* 28(6):351–358.
9. Rollins, B.J. 1997. Chemokines. *Blood* 90(3):909–928.
10. Peveri, P., A. Walz, B. Dewald, *et al.* 1988. A novel neutrophil activating factor produced by human mono-nuclear phagocytes. *Journal of Experimental Medicine* 167(5):1547–1559.
11. Larsen, C.G., A.O. Anderson, E. Appella, *et al.* 1989. The neutrophil-activating protein (NAP-1) is also chemotactic for T lymphocytes. *Science* 243(4897):1464–1466.
12. Schadendorf, D., A. Moller, B. Algermissen, *et al.* 1993. IL-8 produced by human malignant melanoma cells *in vitro* is an essential autocrine growth factor. *Journal of Immunology* 151 (5):2667–2675.
13. Koch, A.E., P.J. Polverini, S.L. Kunkel, *et al.* 1992. Interleukin-8 as a macrophage-derived mediator of angiogenesis. *Science* 258 (5089):1798–1801.
14. Unemori, E.N., E.P. Amento, E.A. Bauer, *et al.* 1993. Melanoma growth-stimulatory activity/GRO decreases collagen expression by human fibroblasts. Regulation by CXC but not CC cytokines. *Journal of Biological Chemistry* 268(2):1338–1342.
15. Sun, A., J.T. Wang, J.S. Chia, *et al.* 2004. Serum interleukin-8 level is a more sensitive marker than serum interleukin-6 level in monitoring the disease activity of recurrent aphthous ulcerations. *Journal of Oral Pathology & Medicine* 33(3):133–139.
16. Sur Toy, G., N. Lenk, B. Yalcin, *et al.* 2005. Serum interleukin-8 as a serologic marker of activity in Behcet's disease. *International Journal of Dermatology* 44(8):657–660.
17. Sun, A., J.T. Wang, J.S. Chia, *et al.* 2005. Serum interleukin-8 level is a more sensitive marker than serum interleukin-6 level in monitoring the disease activity of oral lichen planus. *British Journal of Dermatology* 152(6):1187–1192.
18. Zhang, Y.Y., M. Lin, S.T. Zhang, *et al.* 2008. NF-κB-dependent cytokines in saliva and serum from patients with oral lichen planus: A study in an ethnic Chinese population. *Cytokines* 41(2):144–149.
19. Hull, J., A. Thomson, and D. Kwiatkowski. 2000. Association of respiratory syncytial virus bronchiolitis with the interleukin 8 gene region in UK families. *Thorax* 55(12):1023–1027.
20. Hildebrand, F., M. Stuhrmann, M. Van Griensven, *et al.* 2007. Association of IL-8 −251A/T polymorphism with incidence of acute respiratory distress syndrome (ARDS) and IL-8 synthesis after multiple trauma. *Cytokine* 37(3):192–199.
21. Taguchi, A., N. Ohmiya, K. Shirai, *et al.* 2005. Interleukin-8 promoter polymorphism increases the risk of atrophic gastritis and gastric cancer in Japan. *Cancer Epidemiology, Biomarkers & Prevention* 14(11):2487–2493.
22. Lee, E.B., J.Y. Kim, J. Zhao, *et al.* 2006. Haplotype association of IL-8 gene with Behcet's disease. *Tissue Antigens* 69(2):128–132.
23. Rovin, B.H., L. Lu, and X.L. Zhang. 2002. A novel interleukin-8 polymorphism is associated with severe systemic lupus erythematosus nephritis. *Kidney International* 62(1):261–265.
24. Renzoni, E., P. Lympany, P. Sestini, *et al.* 2000. Distribution of novel polymorphisms of the interleukin-8 and CXC receptor 1 and 2 genes in systemic sclerosis and cryptogenic fibrosing alveolitis. *Arthritis and Rheumatism* 43(7):1633–1640.
25. Van der Meij, E.H., K.P. Schepman, and I. Van der Waal. 2003. The possible premalignant character of oral lichen planus and oral lichenoid lesions: A prospective study. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology & Endodontics* 96 (2):164–171.

26. Zhou, G., H. Liu, M. Lin, *et al.* 2005. The evaluation criteria for the curative effect of the atrophic and erosive oral lichen plans (on probation). *Chinese Journal of Stomatology* 40:92–93.
27. Heinzmann, A., I. Ahlert, T. Kurz, *et al.* 2004. Association study suggests opposite effects of polymorphisms within IL-8 on bronchial asthma and respiratory syncytial virus bronchiolitis. *Journal of Allergy and Clinical Immunology* 114(3):671–676.
28. Al-Hashimi, I., M. Schifter, P.B. Lockhart, *et al.* 2007. Oral lichen planus and oral lichenoid lesions: Diagnostic and therapeutic considerations. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology & Endodontics* 103(S25):e1–e12.
29. Lee, W., D. Tai, K. Lan, *et al.* 2005. The −251T allele of the interleukin-8 promoter is associated with increased risk of gastric carcinoma featuring diffuse-type histopathology in Chinese population. *Clinical Cancer Research* 11(18):6431–6441.
30. Strieter, R.M., S.L. Kunkel, H.J. Showell, *et al.* 1989. Endothelial gene expression of a neutrophil chemotactic factor by TNF-α, LPS and IL-1β. *Science* 243(4897):1467–1469.
31. Sigal, L.H. 2004. Basic science for the clinician 39: NF-kappaB-function, activation, control, and consequences. *Journal of Clinical Rheumatology* 12(4):207–211.
32. Rehman, A.O., and C. Wang. 2009. CXCL12/SDF-1α Activates NF-κB and Promotes Oral Cancer Invasion through the Carma3/Bcl10/Malt1 Complex. *International Journal of Oral Science* 1 (3):105–118.