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Full Length Research Paper

Clinical and molecular genetics association of polymorphisms in interleukin-17A genes with risk of Oral Lichen Planus (OLP) in an Azery population

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Lichen Planus (LP) is a chronic disease that affects the skin and oral mucosa. Although the precise aetiology of LP is not known, it is thought to be comprised of, in majority, genetic and immunological factors. The objective of this study was to assess the association of interleukin-17A (IL-17A) with Oral LP (OLP). 22 adult patients with OLP and 40 healthy controls were genotyped by polymerase chain reaction (PCR) and DNA direct sequence technology for the polymorphism of the IL-17A gene. The genotype frequencies of G1776A (p.Arg29Ter rs139620979) and G3566A (rs7747909) in the IL-17A gene polymorphism were 9 and 13.6% in the OLP group and 0 and 40% in the controls, respectively. Although the proportion of detected polymorphisms did not differ between individuals, a higher prevalence of G3566A (rs7747909) homozygote polymorphism (4.5%) was observed in the OLP patients. Our results show no statistically significant difference in the IL-17A genotype single nucleotide polymorphisms (SNPs) distribution amongst the two groups. Therefore, further studies on a larger population and novel genetic variants are needed to better understand the pathobiology of OLP.

Key words: Oral Lichen Planus (OLP), interleukin-17A (IL-17A) gene, single nucleotide polymorphisms (SNPs), direct sequencing.

INTRODUCTION

Lichen Planus (LP) is a common inflammatory disease of the skin and oral mucosa, commonly found by dentists during routine checkups (Agha-Hosseini et al., 2010). Affecting around 1 to 2% of the general population in the US, approximately one in five people with Oral LP (OLP) have comorbid skin LP (Habif, 2009; Mirowski and Mark, 2010), with severe forms causing painful sores and ulcers (Pavlotsky, 2008).

To follow on, oral lesions which are most commonly found on the tongue and the buccal mucosa are characterized by white or grey streaks, forming a linear or reticular pattern on a violaceous background. These lesions are classified as reticular, plaque like, atrophic, popular, erosive or bulbous (Chuang et al., 1999; Bigby, 2009).

Furthermore, there is a slight increased risk of developing oral cancer in severe cases of LP, in particular when the disease state involves the dorsal aspect of the tongue (Agha-Hosseini et al., 2010; Pavlotsky, 2008). However, this observation may be confounded by other factors, such as smoking or chewing tobacco (Chuang et al., 1999; Bigby, 2009). Despite this, several studies have highlighted the malignant transformation potential of OLP (Sousa and Paradella, 2009).

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The precise understanding of the etiology of LP is in the majority, unknown or incomplete. However, it is likely to be related to an allergic or immune reaction, or a viral or bacterial infection (Bhattacharya et al., 2000; Norris, 1990). Some patients with LP have a positive familial history, thus, LP may be influenced by a genetic predisposition (Chuang et al., 1999; Bigby, 2009). In addition, the role of the immune system is confirmed by the histopathologic appearance of the disease with linear sub-basilar T lymphocytic infiltration, macrophages and basal layer degeneration. These findings can be interpreted as a demonstration of the role of cell-mediated immunity in the pathogenesis of OLP. To follow on, this presentation is created by T cytotoxic lymphocytes, directly produced against the antigen in the basal cell layer (Shengyuan et al., 2009; Bhattacharyya, 1999). T-helper 17 (Th17) cells are a lineage of $CD4^+$T cells, characterized by their production of interleukin-17 (IL-17) (Fouser et al., 2008). IL-17A (OMIM: 603149) was cloned more than 10 years ago (Figure 1), with six IL-17A family members (IL-17A-F) subsequently having been described (Kolls and Lindén, 2004). These findings have helped accumulate support for the role of Th17A cells and such cytokines in inflammatory processes and autoimmunity or inflammation found in animal models. Polymorphisms of IL-17A, G197A (rs2275913) and IL-17F A7488G (p.His161Arg rs763780) have recently been identified as being associated with a susceptibility to rheumatoid arthritis and ulcerative colitis. Emerging data in clinical trials support our understanding of the importance of Th17 cells in inflammatory disease (Fouser et al., 2008). In addition, some studies have shown that IL-17A plays a crucial role in chronic infection by means of several mechanisms (Kolls and Lindén, 2004).

Moreover, there is current experimental and clinical evidence to illustrate that IL-17 family members are involved in specific inflammatory processes leading to the mobilization of granulocytes. To date, most published evidence supports a role for IL-17A, and possibly IL-17F, as a promoter of granulopoeisis, neutrophil accumulation and neutrophil activation in the lungs, joint space, central nervous system and intestinal tissue (Kolls and Lindén, 2004). Due to the importance of LP as a chronic condition with an unknown etiology thought to be involving immunological factors in its progression , we used direct sequencing to study the possible changes in the DNA structure, which allows for the independent evaluation of the role of IL-17A in LP.

MATERIALS AND METHODS

Study subjects

The study population consisted of those who were referred to the Department of Oral Medicine, Tabriz University of Medical Sciences. The Ethics Committee of Tabriz University of Medical Sciences approved the study protocol, with written informed consent obtained from all of the participating subjects. Twenty-two (22) patients were selected from those with the diagnosis of OLP, which was confirmed by a biopsy. All of the patients were between the ages of 18 to 60 years and willing to participate in the study. None of the patients had any potential cause for a lichenoid reaction. In addition, none of the subjects had any congenital or acquired cause for an immune system deficiency such as Acquired Immunodeficiency Syndrome (AIDS), chemotherapy, IV drug addiction, hemophilia or undergoing dialysis. Also, none of the patients were on medication such as anti-inflammatory drugs, or had active infections such as hepatitis, human immunodeficiency virus infection (HIV) or tuberculosis. The subjects were not pregnant at the time of the study and were not using calcium channel blockers. The selected group did not receive medical intervention, so there were no side effects to the treatment. 40 individuals with no history of any oral inflammation were selected as a healthy control group in the study.

DNA extraction

Genomic DNA extraction was performed from 5cc peripheral blood samples with ethylene diamine tetra acetic acid (EDTA) using the salting out method.

Exon amplification and genotyping of polymorphisms

The polymorphisms were genotyped using the direct sequencing method, with three exons of the IL-17A gene and their flanking intronic sequences amplified from genomic DNA. The polymerase chain reaction (PCR) used the following primers: Forward, 5'-CCATCTCATAGCAGGCACAA-3` and Reverse, 5'-CCAAAGCTTTCATTTCCTATCCT-3`(exon 1); Forward, 5'-TGAGAACAATGGTGCAGGAG-3` and Reverse, 5'-CACAGTGGTCCTTCCAGGTT-3`(exon 2); Forward, 5'-TCCTCCTGATTTTTCTCCCC-3` and Reverse, 5'-ATTCCCAAGCCCAGAATCTT-3` (exon 3). The 25 ul reactions were carried out under standard conditions, containing 100 to 200 ng of genomic DNA, 12.5 ul of master mix (Ampliqon), 1 mm each of forward and reverse primers and 5 ul of H_2O. The PCR procedure was as follows: an initial denaturation at 95°C for 5 min followed by 35 cycles of denaturation at 95°C (45 s), annealing at 62°C (45 s) and extension at 72°C (3 min), with a final 10 min extension at 72°C.

The PCR fragments were separated by electrophoresis, composing of 1.5% agarose gel containing ethidium bromide and visualized by ultraviolet illumination. PCR products were purified using a PCR Purification Kit (Core Bio, Korea). The sequencing and analysis of the reactions were then carried out by Macrogen (Korea). DNA sequencing was performed in both directions, initiated from the forward and reverse primers used in the initial PCR of each exon.

Statistical analysis

The distribution of the genotype and alleles between patients and healthy controls were compared using the χ^2 test. A binary logistic regression analysis was used to assess the influence of gender, age and smoking on the association of IL-17A polymorphisms with OLP. Statistical analyses were performed using SPSS 15.0 computer software (SPSS, Inc., Chicago, IL).

RESULTS

The study case group consisted of 14 males and eight

Table 1. Demographics and characteristics of clinical features in OLP patients.

Category	Variable	Case group number (%)	Control group number (%)
Sex	Male	14(63)	25(62.5)
	Female	8(27)	15(37.5)
Cigarette smoking	Male	6(27.2)	12(30)
	Female	0(0)	2(5)

Table 2. The frequency of polymorphisms in the case and control groups.

SNP	Site	Genotype	Case (%)	Control (%)
T569A	Exon1	Heterozygote	2(4.5)	0
G591A	Exon1	Heterozygote	2(4.5)	0
G1776A (p.Arg29Ter rs139620979)	Exon2	Heterozygote	4(9)	0
G1663A	Exon2	Heterozygote	1(2.2)	0
G1578A	Exon2	Heterozygote	2(4.5)	0
T1677A	Exon2	Heterozygote	1(2.2)	0
C3444G	Exon3	Heterozygote	1(2.2)	0
G3566A (rs7747909)	Exon3	Homozygote	2(4.5)	0
G3566A (rs7747909)	Exon3	Heterozygote	6(13.6)	16(40)
A3480G	Exon3	Heterozygote	0	1(2.5)

females, whilst the control group consisted of 25 males and 15 females. The mean ages of the case and control groups were 41 and 43 years respectively. There were no significant differences between the case and control groups with respect to age and gender. The clinical characteristics of the study subjects are summarized and shown in Table 1.

Molecular evaluation and association of IL-17A gene polymorphisms with OLP

The three exons of the IL-17A gene and their flanking intronic sequences were amplified from the extracted genomic DNA samples of the OLP subjects and the healthy controls. These were then genotyped by the direct sequencing method. The distribution of the polymorphisms detected in the IL-17A gene on the 44 chromosomes of cases and 80 chromosomes of controls examined are shown in Table 2 and Figures 2 and 3.

The polymorphisms G1776A (p.Arg29Ter rs139620979) in exon 2 and G3566A (rs7747909) in exon 3 were more prevalent, respectively. The overall predominant polymorphism was G3566A (rs7747909), which was found homozygote on 2% of alleles of OLP patients but not in the control group.

Genotypes and phenotype-genotype correlation

We compared the clinical phenotypes between genotypic groups to determine whether the presence of a polymorphism in an OLP patient was associated with more severe manifestations of the disease. No significant difference was found between the clinical characteristics of those patients with OLP who did not have the IL-7A polymorphism detected, with those who did ($p < 0.05$). Although the proportions of detected polymorphisms did not differ between the patients with OLP and the control group ($p < 0.05$), a higher prevalence of G3566A (rs7747909) homozygote polymorphism was observed in the patients with OLP.

DISCUSSION

In this study, we attempted to detect an association between the single nucleotide polymorphisms (SNPs) of IL-17A with OLP, in a northwest Iranian population. We chose the IL-17A gene as a candidate based predominantly on its recently reported important role in inflammation. The protein encoded by the IL-17A gene is a pro-inflammatory cytokine produced by activated T cells. This cytokine regulates the activities of NF-kappaB and mitogen-activated protein kinases. This cytokine can stimulate the expression of IL-6 and cyclooxygenase-2 (PTGS2/COX-2), as well as enhance the production of nitric oxide (NO). High levels of this cytokine are associated with several chronic inflammatory diseases including rheumatoid arthritis, psoriasis and multiple sclerosis (Fouser et al., 2008; Kolls and Lindén, 2004).

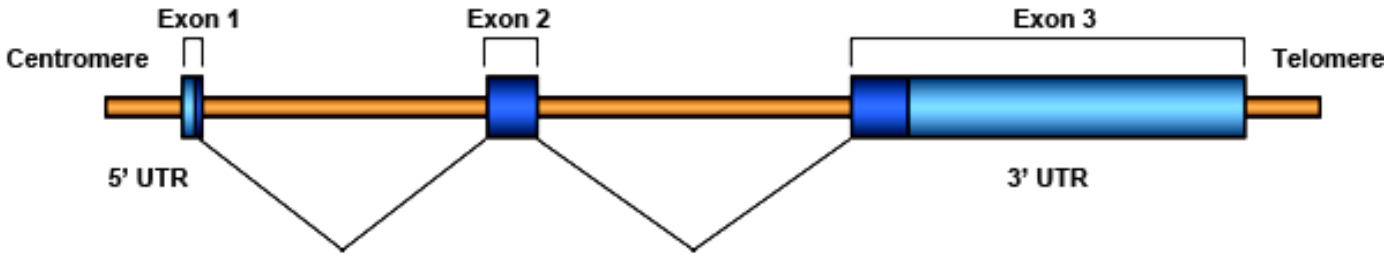

Figure 1. IL17A gene: The IL17A gene spans a region of 4252 bp composed of three exons (untranslated region (UTR), light blue; coding region, blue) and two introns (brown). Exons 1, 2, and 3 are 72 bp (45 bp 5' UTR plus 27 bp coding region), 203 bp (all coding region), and 1584 bp (238 bp coding region plus 1346 bp 3' UTR) in length, respectively. The two introns are 1144 and 1249 bp in length, respectively.

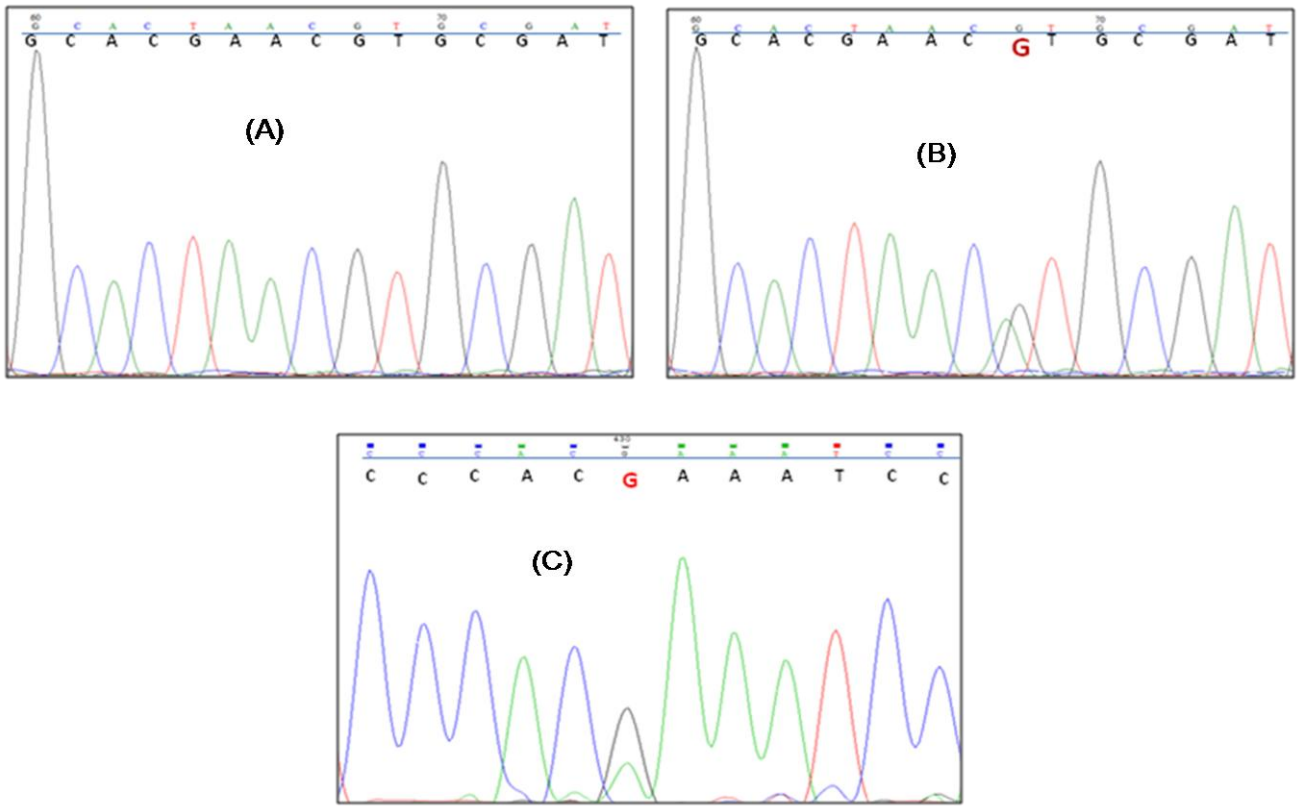

Figure 2. A part of the coding sequence of the IL-17A gene in an OLP patient: A, Normal sequencing of exon 1 of IL-17A. B, A patient with the heterozygous 591 G>GA IN exon 1 SNP. C, A patient with the heterozygous in the R29Q (1776 G>GA) exon 2 SNP.

Polymorphisms of IL-17A, G197A (rs2275913) and IL-17F A7488G (p.His161Arg rs763780) have recently been identified as being associated with susceptibility to rheumatoid arthritis and ulcerative colitis, respectively (Nordang et al., 2009; Arisawa et al., 2008). However, they have not been investigated with respect to OLP and a potential cancer risk. In this study, we investigated the IL-17A polymorphisms in relation to the risk of developing OLP, whilst further studying the association of each SNP with the clinical manifestation of OLP disease in patients who live in the northwest of Iran.

The results of our study show that the IL-17A polymorphisms in exon 1 were present in only 4.5% for T569A and 4.5% for G591A of patients with OLP; a percentage slightly higher than that in the control group (0%). Within exon 2 of the IL-17A gene, the polymorphisms G1776A (p.Arg29Ter rs139620979), G1663A and G1578A were seen in OLP patients; in contrast, these polymorphisms were not detected in the healthy controls. Therefore, the frequencies of these

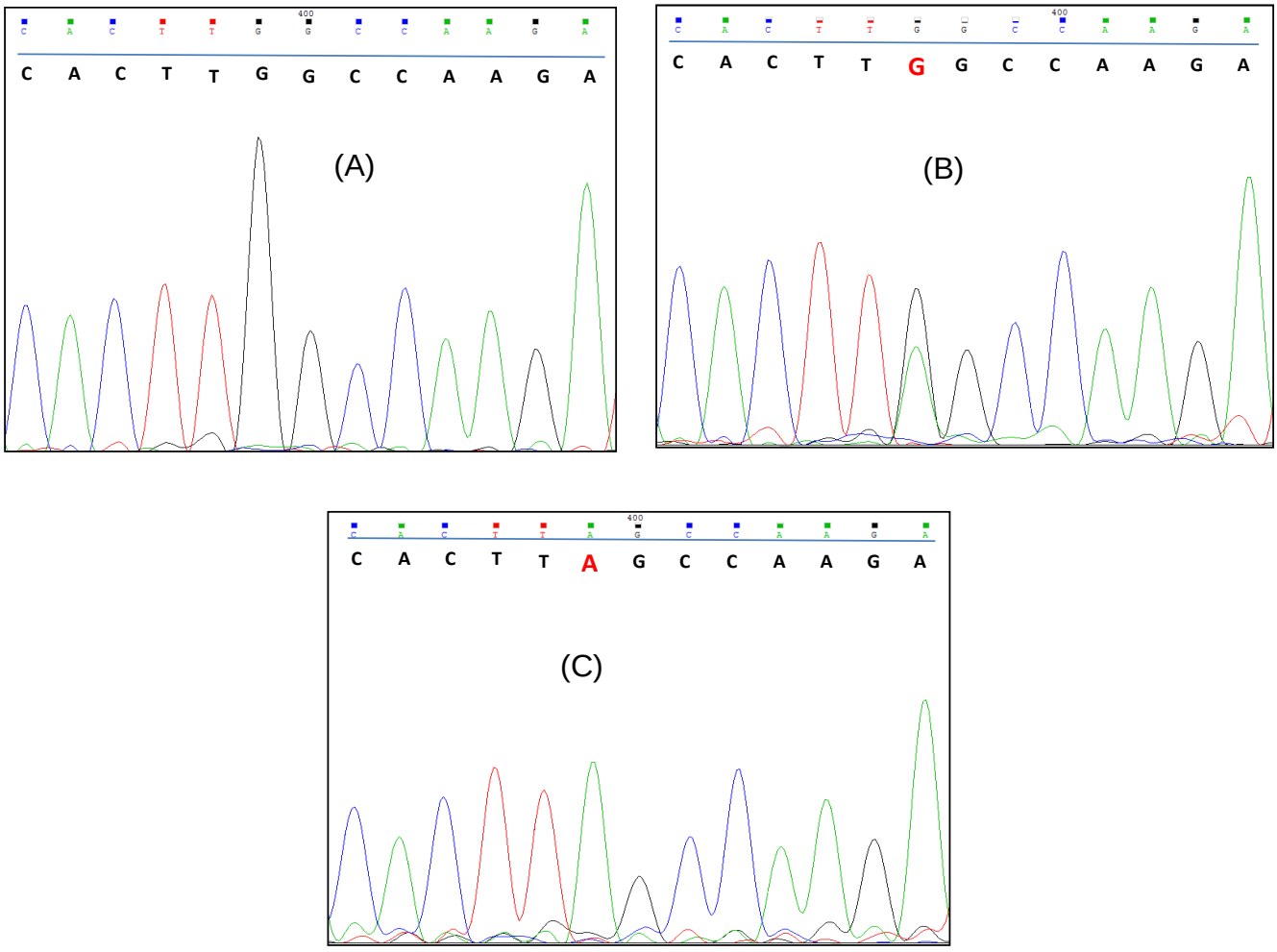

Figure 3. Normal sequencing of exon 3 of IL-17A gene (A), sequencing chromatogram illustrating the 3566 G>GA, c.672 in exon 3 SNP heterozygote (B) and homozygote (C).

polymorphisms are slightly higher in patients with OLP in exon 2 of the IL-17A gene. In addition, we also found that the IL-17A G3566A (rs7747909) heterozygote genotype was significantly increased in the healthy patients in the control group, whereas the IL-17A G3566A (rs7747909) homozygote polymorphism was significantly increased in exon 3 of the IL-17A gene and were probably associated with the development of OLP.

Recently, association between cytokine gene polymorphisms and OLP have been studied. SNPs of IL-4, IL-6, IL-8, IL-18, tumor necrosis factor A (TNFA), tumor necrosis factor receptor 2 (TNFR2) and IFN-α were associated with the development of OLP but SNPs of IL-1, IL-1β, IL-2, IL10, transforming growth factor-beta 1 (TGF- β1) were not associated with its development. (Fujita et al., 2009; Xavier et al., 2007; Bai et al., 2007, 2008; Dan et al., 2010).

Our results demonstrate that the frequency of IL-17A polymorphisms in cases with OLP and those without OLP did not increase with increasing degrees of pain or with the number of cigarettes smoked. Smoking habit was not shown to be associated with significantly higher number of polymorphisms in OLP when comparing long and short-term smokers.

To follow on, association between polymorphisms and clinical data of OLP patients were investigated by Xavier et al. (2007). Results subsequently showed no association between the number of lesions, dermatological manifestation or severity of OLP with gene polymorphisms IL-1β, IL-6 and IL-10 (Xavier et al., 2007).

Finally, the frequencies of the mentioned polymorphisms in the genotype (Table 2) were not positively associated with OLP, whereas the frequency of G3566A (rs7747909) heterozygote genotype was reduced. Our results also show no significant variations in IL-17A genotype distribution among patients who suffered from OLP and the control group. Therefore, further studies on a larger population may be needed to further understand the pathobiology of OLP. Such future

research may need to focus on identifying novel genetic variants and on the interaction of these genetic risk factors with each other and the environment.

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