

# IL17A polymorphism and elevated IL17A serum levels are associated with oral lichen planus

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**Objective:** The aim of this study was to evaluate the association of IL17A G197A polymorphism and serum levels with oral lichen planus (OLP) susceptibility and clinical presentation.

**Subjects and Methods:** Eighty-three individuals diagnosed with OLP and 99 healthy controls (C) were consecutively recruited. All participants had desquamating oral mucosal cells collected and DNA isolated for IL17A (G197A) genotyping. Blood samples of 42 OLP individuals and 23 healthy controls were collected for evaluation of IL17A serum levels.

**Results:** IL17A G197A genotypes were associated with an increased chance of having OLP (GA/AA × GG, OR = 3.44, 95% CI = 1.87–6.33,  $p < .001$ ). Overall A carriers (GA or AA) were more common in OLP (38.1%) than in C (20.2%; OR = 2.43, 95% CI = 1.53–3.87,  $p < .001$ ). Serum levels of IL17A were higher among patients with OLP than in healthy controls (reticular,  $p = .0003$ ; erosive,  $p < .001$ ), but no difference was found among the disease types.

**Conclusions:** IL17A G197A is associated with a higher susceptibility of developing OLP and these patients seem to present a considerable increase in IL17A serum levels. These findings suggest that Th17 cells, and IL17A in particular, may play a pivotal role in OLP pathogenesis.

## KEYWORDS

disease susceptibility, genetic polymorphism, interleukin 17, oral lichen planus

## 1 | INTRODUCTION

Oral lichen planus (OLP) is a mucocutaneous T cell-mediated chronic inflammatory disease of unknown aetiology. Mouth lesions have six clinical presentations, including reticular, papular, plaque-like, erosive, atrophic and bullous, being the presence of white striae a clinical hallmark of the disease (van der Meij, Schepman, & van der Waal, 2003; Roopashree et al., 2010). Microscopically, it is characterised by a dense band-like subepithelial lymphocytic infiltration and degeneration of basal keratinocytes, in a process orchestrated by T cells (Sugerman et al., 2002). In this context, cytotoxic CD8<sup>+</sup> T cells are observed

adjacent to basal keratinocytes and inside the epithelium while CD4<sup>+</sup> T cells are shown to be present in the lamina propria. Besides, the lesional epithelium shows a higher concentration of Langerhans cells (LC), which are responsible for processing and presenting antigen derived to T cells and promote its activation (Gueiros et al., 2012). In this scenario, CD8<sup>+</sup> cytotoxic T cells lead to keratinocyte apoptosis and consequent basal membrane disruption in a process regulated by CD4<sup>+</sup> T cells through the promotion of an altered cytokine profile (Roopashree et al., 2010; Xie, Ding, Xiong, & Zhu, 2012).

Recent evidence supports a central role of immune dysregulation in the pathogenesis of OLP (Lu, Zhang, Sun, Du, & Zhou, 2015). This

process is reflected by an altered production of inflammatory mediators both locally and systemically, being cytokines one of the most relevant mediators of OLP pathogenesis (Rhodus, Cheng, & Ondrey, 2007). T CD4<sup>+</sup> helper cells present two well-known subtypes, Th1 and Th2, which are responsible for cytokine production and immune activation of CD8<sup>+</sup> T cells. OLP seems to have consistent Th1 bias in the orchestration of the inflammatory response, being implicated in the activation of CD8<sup>+</sup> T cells by increased production of INF $\gamma$  (Piccinni et al., 2014). INF $\gamma$  is overexpressed in lesional tissues but decreased in peripheral mononuclear blood cells of OLP patients (Lu et al., 2015). Also, TNF $\alpha$  is another Th1 cytokine acting as a hallmark of OLP, being overproduced in lesional tissues and associated with many pathogenetic mechanisms. TNF $\alpha$  is considered to be directly involved in the important events such as basal cell keratinocyte apoptosis, activation of LCs and basal membrane disruption (Lu et al., 2015). Inasmuch, its expression was shown to be reduced following topical corticosteroid treatment, reinforcing its significant role in the pathogenesis of OLP (Thongprasom, Dhanuthai, Sarideechaigul, Chaiyarit, & Chaimusig, 2006). On the other hand, interleukins 4 and 5 (IL4 and IL5), typical Th2 cytokines, present an inconsistent role in OLP pathogenesis (Lu et al., 2015).

Recent lines of evidence showed that a more recently discovered subset of T helper cell, Th17 cells, also plays a central role in the immune system, acting in both innate and adaptative responses (Yu & Gaffen, 2008) and being involved in the formation and maintenance of a local inflammatory microenvironment. IL17A is the hallmark of Th17 response and its gene is located on chromosome 6p12.1 and contains three exons and two introns (Liu et al., 2015). IL17A G197A (rs2275913) is the most studied IL17A SNP and had been associated with a higher susceptibility to several systemic diseases such as gastric cancer (Liu et al., 2015), Crohn's disease and ulcerative colitis (Zhang et al., 2013), juvenile systemic lupus erythematosus (Hammad et al., 2016), and pulmonary tuberculosis (Shi & Zhang, 2015), but little is known regarding its role in oral diseases.

The available data regarding an IL17A role in OLP pathogenesis point to a upregulation of IL17A expression in lesional tissues and the presence of Th17 cells in OLP lesions (Wang et al., 2013; Xie et al., 2012). Nevertheless, there are no data evaluating IL17A G197A on OLP. Since the available evidence points to a potential role of IL17 in OLP, the aim of this study was to evaluate the association of an IL17A polymorphism and serum levels with OLP and its clinical presentation.

## 2 | MATERIALS AND METHODS

### 2.1 | Subjects

A total of 182 unrelated Brazilian individuals were consecutively recruited at Oral Medicine Unit, Universidade Federal de Pernambuco and Oral Pathology Clinic, Universidade Federal de Minas Gerais from 2009 to 2011. Individuals were distributed into two groups: OLP ( $n = 83$ ) and healthy controls (C,  $n = 99$ ). Healthy controls should present no oral lesions or any inflammatory,

infectious or autoimmune disease, and no history of malignant neoplasia and were selected among Brazilian individuals seeking dental care at the Dental School—UFPE. To be included in OLP group, individuals must present a diagnosis of OLP according to the criteria proposed by van der Meij et al. (2003). Patients diagnosed with oral lichenoid lesions were excluded from the study, so all diagnoses were both clinically and pathologically compatible with OLP. All biopsies were evaluated by the same experienced oral pathologist. All individuals should be 18 years or older at diagnosis. Main clinical features were collected, including clinical subtype, affected sites, the number of sites, the presence of cutaneous lesions and symptoms. The clinical type was classified as reticular, for cases predominantly presenting with white Wickham striae in a reddish background, and non-reticular, when the reticular pattern was not the most significant aspect of the disease. For inclusion in the study, clinical and pathological aspects of the OLP patients were reviewed. Blood samples for IL17 dosing were collected from treatment-naïve individuals.

### 2.2 | Ethical concerns

Each subject has signed an informed consent before entering the study. The study was performed according to the Helsinki statement and was approved by the local Ethics Committee and registered in a Brazilian Registry (Plataforma Brasil) under number CAAE—10221112.3.0000.5208.

### 2.3 | DNA isolation

All subjects were instructed to rinse 5 mL of sucrose solution (3%) for 60 s to collect desquamating oral mucosal cells. The whole saliva for DNA isolation was collected then in a 15-mL centrifuge tube, mixed with 4 mL of PBS and centrifuged at 1,800 g for 5 min. The resulting pellet was stored at  $-20^{\circ}\text{C}$  until processing. Genomic DNA was obtained from the saliva samples using QIAamp Blood Mini Kit (Qiagen, Germany) according to manufacturer's instruction.

### 2.4 | Genotyping analysis

IL17A gene was genotyped by real-time PCR technique with the use of the TaqMan system of probes (Applied Biosystems, Foster City, CA, USA) with the ID assay C\_15879983\_10. The following target sequences were researched for evaluation of single nucleotide polymorphism (SNP): gene IL17A, allele G197A (rs2275913) and forward 5'-ATTCTGCCCTTCCCATTTT-3' and reverse 5'-CCCAGGAGTCATCGTTGTTT-3' sequence.

PCRs were prepared using a set of TaqMan<sup>®</sup> Universal PCR Master Mix (Applied Biosystems) reagents with the following protocol of reaction: 6.25  $\mu\text{L}$  of water, 1.25  $\mu\text{L}$  of primer/probe, 12.5  $\mu\text{L}$  of Master Mix and 5  $\mu\text{L}$  of DNA, with the final volume being 25  $\mu\text{L}$ . As cycling protocol, a total of 40 cycles were used: 10 min at  $95^{\circ}\text{C}$  and cycling of 15 s at  $92^{\circ}\text{C}$  and 1 min at  $60^{\circ}\text{C}$ . The reactions were analysed in the thermal cycler Rotor gene Q (Qiagen).

## 2.5 | IL17A dosing

Blood samples of treatment-naïve patients and controls were collected in a dry vacuum tube with separating gel 30–60 days after biopsy, when the patient returned to discuss about the microscopic report, before initiating any treatment. The tube rested for 30 min for clotting process and was then centrifuged at 1,000 g for 5 min and stored at  $-20^{\circ}\text{C}$  until processing. ELISA was performed with Human IL-17 DuoSet (R&D Systems, EUA) according to manufacturer's instructions. The absorbance of the samples was evaluated at 492 nm in a spectrophotometer (Epoch, Biotek, USA) and the results expressed in pg/mL.

## 2.6 | Statistical analysis

The allele distributions and genotype frequencies were compared between groups with clinicopathological features and serological markers using chi-square ( $\chi^2$ ) or Fisher's exact test, when appropriate. The comparisons of IL17A serum levels between groups were calculated by Student t test and ANOVA (reticular, non-reticular and controls). Odds ratios (OR) and 95% confidence intervals (CI) were calculated for significant associations. Statistical significance was assumed for  $p < .05$ . The statistical analysis of data was performed by using Statistical Package for Social Science software (SPSS, version 17, Chicago, IL, USA).

## 3 | RESULTS

### 3.1 | Clinical features

A total of 182 patients were included in the study. From these, 83 were part of OLP group (23 male, 60 female) and 99 part of C group (22 male, 77 female). The mean age was 48.96 years (ranging from 16 to 78) in OLP and 43.5 years in C (ranging from 19 to 86). The reticular type was the most common presentation ( $n = 61$ , 73.49%), and symptoms were reported by a minority of the OLP group ( $n = 28$ , 33.73%). Most of the patients presented one affected site ( $n = 38$ , 46.3%), and buccal mucosa was more frequently involved ( $n = 73$ , 89.0%; Table 1).

### 3.2 | Association of IL17A polymorphism with disease susceptibility and disease features

Both OLP and control individuals were in Hardy–Weinberg equilibrium. IL17A G197A genotype frequencies were significantly different in OLP (GG, 35.7%; GA, 52.4%; AA, 11.9%) compared to C (GG, 65.6%; GA, 28.3%; AA, 19.7%; GG  $\times$  GA/AA, OR = 3.44, 95% CI = 1.87–6.33,  $p < .0001$ ). In addition, overall A carriers (GA or AA) were higher in OLP (38.1%) than in C (20.2%; OR = 2.43, 95% CI = 1.53–3.87,  $p < .001$ ; Table 2). Nevertheless, IL17A G197A genotype frequencies or allelic distribution were not associated with any clinical features of OLP (Table 3).

### 3.3 | IL17A dosing

Forty-two patients (35 reticular OLP and seven atrophic-erosive OLP) and 23 controls had the serum levels of IL17A measured.

**TABLE 1** Distribution of clinical data among oral lichen planus and control individuals

Variable	Patients	
	OLP n (%)	C n (%)
Overall	83 (45.6)	99 (54.4)
Age		
Mean	48.96	43.5
Range	16–78	19–86
Median	46.5	44.3
Gender		
Male	23 (27.7)	22 (22.22)
Female	60 (72.3)	77 (77.78)
Clinical presentation		
Reticular	61 (73.5)	
Atrophic-erosive	22 (26.5)	
Symptoms		
Yes	28 (33.7)	
No	55 (62.3)	
Number of sites <sup>a</sup>		
1	38 (46.3)	
2	30 (36.6)	
3	14 (17.1)	
Affected sites		
Buccal mucosa	73 (89.0)	
Tongue	31 (37.8)	
Gingiva	21 (25.6)	
Other	15 (18.3)	

OLP, oral lichen planus; C, Control.

<sup>a</sup>Bilateral involvement of the same site were considered one site.

Serum levels of OLP patients were higher than C patients (Table 3). Serum levels of IL17A were higher among patients with reticular ( $16.56 \pm 18.40$  pg/mL) and erosive ( $11.52 \pm 2.60$  pg/mL) OLP than in healthy controls ( $10.211 \pm 5.076$  pg/mL;  $p < .0001$  and  $p = .0005$ , respectively). No difference was found among the OLP groups ( $p = .527$ ; Figure 1). No association between serum levels of IL17A and IL17A G197A genotypes ( $p = .581$ ) or allele frequency ( $p = .156$ ) was demonstrated. Also, symptoms ( $p = .702$ ), clinical type (.228) and number of sites ( $p = .568$ ) were not associated with serum levels of IL17A (Table 4).

## 4 | DISCUSSION

This seems to be the first study to evaluate the role of IL17A polymorphism and serum levels in OLP susceptibility. Importantly, it could be newly observed that GA and AA genotypes and A allele were associated with a higher susceptibility of having OLP, but not with clinical

Variable	OLP		C		Total		p value	OR 95% CI
	n	%	n	%	n	%		
IL17A								
GG	30	35.7	65	65.6	75	41.0	GG × GA+AA $p < .0001^a$	3.44 1.87–6.33
GA	43	52.4	28	28.3	72	39.3		
AA	10	11.9	6	6.1	36	19.7		
Total	83	100	99	100	182	100		
Allele frequency								
G	102	61.9	158	79.8	260	71.6	$p < .001^a$	2.43 1.53–3.87
A	64	38.1	40	20.2	104	28.4		
Total	166	100	198	100	364	100		

OLP, oral lichen planus; C, control; OR, odds ratio; CI, confidence interval.

<sup>a</sup>Chi-square test.

**TABLE 3** IL17A serum levels in oral lichen planus and healthy controls

	IL17A serologic levels		
	OLP	Controls	p value
Age			
Mean	50.02	46.39	.147 <sup>a</sup>
SD	12.31	9.05	
Range	28–78	28–67	
Gender			
Male	11	6	.496 <sup>b</sup>
Female	31	17	
Serum level			
Mean	15.657	10.211	$p < .001^a$
SD	16.735	5.076	
Range	8.242–92.282	8.242–28.663	

<sup>a</sup>Mann–Whitney *U*-test.

<sup>b</sup>Chi-square test.

presentation nor IL17A serum levels. Besides, OLP patients presented a higher serum concentration of IL17A regardless of the clinical disease type. These results suggest that IL17A plays an important role in OLP pathogenesis.

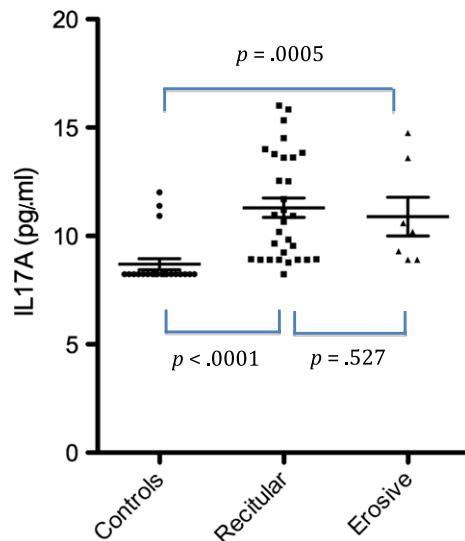
Recent lines of evidence have suggested a possible role of Th17 cells, characterised by IL17A expression and polymorphism, in several diseases initiation and progression (Xie et al., 2012). In Asian individuals with gastric cancer, AA genotype of IL17A polymorphism and A allele were associated with increased cancer risk in a recent meta-analysis (Liu et al., 2015). Also, IL17A was associated with an increased risk of other inflammatory diseases such as Crohn's disease and ulcerative colitis (Zhang et al., 2013), as well as autoimmune diseases such as juvenile systemic lupus erythematosus (Hammad et al., 2016), and infectious diseases such as pulmonary tuberculosis (Shi & Zhang, 2015). Among oral diseases, the periodontal disease seems to be associated with an increased expression and higher serum levels of IL17A, suggesting its possible involvement with periodontal destruction

(Wang et al., 2013, 2014). Interestingly, there seems to be a positive interaction between periodontal disease and OLP, leading a significant increase in IL17A in the serum and inflammatory infiltrate, as well as a higher expression level of mRNA in individuals with both conditions (Wang et al., 2013, 2014). Subsequently, it may lead to polarisation of the immune response and thus influence disease progression.

Some SNPs of Th17 genes have been associated with several diseases and conditions. Of these, IL17A G197A is the most studied, being located in the promoter region of the IL17A gene and described as a functional polymorphism, or capable of altering the function of the gene (Albert, 2011). The IL17A G197A influence on IL17A gene expression and protein secretion was demonstrated in stimulated peripheral blood monocyte culture of patients with graft versus host disease, being the AA genotype responsible for higher levels of protein and mRNA (Espinoza et al., 2011). Additionally, an in vitro study with periodontal disease patients confirmed that A allele was capable of inducing higher levels of IL17A (Linhartova et al., 2016). Nevertheless, these data were not supported in a clinical scenario, and patients with colorectal cancer seem to have a significant increase in IL17A in the sera, but IL17A G197A genotypes did not influence these levels (Nemati, Golmoghaddam, Hosseini, Ghaderi, & Doroudchi, 2015). Based on these data, it seems that IL17A G197A may be functional in some diseases and does not influence protein secretion in others, reinforcing that each clinical situation should be carefully evaluated.

The disease pathway of OLP is still not adequately understood, but some disease mechanisms have been considered to be consistent so far. The hallmark of OLP pathogenesis seems to be the keratinocyte apoptosis by CD8<sup>+</sup> lymphocytes following cytokines regulation by CD4<sup>+</sup> cells. Microscopically, cytotoxic lymphocytes are often present between the epithelial layers, whereas T helper lymphocytes are usually found within lamina propria, in addition to other lymphocytic types such as Tregs (Vered, Fürth, Shalev, & Dayan, 2013). Two main subsets of T helper cells, namely Th1 and Th2, have been implicated in the pathogenesis of OLP. They are identified by signature cytokines, interferon (IFN)- $\gamma$  and IL4, respectively, and a significant Th1 bias has been demonstrated (Khan et al., 2003). Recently, Th17 immune response has gained attention in OLP immune setting. Th17 cells, producing

**TABLE 2** Association of IL17A G197A polymorphism and allele frequency with oral lichen planus susceptibility



**FIGURE 1** Serum levels of IL17A according to clinical types of OLP [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

IL17, characterises a potent proinflammatory response that seems to be uniquely orchestrated by Treg cells (Lu et al., 2014; Xie et al., 2012). Scarce but convergent data have pointed to the role of IL17-mediated immune response in OLP pathogenesis. Similar to other inflammatory diseases, high levels of IL17 can promote an inflammatory response and could be related to worse outcomes.

In this context, the role of Th17 in LP has been recently evaluated only through small studies. Similar to Xie et al., we have demonstrated a higher serum level of IL17A in patients with OLP regardless of the clinical type of the OLP (Xie et al., 2012). Serum levels of IL17A were also described to be elevated in a small study ( $n = 30$ ) that analysed individuals with cutaneous LP (Shaker & Hassan, 2012), reinforcing its relevance to the pathogenesis of the disease. Notwithstanding, some authors have reported a possible distinction among erosive and reticular types of the disease, with the former presenting higher levels of IL17A (Pouralibaba, Babaloo, Pakdel, & Aghazadeh, 2013; Xie et al., 2012). Piccinni et al. (2014) demonstrated an increase in IL17 mRNA

in erosive OLP, associated with an increase in Th0 molecules, which may point to a close relationship between Th0 and Th17 immune responses in OLP. Although there seems to be a clear Th-17 path in OLP, other clinical features rather than clinical presentation did not seem to be influenced by IL17A serum levels in both oral and cutaneous forms (Pouralibaba et al., 2013; Shaker & Hassan, 2012).

The inflammatory infiltrate of OLP is composed of T cells distributed within the epithelium and subepithelial layers. Activated CD8 lymphocytes are located among epithelial cells and seem to be responsible for keratinocyte apoptosis (Roopashree et al., 2010). On the other hand, CD4 lymphocytes are located in the subepithelial layers and seem to orchestrate the pathologic events. Also, Tregs cells also play a major role in OLP and present distinctive function in oral and cutaneous disease, being significantly increased in the oral types. In this scenario, Tregs are positively correlated with IL17 expression, thus forming a unique interplay between Tregs and Th17 cells (Shen et al., 2014). In fact, the overexpression of IL23, the hallmark of Tregs, probably contributes to the induction of Th17 differentiation in OLP. Th17 cells are abundant within inflammatory infiltrate of both reticular and erosive OLP in similar frequency (Vered et al., 2013). As a consequence, increased IL17 expression is observed in the inflammatory infiltrate of both reticular and erosive OLP, but limited IL17+ cells are found in normal oral mucosa (Lu et al., 2014). In fact, once IL17 has been extensively associated with several autoimmune and inflammatory diseases, it was not a surprise to observe its regulatory role in OLP pathogenesis.

Although there is a clear pattern of Th17 response in inflammatory and autoimmune diseases, the relevance of IL17 polymorphism varies according to the disease. We have previously demonstrated a lack of association between IL17A SNP, rheumatoid arthritis and secondary Sjögren's syndrome (Carvalho et al., 2016) in a study with 206 individuals, but a weak association was demonstrated in a larger sample (Nordang et al., 2009). In ankylosing spondylitis, a SNP in the promoter region of IL17RA was associated with an elevated risk, with a stronger association in individuals with severe forms (Vidal-Castiñeira et al., 2016). Inflammatory bowel diseases seem to be influenced by IL17 SNPs, and there seems to be an interaction between the risk

**TABLE 4** Association of IL17A G197A polymorphism and allele frequency with clinical features of oral lichen planus

IL17 G197A Genotypes and alleles	OLP $n = 83$ (%)			OLP $n = 31$ (%)			OLP $n = 82$ (%)		
	Reticular	Erosive	$p$	No symptom	Symptom	$p$	1 site	2+ sites	$p$
GG	23 (38.3)	7 (30.4)	.228 <sup>a</sup>	2 (9.5)	2 (20.0)	.702 <sup>a</sup>	9 (28.1)	19 (38.0)	.568 <sup>a</sup>
GA	28 (46.7)	15 (65.3)		16 (76.2)	7 (70.0)		18 (56.3)	26 (52.0)	
AA	9 (15)	1 (4.3)		3 (14.3)	1 (10.0)		5 (15.6)	5 (10.0)	
GG × GA/AA	23 (38.3) × 37 (61.7)	7 (30.4) × 16 (69.6)	.507 <sup>b</sup>	2 (9.5) × 19 (90.5)	2 (20.0) × 8 (80.0)	.577 <sup>a</sup>	9 (28.1) × 23 (71.8)	19 (38.0) × 31 (62.0)	.357 <sup>b</sup>
G	74 (61.7)	29 (63.0)	.870 <sup>a</sup>	20 (47.6)	11 (55.0)	.786 <sup>b</sup>	36 (56.3)	64 (64.0)	.321 <sup>b</sup>
A	46 (38.3)	17 (37.0)		22 (52.3)	9 (45.0)		28 (43.7)	36 (36.0)	

OLP, oral lichen planus.

<sup>a</sup>Fisher exact test.

<sup>b</sup>Chi-square test.

haplotypes in IL23R and IL17A in individuals with ulcerative colitis (Yu et al., 2012). Also, a high risk of developing gastric cancer was associated with several IL17 SNPs, reinforcing its role in gastrointestinal diseases (Yang et al., 2016).

Nevertheless, to the best of our knowledge, this was the first study to analyse IL17A gene polymorphism G197A in OLP. As reported for other conditions, AA and GA genotypes, as well as the A allele, were strongly associated with OLP susceptibility, reinforcing its role in disease pathogenesis. However, genotypes were not associated with clinical presentation or serum levels, but this should be confirmed in larger studies.

The present results have an important relevance in describing the higher susceptibility related to IL17A genotypes in a considerable number of patients. This finding reinforces the role of Th17 immune response in inflammatory diseases and may suggest that IL17 may become a therapeutic target in similar conditions. Nevertheless, the association between genotyping and serum level could not be properly evaluated due to the limited number of serum samples.

Some other limitations should be addressed. Although not assessed by the van der Meij et al. (2003); diagnostic criteria, distinguishing OLP from chronic ulcerative stomatitis (CUS), may rely on direct immunofluorescence, serologic study or both. Although OLP is much more common than CUS, it is possible that some patients with the latter condition could be included in the present study. Also, IL17A serum levels can be influenced by periodontal disease and smoking status was not assessed in the present study. Although there is no evidence pointing to the influence of smoking on IL17A expression on oral tissues, it was significantly elevated in the bronchial mucosa of asthmatic smokers, supporting its role on the development of neutrophilic inflammation of the airways, characterising severe disease and acute asthma attacks (Huang et al., 2016; Siew, Wu, Ying, & Corrigan, 2017). Inasmuch, recent evidence suggests an increase in IL17A mRNA expression in OLP patients with periodontal disease (Linhartova et al., 2016). These aspects may limit the understanding of serologic results, eventually acting as confounding factors. Another point of concern is the difference between OLP and control individuals in the serologic analysis. Although we included treatment-naïve OLP patients, so far limiting the influence of OLP treatment on IL17A serum levels, only a fraction of healthy controls was included. In this scenario, even having serologic results similar to previous studies (Pouralibaba et al., 2013; Shaker & Hassan, 2012; Xie et al., 2012), one should consider that the present results could be overestimated."

In summary, it could be demonstrated that IL17A G197A is associated with a higher susceptibility of developing OLP and these patients seem to present a considerable increase in IL17A serum levels. Neither susceptibility nor serum levels was influenced by the disease type. These results suggest that OLP may be strongly regulated by Th17 cells, and IL17A seems to play a significant role in its pathogenesis.

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## AUTHOR CONTRIBUTIONS

Drs. Gueiros and Leão have designed the paper. Drs. Arão, Souza and Viera have collected patients samples and performed lab tests. Drs Almeida, Gomez and Lodi have analyzed the data. Drs. Arão, Souza and Viera have drafted the paper. Drs. Gueiros and Leão analyzed final version for submission.

## REFERENCES

- Albert, P. R. (2011). What is a functional genetic polymorphism? Defining classes of functionality. *Journal of Psychiatry and Neuroscience*, 36, 363–365.
- Carvalho, C. N., do Carmo, R. F., Duarte, A. L., Carvalho, A. A., Leão, J. C., & Gueiros, L. A. (2016). IL-17A and IL-17F polymorphisms in rheumatoid arthritis and Sjögren's syndrome. *Clinical Oral Investigations*, 20, 495–502.
- Espinoza, J. L., Takami, A., Nakata, K., Onizuka, M., Kawase, T., Akiyama, H., ... Program, J. M. D. (2011). A genetic variant in the IL-17 promoter is functionally associated with acute graft-versus-host disease after unrelated bone marrow transplantation. *PLoS ONE*, 6, e26229.
- Gueiros, L. A., Gondak, R., Jorge Junior, J., Coletta, R. D., Carvalho, A. A., Leao, J. C., ... Vargas, P. A. (2012). Increased number of Langerhans cells in oral lichen planus and oral lichenoid lesions. *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology*, 113, 661–666.
- Hammad, A., Mosaad, Y. M., Hammad, E. M., Elhanbly, S., El-Bassiony, S. R., Al-Harrass, M. F., ... Fawzy, I. M. (2016). Interleukin-17A rs2275913, Interleukin-17F rs763780 and rs2397084 gene polymorphisms as possible risk factors in Juvenile lupus and lupus related nephritis. *Autoimmunity*, 49, 31–40.
- Huang, C. C., Wang, C. H., Fu, C. H., Huang, C. C., Chang, P. H., Chen, Y. W., ... Lee, T. J. (2016). Association between cigarette smoking and interleukin-17A expression in nasal tissues of patients with chronic rhinosinusitis and asthma. *Medicine (Baltimore)*, 95, e5432.
- Khan, A., Farah, C. S., Savage, N. W., Walsh, L. J., Harbrow, D. J., & Sugeran, P. B. (2003). Th1 cytokines in oral lichen planus. *Journal of Oral Pathology and Medicine*, 32, 77–83.
- Linhartova, P. B., Kastovsky, J., Lucanova, S., Bartova, J., Poskerova, H., Vokurka, J., ... Holla, L. I. (2016). Interleukin-17A gene variability in patients with type 1 diabetes mellitus and chronic periodontitis: Its correlation with IL-17 levels and the occurrence of periodontopathic bacteria. *Mediators of Inflammation*, 2016, 2979846.
- Liu, J., Xu, Q., Yuan, Q., Wang, Z., Xing, C., & Yuan, Y. (2015). Association of IL-17A and IL-17F polymorphisms with gastric cancer risk in Asians: A meta-analysis. *Human Immunology*, 76, 6–12.
- Lu, R., Zeng, X., Han, Q., Lin, M., Long, L., Dan, H., ... Chen, Q. (2014). Overexpression and selectively regulatory roles of IL-23/IL-17 axis in the lesions of oral lichen planus. *Mediators of Inflammation*, 2014, 701094.
- Lu, R., Zhang, J., Sun, W., Du, G., & Zhou, G. (2015). Inflammation-related cytokines in oral lichen planus: An overview. *Journal of Oral Pathology and Medicine*, 44, 1–14.
- van der Meij, E. H., Schepman, K. P., & van der Waal, I. (2003). The possible premalignant character of oral lichen planus and oral lichenoid lesions: A prospective study. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics*, 96, 164–171.
- Nemati, K., Golmoghaddam, H., Hosseini, S. V., Ghaderi, A., & Doroudchi, M. (2015). Interleukin-17FT7488 allele is associated with a decreased risk of colorectal cancer and tumor progression. *Gene*, 561, 88–94.
- Nordang, G. B., Viken, M. K., Hollis-Moffatt, J. E., Merriman, T. R., Førre, Ø., Helgetveit, K., ... Lie, B. A. (2009). Association analysis of the interleukin 17A gene in Caucasian rheumatoid arthritis patients from Norway and New Zealand. *Rheumatology*, 48, 367–370.

- Piccinni, M. P., Lombardelli, L., Logiodice, F., Tesi, D., Kullolli, O., Biagiotti, R., ... Ficarra, G. (2014). Potential pathogenetic role of Th17, Th0, and Th2 cells in erosive and reticular oral lichen planus. *Oral Diseases*, 20, 212–218.
- Pouralibaba, F., Babaloo, Z., Pakdel, F., & Aghazadeh, M. (2013). Serum levels of interleukin-17 in patients with erosive and non-erosive oral lichen planus. *Journal of Dental Research, Dental Clinics, Dental Prospects*, 7, 91–94.
- Rhodus, N. L., Cheng, B., & Ondrey, F. (2007). Th1/Th2 cytokine ratio in tissue transudates from patients with oral lichen planus. *Mediators of Inflammation*, 2007, 19854.
- Roopashree, M. R., Gondhalekar, R. V., Shashikanth, M. C., George, J., Thippeswamy, S. H., & Shukla, A. (2010). Pathogenesis of oral lichen planus – A review. *Journal of Oral Pathology and Medicine*, 39, 729–734.
- Shaker, O., & Hassan, A. S. (2012). Possible role of interleukin-17 in the pathogenesis of lichen planus. *British Journal of Dermatology*, 166, 1367–1368.
- Shen, Z., Gao, X., Ma, L., Zhou, Z., Shen, X., & Liu, W. (2014). Expression of Foxp3 and interleukin-17 in lichen planus lesions with emphasis on difference in oral and cutaneous variants. *Archives of Dermatological Research*, 306, 441–446.
- Shi, G. C., & Zhang, L. G. (2015). Influence of interleukin-17 gene polymorphisms on the development of pulmonary tuberculosis. *Genetics and Molecular Research*, 14, 8526–8531.
- Siew, L. Q. C., Wu, S. Y., Ying, S., & Corrigan, C. J. (2017). Cigarette smoking increases bronchial mucosal IL-17A expression in asthmatics, which acts in concert with environmental aeroallergens to engender neutrophilic inflammation. *Clinical and Experimental Allergy*, 47, 740–750.
- Sugerman, P. B., Savage, N. W., Walsh, L. J., Zhao, Z. Z., Zhou, X. J., Khan, A., ... Bigby, M. (2002). The pathogenesis of oral lichen planus. *Critical Reviews in Oral Biology and Medicine*, 13, 350–365.
- Thongprasom, K., Dhanuthai, K., Sarideechaigul, W., Chaiyarit, P., & Chaimusig, M. (2006). Expression of TNF-alpha in oral lichen planus treated with fluocinolonone acetone 0.1%. *Journal of Oral Pathology and Medicine*, 35, 161–166.
- Vered, M., Fürth, E., Shalev, Y., & Dayan, D. (2013). Inflammatory cells of immunosuppressive phenotypes in oral lichen planus have a proinflammatory pattern of expression and are associated with clinical parameters. *Clinical Oral Investigations*, 17, 1365–1373.
- Vidal-Castiñeira, J. R., López-Vázquez, A., Díaz-Peña, R., Díaz-Bulnes, P., Martínez-Cambor, P., Coto, E., ... Lopez-Larrea, C. (2016). A single nucleotide polymorphism in the IL17ra promoter is associated with functional severity of ankylosing spondylitis. *PLoS ONE*, 11, e0158905.
- Wang, H., Han, Q., Luo, Z., Xu, C., Liu, J., Dan, H., ... Chen, Q. (2014). Oral lichen planus may enhance the expression of Th17-associated cytokines in local lesions of chronic periodontitis. *Clinical Oral Investigations*, 18, 1647–1654.
- Wang, H., Luo, Z., Lei, L., Sun, Z., Zhou, M., Dan, H., ... Chen, Q. (2013). Interaction between oral lichen planus and chronic periodontitis with Th17-associated cytokines in serum. *Inflammation*, 36, 696–704.
- Xie, S., Ding, L., Xiong, Z., & Zhu, S. (2012). Implications of Th1 and Th17 cells in pathogenesis of oral lichen planus. *Journal of Huazhong University of Science and Technology – Medical Sciences*, 32, 451–457.
- Yang, L. J., Gao, W., Bai, J. Y., Zhang, X. K., Han, X., Sun, Y. H., ... Zhang, M. M. (2016). Correlation between Interleukin-17 gene polymorphism and gastric cancer susceptibility in Han Chinese population. *European Review for Medical and Pharmacological Sciences*, 20, 1271–1282.
- Yu, J. J., & Gaffen, S. L. (2008). Interleukin-17: A novel inflammatory cytokine that bridges innate and adaptive immunity. *Frontiers in Bioscience*, 13, 170–177.
- Yu, P., Shen, F., Zhang, X., Cao, R., Zhao, X., Liu, P., ... Zhang, H. (2012). Association of single nucleotide polymorphisms of IL23R and IL17 with ulcerative colitis risk in a Chinese Han population. *PLoS ONE*, 7, e44380.
- Zhang, X., Yu, P., Wang, Y., Jiang, W., Shen, F., Tu, H., ... Zhang, H. (2013). Genetic polymorphisms of interleukin 17A and interleukin 17F and their association with inflammatory bowel disease in a Chinese Han population. *Inflammation Research*, 62, 743–750.

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