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ORIGINAL ARTICLE

Role of cytokine gene (interferon-γ, transforming growth factor-β1, tumor necrosis factor-α, interleukin-6, and interleukin-10) polymorphisms in the risk of oral precancerous lesions in Taiwanese

Han-Jen Hsu [a], Yi-Hsin Yang [b], Tien-Yu Shieh [c], Chung-Ho Chen [a,d], Yu-Hsun Kao [a], Chia-Fu Yang [a], Edward Cheng-Chuan Ko [a,d,*]

[a] *Department of Oral and Maxillofacial Surgery, Chung-Ho Memorial Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan*
[b] *School of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan*
[c] *College of Dental Medicine, Taipei Medical University, Taipei, Taiwan*
[d] *Department of Dentistry, College of Oral Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan*

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Abstract Oral squamous cell carcinoma can be preceded by some benign oral lesions with malignant potential, including leukoplakia, erythroplakia, oral lichen planus, and oral submucous fibrosis. There are different degrees of inflammatory cells infiltration in histopathology. Inflammatory cytokines may play a pathogenic role in the development of oral precancerous lesions (OPCLs). Genetic polymorphisms of cytokine-encoding genes are known to predispose to malignant disease. We hypothesized that the risk of OPCLs might be associated with cytokine gene polymorphisms of interferon (IFN)-γ, transforming growth factor (TGF)-β1, tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-10. In the present study, 42 OPCL patients and 128 controls were analyzed for eight polymorphisms in five different cytokine genes [IFN-γ (+874 T/A), TGF-β1 (codons 10 T/C and 25 G/C), TNF-α (−308 G/A), IL-6 (−174 G/C), and IL-10 (−1082 A/G, −819 T/C, and −592 A/C)]. Cytokine genotyping was determined by the polymerase chain reaction sequence-specific primer technique using commercial primers. Allele and genotype data were analyzed for significance of differences between cases and controls using the Chi-square (χ^2) test. Two-sided $p < 0.05$ were considered to be statistically significant. A series of multivariate logistic regression models, adjusted for age, sex, betel quid chewing, alcohol

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* Corresponding author. Department of Dentistry, College of Oral Medicine, Kaohsiung Medical University, Number 100, Shih-Chuan 1st Road, Kaohsiung 807, Taiwan.
E-mail address: ko.edward.kaseizen@gmail.com (E.C.-C. Ko).

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consumption, and smoking, was constructed in order to access the contribution of homozygous or heterozygous variant genotypes of polymorphisms. The TNF-α (-308) polymorphism was significantly associated with OPCLs. There were significant differences in the distribution of AA, GA, and GG genotypes between OPCL patients and controls ($p = 0.0004$). Patients with the AA or GA genotype had a 3.63-fold increased risk of OPCLs. The TGF-β1 (codon 10 and 25) polymorphism was also significantly associated with OPCLs ($p < 0.001$). The IL-6 polymorphism was significantly associated with OPCLs. There are significant differences in the distribution of CC, GC, and GG genotypes between OPCL patients and controls ($p < 0.001$). Patients with the CC or GC genotype had a 35- or 20.59-fold increased risk of OPCLs. There were no significant differences in the distribution of IL-10 and IFN-γ genotypes between different groups of control individuals and OPCL patients. The IL-6, TGF-β1, and TNF-α gene polymorphisms may have a significant association with the development of OPCLs.

Introduction

Oral squamous cell carcinoma is the eighth most common cancer in the world, with approximately 300,000 new cases diagnosed annually. In Taiwan, this disease is the fourth leading cause of cancer-related deaths in males. Oral squamous cell carcinoma can be preceded by some benign oral lesions with malignant potential, such as leukoplakia, erythroplakia, oral lichen planus, and oral submucous fibrosis. There are chronic inflammatory and immunologic processes. Although the most common oral premalignant lesions appear to be related to the habit of chewing betel quid, tobacco smoking, and heavy alcohol consumption, the shift in the balance between pro- and anti-inflammatory cytokines may affect the inflammation-mediated oncogenesis.

Cytokines are important mediators of inflammatory responses. They may either be involved in the antitumor effector immune mechanisms or may enhance malignant transformation and tumor growth. Th1 type cytokines such as interferon (IFN)-γ are required for antitumor immunity, whereas Th2 type and several inflammatory cytokines such as tumor necrosis factor (TNF)-α and transforming growth factor (TGF)-β1 favor tumor development [1]. Interleukin (IL)-6, another cytokine of Th2 type, plays an important role in different physiologic and pathophysiologic processes such as inflammation, bone metabolism, and carcinogenesis [2]. In a previous study, we found that there were different releasing of cytokines using arecoline cultured peripheral blood mononuclear cells from patients with oral mucous diseases and controls [3].

Single nucleotide polymorphisms (SNPs) in genes encoding for susceptibility factors may influence gene expression, protein function, and disease predisposition. An increasing number of recent reports suggested that SNPs in cytokine genes, such as TNF-α (-308), TGF-β1 (codons 10 and 25), IL-10 (-1082, -819, and -592), IL-6 (-174), and IFN-γ ($+874$), have been correlated with an increased risk for developing oral malignancies [4–6]. However, reports discussing about the association of cytokine polymorphism and oral precancerous lesion are limited. The aim of this study was to determine any possible gene polymorphisms of TNF-α (-308), TGF-β1 (codons 10 and 25), IL-10 (-1082, -819, and -592), IL-6 (-174), and IFN-γ ($+874$) that may influence any susceptibility to oral precancerous lesions.

Materials and methods

Recruitment of study participants

Of the study participants, the oral precancerous lesion (OPL) patients were recruited between April 2004 and November 2006 and control individuals between March 2006 and September 2006; informed consent was obtained from all participants prior to being enrolled in the study. A total of 42 patients were treated at the Department of Oral and Maxillofacial Surgery of Chung-Ho Memorial Hospital, Kaohsiung Medical University in Kaohsiung, Taiwan. The healthy control group comprised 128 consecutive volunteers who attended the yearly health check-up. All patients were diagnosed histologically by incisional biopsy or surgical specimen. After obtaining written informed consent, 10 mL of peripheral blood was collected for DNA extraction. Each participant was interviewed using a standard questionnaire by a trained nurse, to collect medical histories, demographic characteristics, and information on the history of exposure to risk factors such as betel quid chewing, cigarette smoking, and alcohol consumption. The experimental protocol was approved by the Institutional Review Board of Kaohsiung Medical University Hospital.

DNA isolation and cytokine genotyping

Genomic DNA was extracted from whole EDTA-treated peripheral blood using a QIAamp Blood Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. SNPs were analyzed in five cytokines (IFN-γ, TGF-β1, TNF-α, IL-6, and IL-10) for genotype assignment. A coding sequence mutation (G vs. C) at position -174 of the promoter region was analyzed for the IL-6 (-174, rs1800795). Three different polymorphisms were examined for the IL-10 promoter region: positions -1082 (G vs. A, rs1800896), -819 (C vs. T, rs1800871), and -592 (A vs. C, rs1800872). The presence of a single nucleotide modification at position $+874$ (rs2430561) was examined for IFN-γ. Two single nucleotide mutations in

the coding region were analyzed for TGF-β1: codon 10 can be either T or C (rs1982073), and codon 25 (1800471) can be either C or G. SNPs at position −308 of the promoter region (either A or C or both, rs1800629) were surveyed for TNF-α. Cytokine genotypes were determined using the polymerase chain reaction sequence-specific primer method employing a commercially available kit (One Lambda, Inc., Canoga Park, CA, USA) in accordance with the manufacturer's instructions. All polymerase chain reaction products were visualized after electrophoresis on a 2% agarose gel and ethidium bromide staging. DNA extractions and polymerase chain reaction amplifications were performed by a technician blinded to the study groups.

Statistical analysis

Statistical analysis was conducted using JMP software version 9 (SAS Institute Inc., Cary, NC, USA) for Microsoft Windows. The Pearson χ^2 test or Fisher's exact test was used to analyze the difference in frequencies between two or more groups. Hardy–Weinberg equilibrium was tested using the goodness-of-fit χ^2 test to compare the observed genotype frequencies with the expected ones in the healthy controls. To determine whether an association existed between cytokine genotypes and allele frequencies associated with cancer risk, the significance of the difference in the distribution of genotypes and alleles between patients and control individuals was calculated by χ^2 statistics or Fisher's exact test and shown by p values. All p values were two sided. A p value of < 0.05 was considered statistically significant. To control the potential confounding effects (age, smoking, drinking, and betel quid chewing status), logistic regression was used to adjust for possible confounders and to test for associations between predictors and outcomes for odds ratio (OR) and 95% confidence interval (CI).

Results

Table 1 presents clinical characteristics of the study participants. Although there were differences in the age group, sex distribution, betel quid chewing habit, drinking habit, and smoking habit between the cases and controls, these were taken into consideration during the computation of ORs to compare between genotype. The age range of the patients with OPL was 26–79 years, with a median age of 50.83 years and a 95% CI of 36.69–63.97 years. The respective age range of the healthy controls was 23–83 years, with a median age of 59.20 years and a 95% CI of 46.29–72.11 years. Table 2 presents the histological diagnosis of OPLs. We included the patient with OPL among leukoplakia, erythroplakia, oral lichen planus, and oral submucous fibrosis. Genotype distribution of the controls satisfied the Hardy–Weinberg equilibrium.

Allele frequency and genotype distribution

Allele frequencies and genotype distributions of IL-6, IL-10, IFN-γ, TGF-β1, and TNF-α are shown in Tables 3 and 4.

SNP analysis for TNF-α (−308) position revealed that the "G" allele was more common than the "A" allele in both the OPL and the control groups. Frequencies of the allele "G" (0.75 vs. 0.63, $p = 0.0433$) and of the homozygous form (G/G) were found to be significantly higher in the healthy control individuals compared to those in OPL patients (51.6% vs. 26.2%, $p = 0.0004$; Table 3). Moreover, as shown in Table 5, the high-producing genotypes (G/A and A/A) were found much more commonly among the OPL patients in comparison to low-producing ones (OR = 3.63, 95% CI 1.65–7.97, $p < 0.001$).

Frequency of the T allele in the OPL patient population (46.4%) was not different from that in the control group (50.0%) in TGF-β1 codon 10 (Table 3). Genotype frequencies (TT, TC, and CC) at codon 10 among both the OPL patients and the healthy controls showed similar distribution.

Table 1 Characteristics of the study population.

Variable	Cases ($n = 42$)	Controls ($n = 128$)	p
Age (y)			
≤55	28 (67)	49 (38.3)	0.0013*
>55	14 (33)	79 (61.7)	
Sex			
Male	9 (21.4)	77 (60.2)	<0.0001*
Female	33 (78.6)	51 (39.8)	
Betel quid chewing status			
Ever	11 (26.2)	22 (17.2)	0.2118
Never	31 (73.8)	106 (82.8)	
Alcohol drinking consumption status			
Ever	13 (31)	32 (25)	0.4530
Never	29 (69)	96 (75)	
Smoking status			
Ever	13 (31)	38 (29.7)	0.2118
Never	29 (69)	90 (70.3)	

Data are presented as n (%).
* Statistically significant difference between patients and control individuals ($p < 0.05$).

Table 2 Diagnosis of oral mucosa disease.

Diagnosis	Number
Epithelium hyperplasia and epithelium dysplasia	2
Epithelium hyperplasia	1
Hyperorthokeratosis	3
Hyperparakeratosis	5
Hyperparakeratosis and epithelium dysplasia	2
Hyperorthokeratosis and oral submucous fibrosis	3
Oral submucous fibrosis	2
Verrucous hyperplasia	2
Verrucous hyperplasia and epithelium dysplasia	1
Verrucous hyperplasia and oral submucous fibrosis	1
Verrucous hyperplasia, oral submucous fibrosis, and epithelium dysplasia	2
Lichen planus	18
Total	42

Table 3 Distribution and allele frequency of TNF-α (−308), TGF-β1 (codon 10 and 25), IL-10 (−1082, −819, −592), IL-6 (−174), IFN-γ (+874) among BOMDP and healthy controls.

Cytokine gene	Genotype frequency (%)	BOMDP	Controls	*p*	Adjusted OR (age, sex, habits)	95% CI	*p*
TNF-α (−308)[a]	AA	1 (2.4)	10 (7.8)	<0.001*			n.s.
	GA	28 (66.7)	38 (29.7)		4.03	(1.61, 10.73)	0.0026*
	GG	11 (26.2)	66 (51.6)		1.00		
	A	30 (37.5)	58 (25.4)	0.0433*			n.s.
	G	50 (62.5)	170 (74.6)				
TGF-β1 (codon 10)							
	TT	1 (2.4)	8 (6.3)				
	TC	37 (88.1)	113 (88.3)	n.s.			
	CC	4 (9.5)	3 (2.34)				
	T	39 (46.4)	129 (50.0)	n.s.			
	C	45 (53.6)	119 (48.0)				
TGF-β1 (codon 25)							
	CC	0 (0)	1 (0.8)	<0.001*	Unstable		
	GC	17 (40.5)	8 (6.5)		23.00	(6.16, 113.23)	<0.001*
	GG	25 (59.5)	115 (92.7)		1.00		
	C	17 (20.2)	10 (4.3)	<0.001*	12.10	(3.96, 41.74)	<0.001*
	G	67 (79.8)	238 (96.7)		1.00		
IL-10 (−1082)[b]							
	GG	0 (0)	0 (0)				
	GA	4 (11.8)	16 (14.3)	n.s.			
	AA	30 (88.2)	96 (85.7)				
	G	4 (5.9)	16 (7.1)	n.s.			
	A	64 (94.1)	208 (92.9)				
IL-10 (−819)[b]							
	TT	10 (29.4)	53 (47.3)				
	CT	22 (64.7)	51 (45.5)	n.s.			
	CC	2 (5.9)	8 (7.1)				
	T	42 (61.8)	157 (70.1)	n.s.			
	C	26 (38.2)	67 (29.9)				
IL-10 (−592)[b]							
	AA	10 (29.4)	53 (47.3)				
	CA	22 (64.7)	51 (45.5)	n.s.			
	CC	2 (5.9)	8 (7.1)				
	A	42 (61.8)	157 (70.1)	n.s.			
	C	26 (38.2)	67 (29.9)				
IL-6 (−174)[a]							
	CC	5 (11.9)	4 (3.70)	<0.0001*	35.00	(5.28, 266.90)	<0.001*
	GC	30 (71.4)	26 (24.0)		20.59	(6.44, 81.73)	<0.001*
	GG	5 (11.9)	78 (72.2)		1.00		
	C	40 (50)	34 (15.7)	<0.0001*	5.05	(2.49, 10.72)	<0.001*
	G	40 (50)	182 (84.3)		1.00		
IFN-γ (+874)[c]							
	AA	28 (66.7)	81 (63.3)	n.s.			
	TA	11 (26.2)	35 (27.3)				
	TT	0 (0)	5 (3.90)				
	A	67 (85.9)	197 (81.4)	n.s.			
	T	11 (14.1)	45 (18.6)				

Data are presented as *n* (%).
BOMDP = benign oral mucosa disease patients; CI = confidence interval; IFN = interferon; IL = interleukin; n.s. = not statistically significant ($p > 0.05$); OR = odds ratio; TGF = transforming growth factor; TNF = tumor necrosis factor.
* Statistically significant difference between patients and control individuals ($p < 0.05$).
[a] Due to some technical problems, the genotyping results of 40 patients were included.
[b] Due to some technical problems, the genotyping results of 34 patients were included.
[c] Due to some technical problems, the genotyping results of 39 patients were included.

Table 4 Distributions of combined polymorphisms at different positions of TGF-β1 and IL-10.

Cytokine gene polymorphisms	Patients, *n* (%)	Controls, *n* (%)	*p**
TGF-β1 (codon 10 and 25)			
T/T-G/G	1 (2.4)	7 (5.7)	<0.001*
T/C-G/G	22 (52.4)	106 (85.5)	
T/C-G/C	15 (35.7)	7 (5.7)	
C/C-G/G	2 (4.8)	2 (1.6)	
T/T-G/C	0 (0.0)	0 (0.0)	
C/C-G/C	2 (4.8)	1 (0.8)	
C/C-C/C	0 (0.0)	0 (0.0)	
T/T-C/C	0 (0.0)	1 (0.8)	
T/C-C/C	0 (0.0)	0 (0.0)	
IL-10 (−1082, −819, −592)[a]			
GCC/GCC	0 (0.0)	0 (0.0)	
GCC/ACC	0 (0.0)	5 (4.5)	
GCC/ATA	4 (11.8)	11 (9.8)	
ACC/ACC	2 (5.9)	3 (2.7)	n.s.
ACC/ATA	18 (52.9)	40 (35.7)	
ATA/ATA	10 (29.4)	53 (47.3)	
ACA/ATA	0 (0.0)	0 (0.0)	
ATC/ATA	0 (0.0)	0 (0.0)	
GCC/ATC	0 (0.0)	0 (0.0)	

* Statistically significant difference between patients and control individuals ($p < 0.05$).
IL = interleukin; n.s. = not statistically significant ($p > 0.05$); TGF = transforming growth factor.
[a] Due to some technical problems, the genotyping results of 34 patients were included.

Fifty-nine percent (25/42) of OPL patients and 92.7% of the healthy controls had the high-producer GG genotype at codon 25 (Table 3). The intermediate-producer GC genotype was present in 40.5% (17/42) of the OPL patients, and in 6.5% (8/124) of the healthy controls. Only one in the control group and none of the OPL patients had the low-producer CC (Pro/Pro) genotype. In case of the high-producer G (Arg) allele, the percentages of OPL patients (79.8%) and controls (96.0%) were different ($p < 0.0001$). The respective frequencies of the C allele were 23.4% for patients with oral squamous cell carcinoma, 20.2% for patients with OPL, and 4.3% for the healthy controls. There was a difference in the polymorphism at codon 25 of TGF-β1 gene between patients and controls ($p < 0.0001$). People with TGF-β1 codon 25 C-allele containing genotypes (GC or CC) posed an increased risk of OPL (adjusted OR = 23.0, 95% CI = 6.16—113.23, $p < 0.0001$). The different genotypes of TGF-β1 in codon 10—25 are associated with the production of different cytokines. High-production genotypes are TTGG and TCGG; intermediate-production genotypes TCGC, TTGC, and CCGG; and low-production genotypes TTCC and CCGC. The group of intermediate- and low-production genotypes were significantly higher in the patient group than those in the control group (OPL patients:controls = 45.2%:11.72%, $p < 0.001$). People with intermediate- or low-production genotypes posed an increased risk of OPL (adjusted OR = 13.40, 95% CI = 3.81—56.66, $p < 0.0001$).

Of the OPL patients, 12.5% (5/40) and 72.2% of the healthy controls had the high-producer GG (Arg/Arg) genotype at IL-6 −174 gene (Table 3). The high-producer GC (Arg/Pro) genotype was present in 75.00% (30/40) of the OPL patients, and 24.1% (26/108) of the healthy controls. Only four in the control group had the low-producer CC (Pro/Pro) genotype. There was a difference in the polymorphism at IL-6 −174 gene between patients and controls ($p < 0.0001$). In case of the high-producer G (Arg) allele, the percentages of OPL patients (50.00%) and controls (84.3%) were different ($p < 0.0001$). The respective frequencies of the C allele were 50.00% for the patients with OPL and 15.74% for the healthy controls. People with IL-6 −174 C-allele containing genotypes (GC or CC) posed an increased risk of OPL (GC/GG: adjusted OR = 20.6, 95% CI = 6.44—81.73, $p < 0.0001$; CC/GG: adjusted OR = 35, 95% CI = 5.28—266.90, $p < 0.0001$) (Table 3).

Allele and genotype frequencies of the OPL patients in IL-10 (−1082, −819, and −592) and IFN-γ (+874) showed a similar distribution pattern to that in the healthy control individuals (Tables 3—5).

Discussion

In the present study, we found that functional polymorphisms at TNF-α −308 G > A, TGF-β1 codon 25 G > C, and IL-6 −174 G > C have significant effects on the risk of OPL development. The female patients were more than males in the study because the blood examination doesn't be involved in the routine protocol of treatment of the oral precancerous lesions and female patients were more willing to join the study. We assume that there is no difference between females and males regarding the distribution of polymorphisms of those cytokines.

Expressions of these cytokines in different polymorphisms are shown in Table 5. A G-to-A transition in the promoter region (−308) of the TNF-α gene results in higher expression of TNF-α *in vitro* and *in vivo* [7]. TNF-α high-expression allele increases the risk of oral cancer [7]. TNF-α is a proinflammatory multifunctional cytokine produced by macrophages. It plays an important role in the regulation of immune response, because its increase after traumatic injury generates a cytokine cascade resulting in activation, proliferation, and hypertrophy of mononuclear and phagocytic cells. TNF-α-initiated signaling pathways activate proinflammatory gene expression via the transcription factor nuclear factor-kappa B. TNF-α has been implicated in the pathogenesis and progression of various malignancies. In comparison to healthy individuals, increased serum levels of TNF-α have been observed in European patients with oral cancer [8]. Significantly higher levels of salivary IL-6 and TNF-α were found in patients with oral leukoplakia when compared with healthy controls [9]. SNPs in inflammation genes have been shown to alter their expression and functions. TNF-α and IFN-γ polymorphisms contribute to susceptibility to oral lichen planus [10]. A high-producing G/A genotype of TNF-α at position −308 was more commonly found among the OPL patients of this study.

IL-6 is an anti-inflammatory cytokine [11]. It has already been well discussed that cigarette smoking affects the

Table 5 Cytokine gene polymorphisms and their associated phenotypes.

Cytokine gene polymorphisms		Patients, *n* (%)	Controls, *n* (%)	*p*	Adjusted OR (95% CI)	*p**
TNF-α (−308)[a]						
High	G/A, A/A	29 (72.5)	48 (42.1)	<0.001*	3.63 (1.65−7.97)	<0.001
Low	G/G	11 (27.5)	66 (57.9)		1	
TGF-β1 (codon 10−25)						
High	T/T-G/G, T/C-G/G	23 (54.8)	113 (88.3)	<0.001*	1	
Intermediate/low	(T/C-G/C, C/C-G/G, T/T-G/C)/(C/C-G/C, C/C-C/C, T/T-C/C, T/C-C/C)	19 (45.2)	15 (11.7)		13.40 (3.81−56.66)	<0.001
IL-10 (−1082, −819, −592)[b]						
High/intermediate	(GCC/GCC)/(GCC/ACC, GCC/ATA, GCC/ATC)	4 (11.8)	16 (14.3)	n.s.		
Low	ACC/ACC, ACC/ATA, ATA/ATA, ATC/ATA	30 (88.2)	96 (85.7)			
IL-6 (−174)[a]						
High	G/G, G/C	35 (87.5)	104 (96.3)	n.s. (0.062)		
Low	C/C	5 (12.5)	4 (3.7)			
IFN-γ (+874)[c]						
High	T/T	0 (0.0)	5 (4.1)	n.s.		
Intermediate	T/A	11 (28.2)	35 (28.9)			
Low	A/A	28 (71.8)	81 (66.9)			

* Statistically significant difference between patients and control individuals ($p < 0.05$).
CI = confidence interval; IFN = interferon; IL = interleukin; n.s. = not statistically significant ($p > 0.05$); OR = odds ratio; TGF = transforming growth factor; TNF = tumor necrosis factor.
[a] Due to some technical problems the genotyping results of 40 patients were included.
[b] Due to some technical problems, the genotyping results of 34 patients were included.
[c] Due to some technical problems, the genotyping results of 39 patients were included.

immune system [12]. Cigarette smoking decreases bioactive IL-6 secretion by alveolar macrophages. Betel quid component also influences inflammatory reaction. Areca nut extract induces IL-6 production by gingival keratinocyte and also induces Cox-2 and IL-6 mRNA expression [13]. Betel quid chewing contributes to the pathogenesis of cancer and oral submucous fibrosis by impairing T cell activation and inducing IL-6 production, which affect oral mucosal inflammation and growth of oral fibroblast and oral epithelial cells [14].

IL-6 activates mainly the JAK1/STAT3 signal pathway and functions as a proinflammatory factor [15]. Expression rates of IL-6R and IL-6 mRNA transcripts in tumor specimens were much higher than those in nontumor mucosa [16]. A G/C SNP in the promoter region (−174) of IL-6 was shown to affect transcription and alter plasma IL-6 levels. As reported in the literature, the IL-6 variant genotype (C/C) frequency varied dramatically among different ethnic groups; for example, it is very low in Asian populations [17]. Our control frequency, compared with other studies about Southern Chinese population [18], is slightly higher. The IL-6 polymorphisms have been linked to various malignancies such as gastric, hepatocellular, lung, colorectal, cervical, breast [19−21], and bladder cancers [22]. Significantly higher levels of salivary IL-6 were found in patients with oral leukoplakia when compared to healthy controls [9]. In this study, we found an association between the variant C allele of the −174 G > C SNP in the IL-6 promoter region with an increasing OPL risk.

TGF-β1 induces tumor regression *in vivo* by Smad4-independent pathways that sensitize keratinocytes to mitochondrial-mediated apoptosis [23]. TGF-β1 gene is located on chromosome 19q13. SNPs in cytokine genes may influence the expression or function of cytokines via cytokine network, and many have been evaluated for their role in inflammatory diseases and cancer predisposition. Two polymorphisms of TGF-β1 are located on codon 10 (869T > C::Leu/Pro) and codon 25 (915 G > C::Arg/Pro) of exon 1 that encodes leader sequence of the protein and have functional importance in modulating its transmembrane transport. TGF-β1 low-production genotypes (TGF-β1 codon 10 CC) were associated with not only an increased risk of breast cancer [24], but also an increased odd of breast cancer relapse and reduced 5-year disease-free survival rate [25]. The present study revealed that the codon 10 genotype is not a critical factor in the development of OPL. Codon 25 of TGF-β1 increased serum levels of TGF-β1 in the presence of the G allele, which might contribute to long-term suppression of epithelium proliferation, leading to a decrease in the risk of OPL. The C allele at codon 25 may contribute to weaker suppression of the epithelium proliferation, thereby increasing the risk of OPL. TGF-β1 codon 25, not codon 10, is an important risk factor of OPL, as found in the study. TGF-β1 is the major cytokine responsible for the regulation of fibroblast proliferation and differentiation [26]. The rough fibers present in betel nuts may cause multiple invisible tiny abrasive wounds during betel quid chewing. Wound healing was affected by the chemical component, which may modulate cytokine secretion.

Polymorphism analysis of IFN-γ, an important cytokine in antitumor host defense, showed no genotype difference at

position +874 in OPL patients and controls. However, the higher-producer T/T genotype at position +874 was reported to be significantly higher in hepatocellular carcinoma in Brazil [27].

IL-10 is a multifunctional cytokine with both immunosuppressive and antiangiogenic functions, and consequently has both tumor-promoting and tumor-inhibiting properties [28]. Raised levels of serum and peritumoral IL-10 production have been reported in many malignancies, which have been interpreted in support of a role for IL-10 in tumor escape from the immune response [29]. The IL-10 SNP and haplotype have been reported to be associated with differential IL-10 expression *in vitro*, with the −1082 A, −819 T, and −592 A haplotypes being associated with a decreased IL-10 expression, compared with the −1082 G, −819 C, and −592 C haplotypes. SNPs at position −1082 A/G in the IL-10 gene promoter are significantly associated with the risk of oral cancer [30]. However, it has not been found to be associated with OPL in our study.

Limitations of the present study need to be addressed. It has been suggested that the sample size is always an important issue. Statistical power of the analysis would generally increase with increasing size of sample. Because of the relatively small sample size, this study may not be powerful enough to test the interaction among genes with low frequencies.

This study provides evidence for the association between the variants IL-6, TNF-α (−308), and TGF-β1 codon 10 genotype, and an overall increased OPL risk. While these findings need to be replicated in larger, prospective studies, they support the role of chronic inflammation as an etiologic factor in this population.

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