BMC Oral Health

RESEARCH ARTICLE Open Access

Association of genetic polymorphisms in interferon-γ, interleukin-6 and transforming growth factor-β1 gene with oral lichen planus susceptibility

Maha Ali M. Al-Mohaya[1], Lubna Al-Otaibi[1], Fahad Al-Harthi[2], Ebtissam Al Bakr[1], Misbahul Arfin[3] and Abdulrahman Al-Asmari[3*]

Abstract

Background: Oral lichen planus (OLP) is a premalignant mucocutaneous disease in which genetic factors and immune responses play a major role. Cytokines play an important role in the pathogenesis and disease progression of OLP. The aim of this study was to investigate the impact of gene polymorphisms of T helper cell subtype Th1 and Th2 cytokines, interferon-gamma (IFN-γ), interleukin-6 (IL-6) and transforming growth factor (TGF)-β1 on OLP susceptibility in a Saudi cohort.

Methods: Forty two unrelated patients with OLP and 195 healthy controls were genotyped for IFN-γ (874A/T), IL-6 (174G/C) and TGF-β1 (509C/T) polymorphisms.

Results: The frequency of genotype AT of IFN-γ (874A/T) was significantly higher while genotype AA was lower in OLP patients as compared to controls ($P < 0.05$). The frequency of T containing genotypes (AT + TT) was also higher in OLP patients as compared to that in controls ($P = 0.003$). The frequencies of allele T was higher while that of allele A lower in patients than the controls however the difference was not statistically significant ($P = 0.07$). There was no significant difference in the frequencies of alleles and genotypes of IL-6 (174G/C) and TGF-β1 (509C/T) polymorphisms between patient and control groups. These results indicated that genotype AT of IFN-γ (874A/T) polymorphism is associated with OLP risk and genotype AA is protective to OLP. On the other hand the polymorphisms IL-6 (174G/C) and TGF-β1 (509C/T) may not be associated with OLP risk in our population.

Conclusion: It is concluded that IFN-γ (874A/T) polymorphism is associated with the susceptibility of OLP, however further studies with large sample size involving different ethnic populations should be conducted to strengthen our results.

Keywords: Oral lichen planus, Interferon-γ, Interleukin -6, Transforming growth factor -β1, Polymorphism, Saudis

Abbreviations: EF, Etiological fraction; IFN-γ, Interferon gamma; IL-6, Interleukin-6; OLP, Oral lichen planus; OPCLs, Oral precancerous cells; PCR-RFLP, Polymerase chain reaction -restriction fragment length polymorphism; PF, Preventive fraction; RR, Relative risk; TGF- β, Transforming growth factor beta

* Correspondence: abdulrahman.alasmari@gmail.com
[3]Research Center, Prince Sultan Military Medical City, P. O. Box 7897, Riyadh 11159, Saudi Arabia
Full list of author information is available at the end of the article

© 2016 The Author(s). **Open Access** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.

Background

Oral lichen planus (OLP) is a premalignant mucocutaneous disease in which genetic factors and immune responses play a major role. It is a chronic inflammatory, prototype of oral lichenoid lesions characterized by T-cell mediated immune response and abnormal epithelial keratinization cycle in which Th1 is considered to play a predominant role [1–4]. The OLP lesions may coexist with cutaneous and genital lesions, or may be the only disease manifestations. The OLP with complex pathogenesis, involves antigen presentation by the oral keratinocytes either of an exogenous or an endogenous origin [5]. This immune response is accompanied by a mixed inflammatory response of T-cells, macrophages, and mast cells, together with the associated cytokines and cytotoxic molecules [5, 6]. OLP is more common in middle aged women [7]. The genetic factors influencing immune function have been indicated to contribute to the OLP etiology [4, 8]. Cytokines play an important role in the progression/ pathogenesis of OLP and polymorphisms in cytokines genes such as IFN-γ, TNF-α, TNF-β, IL-4, IL-10 have been associated with the susceptibility of OLP [9, 10].

Interferon gamma (IFN-γ), a proinflammatory cytokine has also been reported to play an important role in host defense and immune regulation. The gene encoding IFN-γ is located on chromosome 12q24 and consists of four exons with three intervening regions [11]. A polymorphism in the first intron of IFN-γ gene at position 874 (rs2430561) directly influences IFN-γ production level [12]. The IFN-γ (874 A/T) is located within a putative nuclear factor-kB (NF-kB) binding site, and T allele might be responsible for the induction of IFN-γ production at a higher level.

Interleukin -6 (IL-6) is one of the major inflammation related cytokines [13]. It is responsible for the synthesis of acute-phase reactants by the liver and regulates inflammatory/ immune pathways, bone metabolism and endocrine functions. The human IL-6 gene is located on chromosome 7p21. Among the polymorphic sites described in the IL-6 gene promoter, two biallelic polymorphisms, 572G/C (rs1800796) and −174G/C (rs1800795) have been associated with differences in cytokine production. These polymorphisms consist of a single nucleotide change from guanine (G) to cytosine (C) at positions −572 and −174 in the promoter region, respectively [14, 15]. Some researchers have investigated a possible association between IL-6 (−174G/C) polymorphism and OLP in Taiwanese and Brazilian patients [16, 17].

Another cytokine, transforming growth factor (TGF)-β1 is produced by both immune and non-immune cells and exhibits a broad range of functions including tissue repair and immune response [18]. The TGF-β1 gene is located on chromosome 19q13 [19] and its production is under genetic control [20]. TGF-β1 gene polymorphisms at codon 10 and 25 regulate the TGF-β1 production in vivo and in vitro [21]. The most thoroughly studied TGF-β1 (509C/T) polymorphism (rs1800469) is located within a Yin-Yang1 consensus binding site [22] and T allele has been associated with increased level of TGF-β1 in plasma [20] and reduced T cell proliferation [21].

The cytokines involved in inflammation- and immune regulation has been suggested to play an important role in the pathogenesis of OLP. The genotype frequencies of polymorphisms are known to vary according to race or ethnicity. To date, no studies have been performed in Saudi Arabian patients to evaluate whether the IL-6, IFN-γ and TGF- β1 gene polymorphisms are associated with OLP susceptibility. We evaluated the association of IFN-γ (874A/T), IL-6 (174G/C) and TGF-β1 (509C/T) polymorphisms with OLP risk in Saudi patients.

Methods

Two hundred thirty seven Saudi subjects visiting Prince Sultan Military Medical City (PSMMC), Riyadh Saudi Arabia were recruited for this study. Forty two unrelated OLP patients (16 male, 26 female) aged 27–72 years and 195 unrelated healthy matched controls from the same ethnicity (100 male, 95 female) aged 20–65 years were genotyped for polymorphisms in IFN-γ and TGF- β1 and IL-6 genes. Patients and controls with the history of any other inflammatory/ autoimmune diseases were excluded from the study. The study protocol was approved by the research and ethical committee of PSMMC, Riyadh and written informed consent was obtained from each subject before recruitment.

The diagnosis of OLP was based on the clinical manifestations and histopathological criteria of the World Health Organization. Senior oral pathologists examined the patients, reviewed histological findings and the patients history to diagnose OLP as described elsewhere [9]. The patient suspected to have drug or restoration related lichenoid lesions, and with any histologic signs of dysplasia were excluded.

PCR amplification

Genomic DNA was extracted from the peripheral blood samples of OLP patients and controls using QIAamp^{R} DNA mini kit (Qiagen Hilden, Germany). IFN-γ gene was amplified using amplification refractory mutation systems (ARMS)-PCR methodology to detect polymorphisms at position 874 of IFN-γ. DNA was amplified in two different PCRs with a generic antisense primer and one of the two allele specific sense primers (antisense: 5’- TCA ACA AAG CTG ATA CTC CA-3’ T- allele: 5’- TTC TTA CAA CAC AAA ATC AAA TCT-3’ and A-allele: 5’- TTC TTA CAA CAC AAA ATC AAA TCA-3’). For quality control and to check the success of PCR amplification in both the reactions, an internal control of 426 bp was amplified

using a pair of primers for the human growth hormone (HGH). PCR amplification was carried out using 5× FIREPol Master Mix (Solis Biodyne, Tartu, Estonia) with specific optimized reaction conditions. The amplified products were separated on the 1.5 % agarose gel, stained with ethidium bromide and photographed.

IL-6 (174 G/C) polymorphism was detected by PCR-RFLP technique using a set of forward and reverse primers [23]. Amplification of genomic DNA using specific protocol yielded a198 bp DNA. The PCR product (198 bp) DNA was digested with SfaNI restriction enzyme (New England BioLabs, Beverly, MA) at 37 ° C for 3 h. Resulting into two fragment of 140 and 58 bp indicating GG genotype, while three fragment of 198, 140 and 58 bp indicating GC genotype. Undigested single band of 198 bp indicated CC genotype. The amplified products and digested products for various samples were separated on the 2.5 % agarose gel, stained with ethidium bromide and photographed.

Genotyping for TGF-β1 (509C/T) polymorphisms was performed using PCR-RFLP technique. Amplification of a TGF-β1 using specific primers was performed to get 441 bp PCR product which was digested with restriction enzyme Bsu36 I to get two fragments of 251 and 190 bp for CC, three fragments of 441,251 and 190 bp for CT and uncut DNA of 441 bp for TT genotype. The alleles and genotypes frequencies were calculated in patients and control groups. Hardy-Weinberg equilibrium was determined as described earlier [9].

Statistical analysis

The differences in allele/genotype frequencies between patient and control groups were analyzed by the Fisher's exact test using the CalcFisher software (www.jstatsoft.org/article/view/V008i21/Article-JSS). *P* values ≤ 0.05 were considered significant. The odd ratio interpreted as *relative risk* (RR) was calculated following the Woolf's method as out lined by Schallreuter et al. [24]. Etiologic fraction (EF) indicating the hypothetical genetic component of the disease and preventive fraction (PF) showing the hypothetical protective effect of one specific allele/ genotype for the disease were calculated using formulas given by Svejgaard [25].

Results

The genotype and allele frequencies of IFN-γ (874A/T), IL-6(174G/C) and TGF-β1 (509C/T) polymorphisms are presented in (Tables 1, 2 and 3). The representative gel pictures of amplification or after restriction enzyme digestion for IFN-γ, IL-6 and TGF-β1 are shown in Figs. 1, 2 and 3. The results of the genotyping repeated for 30 % of the random blind sample were compared with 100 % success rate. The genotype distributions were in Hardy-Weinberg equilibrium in both, OLP patient and control groups.

Table 1 Genotype and allele frequencies of IFN-γ (874A/T) polymorphism in OLP patients and matched controls

Genotypes/ Allele	OLP (*N* = 42)		Control (*N* = 195)		*P*- value	*RR*	*EF*[b]/*PF*
	N	%	*N*	%			
AA	2	4.76	49	25.13	0.003[a]	0.149	0.182
AT	27	64.29	91	46.66	0.04[a]	2.057	0.117[b]
TT	13	30.95	55	28.21	0.71	1.141	0.023[b]
AT + TT	40	95.24	146	74.87	0.003[a]	6.712	0.183[b]
A-allele	31	36.90	189	48.46	0.07	0.622	0.070
T-allele	53	63.10	201	51.54	0.07	1.607	0.078[b]

N number of subjects, *RR* relative risk, *EF* = etiological fraction, *PF* preventive fraction
[a]statistically significant
[b]data for EF

The frequencies of genotypes and alleles of IFN-γ (874A/T) polymorphism differed in OLP patients and controls (Table 1). The frequency of heterozygous genotype (AT) of IFN-γ was significantly higher in OLP patients than control ($P = 0.04$) whereas the frequency of homozygous genotypes (AA) was significantly lower in OLP patients than controls ($P = 0.003$). The frequency of T-containing genotypes (AT + TT) were significantly higher in OLP patients as compared to that in controls [$P = 0.003$ and relative risk (RR) = 6.712]. The frequency of allele T was higher and that of allele-A was lower in OLP patients than control subjects however, the difference was slightly short of significant ($P = 0.07$). On the other hand the difference in frequency of TT genotype among the two groups was not statistically significant ($P = 0.71$).

The genotype and allele frequencies of IL-6 (174G/C) polymorphism did not differ between patients and controls (Table 2). Both the homozygous genotypes CC and GG were absent in OLP patients. The heterozygous genotype GC was present in all OLP patients while 98.98 % of the controls had this genotype. The frequencies of allele G and C were exactly similar in controls and OLP patients.

The distribution of frequencies of alleles and genotype of TGF-β1 (509C/T) polymorphism did not differ

Table 2 Allele and genotype frequencies of IL-6 (174G/C) polymorphism in oral lichen planus and controls

Genotype/Allele	OLP (*N* = 42)		Control (*N* = 195)		*P*-value
	N	%	*N*	%	
GG	0	0	1	0.51	0.99
GC	42	100	193	98.98	0.99
CC	0	0	1	0.51	0.99
G-allele	42	50	195	50	0.99
C-allele	42	50	195	50	0.99

N number of subjects

Table 3 Allele and genotype frequencies of TGF-β1 (509C/T) polymorphism in OLP and controls

Genotype/allele	OLP (*N* = 42)		Control (*N* = 195)		*P*-value
	N	%	*N*	%	
CC	11	26.19	40	20.51	0.41
CT	27	64.29	115	58.98	0.60
TT	4	9.52	40	20.51	0.12
C-containing	38	90.48	155	79.49	0.12
C-allele	49	58.33	195	50	0.18
T-allele	35	41.67	195	50	0.18

N number of subjects

significantly between OLP patients and healthy controls (Table 3). The frequencies of genotypes CC, CT and AA were 26.19, 64.29 and 9.52 % in OLP patients as compared to 20.51, 58.98 and 20.51 in controls respectively. The distribution of allele C and T was quite similar, and it was respectively 58.33 and 41.67 % in OLP patient group and 50 % each in healthy control group ($P > 0.05$).

The frequency distribution of genotypes of IFN-γ (874A/T) polymorphism in various healthy ethnic populations is summarized in Table 4 which clearly indicated ethnic variations.

Discussion

Genotyping results of IFN- γ (874A/T) polymorphism indicated that T-containing genotypes are significantly associated with the susceptibility to OLP in Saudis ($RR = 6.712$). The IFN-γ is an important predominant cytokine in the pathogenesis of OLP. The role of IFN-γ gene polymorphism on OLP susceptibility has been investigated in various populations. Studies from China, Thailand and Italy indicted an association between IFN-γ (874A/T) polymorphism and OLP [26–28] and it has been suggested that IFN-γ (874A/T) polymorphism may be a risk factor to OLP development.

Carrozzo et al. [26] found significant increase in number of TT homozygous genotype of IFN-γ (874A/T) polymorphism in OLP patients compared with controls and suggested that IFN-γ genetic polymorphism may be an important risk factor to develop OLP in Italian. Bai et al. [27] suggested that IFN-γ (874A/T) polymorphism is associated with susceptibility and influence progression of OLP in a Chinese cohort. Recently Kimkong et al. [28] also reported an association between IFN-γ (874A/T) polymorphism and susceptibility to OLP in Thai population and suggested that the T allele is significantly associated with an increased risk of OLP development as compared to the A allele. Several other studies have also shown that IFN-γ +874 T/A polymorphism is associated with the development of several autoimmune diseases while others showed no such associations [29]. These disparities are probably caused by small sample sizes, low statistical power, ethnic difference, and/or clinical heterogeneity as suggested by Lee and Bae [29].

The results of present study on the associations of the IFN-γ 874 T/A polymorphism with OLP are consistent with the functional effect of the IFN-γ 874 T/A polymorphism as genotypes of the IFN-γ 874 T/A polymorphism have been associated with low (AA), medium (AT), and high (TT) cytokine production [12]. The IFN-γ 874 T/A polymorphism has been associated with autoimmune diseases in Caucasian, Latin American, and Middle Eastern, but not Asian, populations as shown in a recent meta-analysis [29]. The reason why a particular polymorphism was found to be associated with one population and not another may be due to ethnic differences in the frequency of IFN-γ 874 T/A polymorphism among these ethnic groups. The frequency distribution of genotypes of IFN-γ (874A/T) polymorphism in

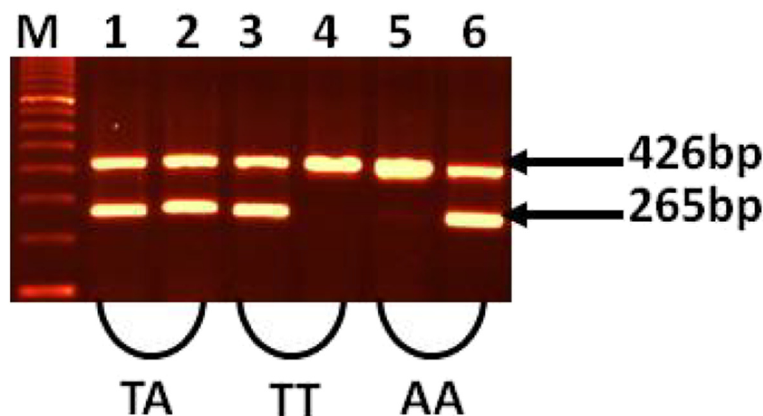

Fig. 1 Shows the amplification of IFN-γ (874A/T) genotypes (TT, TA and AA). Lane M: 100 bp DNA marker, Lane 1 and 3: amplification of allele T, Lane 2 and 6: amplification of allele A (taking both alleles together: lanes 1 and 2 indicate TA genotype, lanes 3 and 4, TT genotype and lanes 5 and 6 AA genotype), 265 bp band for target DNA, 426 bp band for internal control

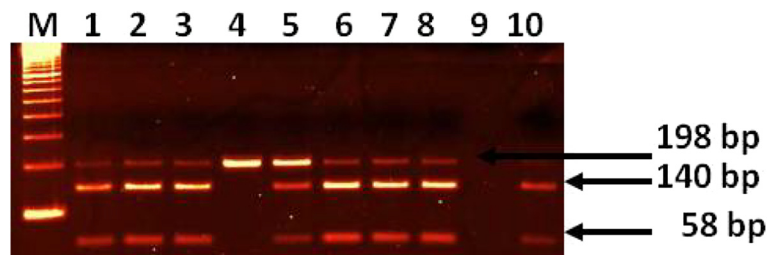

Fig. 2 Amplified DNA digested with SfaNI showing genotypes of IL-6(174G/C). Lane M: 100 bp DNA marker, Lane 1, 2, 3,5,6,7 and 8 for genotype GC (3 bands of 198, 140 and 58 bp), Lane 4 for genotype CC (uncut DNA of 198 bp), Lane 10 for genotype GG (2 bands of 140 and 58 bp)

various healthy ethnic populations clearly indicated ethnic variations (Table 4). The frequency of AA genotype varies from 1.60 % in German to 83.51 % in Japanese while frequency of AT genotype from 16.49 % in Japanese to 66.22 % in Polish population. On the other hand the frequency of TT genotype varies from 0.78 % in Chinese to 74.15 % in German healthy population however it was totally absent in Japanese healthy population. Obviously, there is a difference in the frequency of the T allele of the polymorphism among the healthy ethnic groups. The T allele was a minor allele in Asian populations, whereas it was a major allele in Caucasian and Latin American populations [29]. Our previous study dealing with the polymorphisms of pro- and anti-inflammatory cytokines showed that the OLP patients has significantly higher frequency of the A allele of TNF-α (−308) polymorphism [9]. Notably, both the polymorphisms (IFN-γ and TNF-α are known to increase the production of the respective cytokine [30, 31]. Significantly, several studies demonstrate a high level of IFN-γ and TNF-α in the oral mucosa [32] and serum of the patients with OLP [33].

INF-γ is one of the most critical mediators of immunity and inflammation and plays a pivotal role in both innate and adaptive immune responses [34]. It has been shown to promote innate immune responses by activating macrophages. Further by disrupting several anti-inflammatory feedback loops, INF-γ also up-regulates various proinflammatory mediators [35]. IFN-γ is known to enhance Th1 responses by activating NK cells and macrophages. It also promotes the specific cytotoxic immunity via T cell and APC interaction [36].

Moreover, IFN-γ is among the most extensively studied cytokines in OLP. Higher expression of IFN-γ has been reported in the isolated T-cell lines from the OLP biopsies [2, 37] and in erosive OLP lesions [3, 37]. Its expressions have been located on the CD4 + Th cells in OLP lesions [38]. Sugerman et al. [1] suggested that the high expression of IFN-γ at the advanced stage of OLP development may be involved in the activation of CD8 + T cells and help in maintaining the expression of major histocompatibility class on the keratinocyte. The increased expressions of IFN-γ in OLP influence the clinical outcome and has been associated with the clinical manifestations of OLP lesions [39].

On the other hand, in this study no significant difference in the allele or genotype frequencies of IL-6 (174G/C) were observed between the OLP subjects and healthy controls (Table 2). In contrast to our results some reports have indicated an association of IL-6 (−174 (G/C) with the OLP susceptibility. Xavier et al. [17] found a significant higher frequency of IL-6 174 G/G genotype in a cohort of Brazilian patients with OLP and suggested an association with the susceptibility of OLP and involvement of this polymorphism in the genetic basis of this disease.

Recently the IL-6 (174G/C) polymorphism has also been significantly associated with oral precancerous

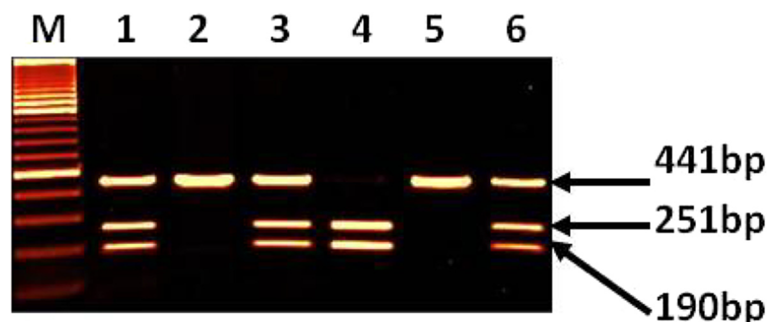

Fig. 3 Amplified DNA digested with Bsu36 I showing genotypes of TGF-β1 (509C/T). Lane M: 100 bp DNA marker, Lane 1, 3 and 6 for genotype CT(3 bands of 441, 251and190bp), Lane 2 and 5 for genotype TT (uncut DNA of 441 bp), Lane 4 for genotype CC (2 bands of 251 and 190 bp)

Table 4 Genotypes frequencies of IFN-γ (874A/T) polymorphism in different healthy populations

Population	Subjects number	Genotypes			References
		AA	AT	TT	
Saudis	195	49 (25.13)	91 (46.66)	55 (28.21)	Present study
Brazilian	186	64 (34.41)	77 (41.40)	45 (24.19)	[46]
Brazilian	76	17 (22.37)	46 (60.53)	13 (17.10)	[47]
Brazilian	191	60 (31.41)	34 (17.80)	97 (50.79)	[48]
Canadian	91	27 (29.67)	41 (45.06)	23 (25.27)	[49]
Chinese	480	212 (44.17)	201 (41.87)	67 (13.96)	[50]
Chinese	97	77 (79.38)	17 (17.53)	3 (3.09)	[51]
Chinese	128	101 (78.91)	26 (20.31)	1 (0.78)	[52]
Egyptian	118	6 (5.08)	60 (50.85)	52 (44.07)	[53]
Egyptian	106	8 (7.5)	92 (86.8)	6 (5.7)	[54]
Finnish	63	23 (36.51)	31 (49.21)	9 (14.28)	[55]
German	561	9 (1.60)	136 (24.24)	416 (74.15)	[56]
Greek	39	5 (12.82)	23 (58.97)	11 (28.21)	[57]
Indian	881	281 (31.90)	435 (49.37)	165 (18.73)	[58]
Indian	374	90 (24.07)	178 (47.59)	106 (28.34)	[59]
Indian	150	66 (44)	63 (42)	21 (14)	[60]
Iranian	354	123 (34.75)	134 (37.85)	97 (27.40)	[61]
Iranian	539	138 (25.60)	248 (46.01)	153 (28.39)	[62]
Israeli	48	18 (37.5)	24 (50)	6 (12.5)	[63]
Italian	140	42 (30)	66 (47.14)	32 (22.86)	[26]
Italian	96	30 (31.25)	51 (53.13)	15 (15.62)	[64]
Japanese	188	157 (83.51)	31 (16.49)	0 (0)	[65]
Korean	201	151 (75.12)	47 (23.38)	3 (1.50)	[66]
Polish	74	10 (13.51)	49 (66.22)	15 (20.27)	[67]
Thai	154	92 (59.74)	53 (34.42)	9 (5.84)	[68]
Thai	137	83 (60.58)	46 (33.58)	8 (5.84)	[69]
Tunisian	113	33 (29.20)	47 (41.60)	33 (29.20)	[70]
Turkish	40	15 (37.5)	21 (52.5)	4 (10)	[71]
Turkish	71	31 (43.66)	31 (43.66)	9 (12.68)	[72]
Turkish	99	16 (16.16)	56 (56.57)	27 (27.27)	[73]

lesions (OPCLs) in Taiwanese patients [16]. These differences in the results can be attributed to the ethnic variations in the distribution of polymorphic variants as the genotypes/alleles frequencies of polymorphisms are known to vary according to race or ethnicity.

Further, we did not notice any significant difference in the allele or genotype frequencies of TGF-β1 polymorphism between the OLP patients and healthy controls in this study. Frequency of the T allele of TGF- β 1 (509 C/T) polymorphism in the OLP patient group was 41.67 % as compared to 50.0 % in the control group. Genotypes (TT, TC, and CC) frequencies of TGF-β 1 (509 C/T) polymorphism among the patients and the healthy controls though differed but the difference was not statistically significant (Table 3). In accordance with our results, a study from Italy also repoted no significant difference in the allele or genotype frequencies of TGF-β1 between the OLP subjects and healthy controls [26]. Recently Hsu et al. [16] suggested that polymorphism in TGF-β 1 (C/T) (rs1800469) is not associated with OPCLs but another polymorphism TGF-β 1 (C/G) at codon 25 is associated significantly with the development of OPCLs in Taiwanese.

TGF-β plays highly significant role in the immune system. It regulates the IFN-γ production by NK cells. It can help accumulate the pro-inflammatory macrophage

(M1) to the anti-inflammatory type (M2) [40]. On the other hand inhibition of TGF-β pathway in the lymphocytes contributes to the chronic inflammation in OLP lesions [41] which is partly attributed to the over productions of IFN-γ, leading to the blockage of the phosphorylation of Smad3 [41]. The balance between TGF-β and IFN-γ signaling determines the immunological status and can be a therapeutic target in OLP patients [42]. The available literature on *TGF-β* 1 polymorphism is inconsistent, as some reports support the fact that the polymorphism is associated with susceptibility to the disease while others suggest that it may be protective [43–45].

Conclusion

It is concluded that the IFN-γ (874A/T) polymorphism is associated with the susceptibility to OLP. On the other hand the polymorphisms IL-6 (174G/C) and TGF-β1 (509C/T) may not be associated with OLP risk in our population, however further studies with large sample size involving different ethnic populations should be conducted to strengthen our results.

Acknowledgements
The authors thank S. Sadaf Rizvi and Mohammad Al-Asmari for their help with laboratory work.

Funding
This study was not supported by any external funding agency and no grant was received from any source and the required materials/chemicals were supplied by PSMMC.

Availability of data and materials
All data related to this study especially the genotyping gel photographs for all the subjects are available in the Research Center, Prince Sultan Military Medical City, Riyadh, Saudi Arabia and can provided upon request.

Authors' contribution
MAMA, LA, FA and EAB performed clinical examinations, collected demographic data, extracted DNA and have been involved in revising the manuscript. MA, performed genotyping, analysed, interpreted the data and drafted the manuscript. AAA, designed the study, supervised, edited and gave final approval for the manuscript to be published. All authors read and approved the final manuscript.

Competing interest
The authors declare that they have no competing interest.

Consent for publication
Not applicable.

Ethics approval and consent to participate
This study was approved by the research and ethical committee of Prince Sultan Military Medical City (PSMMC) Riyadh and written informed consent was obtained from each subject before recruitment.

Author details
[1]Department of Dentistry, Prince Sultan Military Medical City, Riyadh, Saudi Arabia. [2]Department of Dermatology, Prince Sultan Military Medical City, Riyadh, Saudi Arabia. [3]Research Center, Prince Sultan Military Medical City, P. O. Box 7897, Riyadh 11159, Saudi Arabia.

Received: 9 March 2016 Accepted: 12 August 2016
Published online: 20 August 2016

References

1. Sugerman PB, Savage NW, Walsh LJ, Zhao ZZ, Zhou XJ, Khan A, et al. The pathogenesis of oral lichen planus. Crit Rev Oral Biol Med. 2002;13(4):350–65.
2. Khan A, Farah CS, Savage NW, Walsh LJ, Harbrow DJ, Sugerman PB. Th1 cytokines in oral lichen planus. J Oral Pathol Med. 2003;32(2):77–83.
3. Tao X, Li C, Rhodus NL, Xia J, Yang X, Cheng B. Simultaneous detection of IFN-gamma and IL-4 in lesional tissues and whole unstimulated saliva from patients with oral lichen planus. J Oral Pathol Med. 2008;37(2):83–7.
4. Carrozzo M. Understanding the pathobiology of oral lichen planus. Curr Oral Health Rep. 2014;1:173–9.
5. Farhi D, Dupin N. Pathophysiology, etiologic factors, and clinical management of oral lichen planus, part I: facts and controversies. Clin Dermatol. 2010;28(1):100–8.
6. Gonzalez-Moles MA, Scully C, Gil-Montoya JA. Oral lichen planus: controversies surrounding malignant transformation. Oral Dis. 2008;14(3):229–43.
7. Bermejo-Fenoll A, Sánchez-Siles M, López-Jornet P, Camacho-Alonso F, Salazar-Sánchez NJ. A retrospective clinicopathological study of 550 patients with oral lichen planus in south-eastern Spain. Oral Pathol Med. 2010;39(6):49–6.
8. Jin X, Wang J, Zhu L, Wang L, Dan X, Zeng X, et al. Association between −308 G/A polymorphism in TNF-α gene and lichen planus: a meta-analysis. J Dermatol Sci. 2012;68(3):127–34.
9. Al-Mohaya MA, Al-Harthi F, Arfin M, Al-Asmari A. TNF-α, TNF-β and IL-10 gene polymorphism and association with oral lichen planus risk in Saudi patients. J Appl Oral Sci. 2015;23(3):295–301.
10. Lu R, Zhang J, Sun W, Du G, Zhou G. Inflammation-related cytokines in oral lichen planus: an overview. J Oral Pathol Med. 2015;44(1):1–14.
11. Calvo J, Martínez N, Etxagibel A, Calleja S, Sáez-torres C, Sedeño M, Julià R, Muncunill J, Matamoros N, Gayà A. Allelic frequencies of polymorphic variants of cytokine genes (IL1A, IL1B, IL1RN, IL6, IL10, IL12p40, and IFNG) in a Spanish population. Inmunologia. 2002;21(2):76–86.
12. Pravica V, Perrey C, Stevens A, Lee J-H, Hutchinson IV. A single nucleotide polymorphism in the first intron of the human IFN-g gene: absolute correlation with a polymorphic CA microsatellite marker of high IFN-g production. Hum Immunol. 2000;61(9):863–66.
13. Choy EH, Panayi GS. Cytokine pathways and joint inflammation in rheumatoid arthritis. N Engl J Med. 2001;344(12):907–16.
14. Fishman D, Faulds G, Jeffery R, Mohamed-Ali V, Yudkin JS, Humphries S, Woo P. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J Clin Invest. 1998;102(7):1369–76.
15. Terry CF, Loukaci V, Green FR. Cooperative influence of genetic polymorphisms on interleukin 6 transcriptional regulation. J Biol Chem. 2000;275(24):18138–44.
16. Hsu HJ, Yang YH, Shieh TY, Chen CH, Kao YH, Yang CF, Ko EC. Role of cytokine gene (interferon-γ, transforming growth factor-β1, tumor necrosis factor-α, interleukin-6, and interleukin-10) polymorphisms in the risk of oralprecancerous lesions in Taiwanese. Kaohsiung J Med Sci. 2014;30(11): 551–8.
17. Xavier GM, de Sá AR, Guimarães AL, da Silva TA, Gomez RS. Investigation of functional gene polymorphisms interleukin-1beta, interleukin-6, interleukin-10 and tumor necrosis factor in individuals with oral lichen planus. J Oral Pathol Med. 2007;36(8):476–81.
18. Smith GH. TGF-beta and functional differentiation. J Mammary Gland Biol Neoplasia. 1996;1(4):343–52.
19. Clark DA, Coker R. Transforming growth factor beta (TGT-beta). Int J Biochem Cell Biol. 1998;30(3):293–98.
20. Shah R, Hurley CK, Posch PE. A molecular mechanism for the differential regulation of TGF-beta1 expression due to the common SNP – 509C-T (c-1347C > T). Hum Genet. 2006;120(4):461–9.
21. Meng J, Thongngarm T, Nakajima M, Yamashita N, Ohta K, Bates CA. Association of transforming growth factorbeta1 single nucleotide polymorphism C-509T with allergy and immunological activities. Arch Allergy Immunol. 2005;138(2):151–60.
22. Pulleyn LJ, Newton R, Adcock IM, Barnes PJ. TGFbeta1 allele association with asthma severity. Hum Genet. 2001;109(6):623–7.
23. Karadeniz M, Erdogan M, Berdeli A, Yilmaz C. Association of interleukin-6 –174 G > C promoter polymorphism with increased risk of type 2 diabetes

mellitus patients with diabetic nephropathy in Turkey. Genet Test Mol Bioma. 2014;18(1):62–5.
24. Schallreuter KU, Levenig C, Kühnl P, Löliger C, Hohl-Tehari M, Berger J. Histocompatibility antigens in vitiligo: Hamburg study on 102 patients from northern Germany. Dermatology. 1993;187(3):186–92.
25. Svejgaard A, Platz P, Ryder LP. HLA and disease 1982 - a survey. Immunol Rev. 1983;70:193–218.
26. Carrozzo M, Uboldi de Capei M, Dametto E, Fasano ME, Arduino P, Broccoletti R, et al. Tumor necrosis factor-alpha and interferon-gamma polymorphisms contribute to susceptibility to oral lichen planus. J Invest Dermatol. 2004;122(1):87–94.
27. Bai J, Lin M, Zeng X, Zhang Y, Wang Z, Shen J, Jiang L, Gao F, Chen Q. Association of polymorphisms in the human IFN-gamma and IL-4 gene with oral lichen planus: a study in an ethnic Chinese cohort. J Interferon Cytokine Res. 2008;28(6):351–8.
28. Kimkong I, Nakkuntod J, Sodsai P, Hirankarn N, Kitkumthorn N. Association of interferon-gamma gene polymorphisms with susceptibility to oral lichen planus in the Thai population. Arch Oral Biol. 2012;57(5):491–4.
29. Lee YH, Bae SC. Association between interferon-c þ874 T/A polymorphism and susceptibility to autoimmune diseases: a meta-analysis. Lupus. 2015;0:1–9.
30. Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. Proc Natl Acad Sci U S A. 1997;94(7):3195–9.
31. Hoffmann SC, Stanley EM, Darrin Cox E, Craighead N, DiMercurio BS, Koziol DE, et al. Association of cytokine polymorphic inheritance, in vitro cytokine production in anti-CD3/CD28-stimulated peripheral blood lymphocytes. Transplantation. 2001;72(8):1444–50.
32. Fayyazi A, Schweyer S, Soruri A, Duong LQ, Radzun HJ, Peters J, Parwaresch R, Berger H. T lymphocytes and altered keratinocytes express interferon-gamma and interleukin 6 in lichen planus. Arch Dermatol Res. 1999;291(9): 485–90.
33. Karagouni EE, Dotsika EN, Sklavounou A. Alteration in peripheral blood mononuclear cell function and serum cytokines in oral lichen planus. J Oral Pathol Med. 1994;23(1):28–35.
34. Halma MA, Wheelhouse NM, Barber MD, Powell JJ, Fearon KC, Ross JA. Interferon-gamma polymorphisms correlate with duration of survival in pancreatic cancer. Hum Immunol. 2004;65(11):1405–8.
35. Hu X, Ivashkiv LB. Cross-regulation of signaling pathways by interferon-gamma: implications for immune responses and autoimmune diseases. Immunity. 2009;31(4):539–50.
36. Szabo SJ, Sullivan BM, Peng SL, Glimcher LH. Molecular mechanisms regulating Th1 immune responses. Annu Rev Immunol. 2003;21:713–58.
37. Piccinni MP, Lombardelli L, Logiodice F, Tesi D, Kullolli O, Biagiotti R, et al. Potential pathogenetic role of Th17, Th0, and Th2 cells in erosive and reticular oral lichen planus. Oral Dis. 2014;20(2):212–8.
38. Xie S, Ding L, Xiong Z, Zhu S. Implications of Th1 and Th17 cells in pathogenesis of oral lichen planus. J Huazhong Univ Sci Technolog Med Sci. 2012;32(3):451–7.
39. Youngnak-Piboonratanakit P, Dhanuthai K, Thongprasom K, et al. Expression of IFN-gamma before and after treatment of oral lichen planus with 0.1% fluocinolone acetonide in orabase. J Oral Pathol Med. 2009;38(9):689–94.
40. Akhurst RJ, Hata A. Targeting the TGFb signalling pathway in disease. Nat Rev Drug Discov. 2012;11(10):790–811.
41. Prime SS, Pring M, Davies M, Paterson IC. TGF-beta signal transduction in oro-facial health and non-malignant disease (part I). Crit Rev Oral Biol Med. 2004;15(6):324–36.
42. Zhang J, Zhou G. Green tea consumption: an alternative approach to managing oral lichen planus. Inflamm Res. 2012;61(6):535–9.
43. Noori-Daloii MR, Rashidi-Nezhad A, Izadi P, Hossein-Nezhad A, Sobhani M, Derakhshandeh-Peykar P, et al. Transforming growth factor-beta1 codon 10 polymorphism is associated with acute GVHD after allogenic BMT in Iranian population. Ann Transplant. 2007;12(4):5–10.
44. Castillejo A, Mata-Balaguer T, Guarinos C, Castillejo MI, Martínez-Cantó A, Barberá VM, et al. The Int7G24A variant of transforming growth factor-beta receptor type I is a risk factor for colorectal cancer in the male Spanish population: a case–control study. BMC Cancer. 2009;9:406. doi:10.1186/1471-2407-9-406.
45. Qian B, Katsaros D, Lu L, Preti M, Durando A, Arisio R, et al. High miR-21 expression in breast cancer associated with poor disease-free survival in early stage disease and high TGF-beta1. Breast Cancer Res Treat. 2009; 117(1):131–40.
46. Angelo HD, Gomes Silva IIF, Oliveira RD, Louzada-Júnior P, Donadi EA, Crovella S, et al. Interleukin-18, interleukin- 12B and interferon gamma gene polymorphisms in Brazilian patients with rheumatoid arthritis: a pilot study. Tissue Antigens. 2015;86:276–8.
47. Silva GA, Naveca FG, Ramasawmy R, Boechat AL. Association between the IFNG þ874A/T gene polymorphism and leprosy resistance: a meta-analysis. Cytokine. 2014;65(2):130–3.
48. Wastowski IJ, Sampaio-Barros PD, Martelli-Palomino G, Marques-Neto JF, Crispim JC, Rassi DM, et al. Association of interferon-gamma gene polymorphism (þ874 T/A) with systemic sclerosis. Dis Markers. 2009;27(2):93–5.
49. Cantor MJ, Nickerson P, Bernstein CN. The role of cytokine gene polymorphisms in determining disease susceptibility and phenotype in inflammatory bowel disease. Am J Gastroenterol. 2005;100(5):1134–42.
50. Hu Y, Wu L, Li D, Zhao Q, Jiang W, Xu B. Association between cytokine gene polymorphisms and tuberculosis in a Chinese population in Shanghai: a case–control study. BMC Immunol. 2015;16:8. doi:10.1186/s12865-015-0071-6.
51. Xu H, Li W, Fu H, Jiang G. Interferon-gamma gene polymorphism +874 (a/t) in Chinese children with Henoch-Schonlein purpura. Iran J Allergy Asthma Immunol. 2014;13(3):184–9.
52. Chen X, Xu J, Chen Z, Zhou Z, Feng X, Zhou Y, et al. Interferon-gamma þ874A/T and interleukin-4 intron3 VNTR gene polymorphisms in Chinese patients with idiopathic thrombocytopenic purpura. Eur J Haematol. 2007; 79(3):191–7.
53. Mosaad YM, Soliman OE, Tawhid ZE, Sherif DM. Interferon-gamma +874 T/A and interleukin-10 - 1082 A/G single nucleotide polymorphism in Egyptian children with tuberculosis. Scand J Immunol. 2010;72(4):358–64.
54. Elsaid A, Helaly MA, Hatata EZ, Fouda O, Settin A. TNF-α-308 and INF-γ + 874 gene polymorphisms in relation to susceptibility and severity of type 2 diabetes mellitus among Egyptian cases. Eur J Gen Med. 2012;9(3):173–7.
55. Pertovaara M, Antonen J, Hurme M. Th2 cytokine genotypes are associated with a milder form of primary Sjogren's syndrome. Ann Rheum Dis. 2006; 65(5):666–70.
56. Spriewald BM, Witzke O, Wassmuth R, Wenze RR, Arnold ML, Philipp T, et al. Distinct tumour necrosis factor alpha, interferon gamma, interleukin 10, and cytotoxic T cell antigen 4 gene polymorphisms in disease occurrence and end stage renal disease in Wegener's granulomatosis. Ann Rheum Dis. 2005; 64(3):457–61.
57. Tsiavou A, Hatziagelaki E, Chaidaroglou A, Koniavitou K, Degiannis D, Raptis SA. Correlation between intracellular interferon- gamma (IFN-gamma) production by CD4þ and CD8þ lymphocytes and IFN-gamma gene polymorphism in patients with type 2 diabetes mellitus and latent autoimmune diabetes of adults (LADA). Cytokine. 2005;31(2):135–41.
58. Dwivedi M, Laddha NC, Shah K, Shah BJ, Begum R. Involvement of interferon-gamma genetic variants and intercellular adhesion molecule-1 in onset and progression of generalized vitiligo. J Interferon Cytokine Res. 2013;33(11):646–59.
59. Mishra N, Arankalle VA. Association of polymorphisms in the promoter regions of TNF-α (−308) with susceptibility to hepatitis E virus and TNF-α (−1031) and 16 IFN-γ (+874) genes with clinical outcome of hepatitis E infection in India. J Hepatol. 2011;55(6):1227–34.
60. Rekha PL, Ishaq M, Valluri V. A differential association of interferon-gamma high-producing allele T and low-producing allele A (þ874 A/T) with Hashimoto's thyroiditis and Graves' disease. Scand J Immunol. 2006;64(4): 438–43.
61. Shokrgozar MA, Sarial S, Amirzargar A, Shokri F, Rezaei N, Arjang Z, et al. IL-2, IFN-gamma, and IL-12 gene polymorphisms and susceptibility to multiple sclerosis. J Clin Immunol. 2009;29(6):747–51.
62. Namian AM, Shahbaz S, Salmanpoor R, Namazi MR, Dehghani F, Kamali-Sarvestani E. Association of interferon-gamma and tumor necrosis factor alpha polymorphisms with susceptibility to vitiligo in Iranian patients. Arch Dermatol Res. 2009;301(1):21–5.
63. Ben-Ari Z, Mor E, Papo O, Kfir B, Sulkes J, Tambur AR, et al. Cytokine gene polymorphisms in patients infected with hepatitis B virus. Am J Gastroenterol. 2003;98(1):144–50.
64. Falleti E, Fabris C, Toniutto P, Fontanini E, Cussigh A, Caldato M, et al. Genetic polymorphisms of inflammatory cytokines and liver fibrosis progression due to recurrent hepatitis C. J Interferon Cytokine Res. 2007; 27(3):239–46.
65. Migita K, Miyazoe S, Maeda Y, Daikoku M, Abiru S, Ueki T, et al. Cytokine gene polymorphisms in Japanese patients with hepatitis B virus infection –

association between TGF-beta1 polymorphisms and hepatocellular carcinoma. J Hepatol. 2005;42(4):505–10.
66. Cheong JY, Cho SW, Chung SG, Lee JA, Yeo M, Wang HJ, et al. Genetic polymorphism of interferon-gamma, interferon-gamma receptor, and interferon regulatory factor-1 genes in patients with hepatitis B virus infection. Biochem Genet. 2006;44(5–6):246–55.
67. Baran W, Szepietowski JC, Mazur G, Baran E. IFN-gamma promoter gene polymorphism in psoriasis vulgaris. Biomarkers. 2008;13(1):52–8.
68. Tangwattanachuleeporn M, Sodsai P, Avihingsanon Y, Wongpiyabovorn J, Wongchinsri J, Hirankarn N. Association of interferon-gamma gene polymorphism (+874A) with arthritis manifestation in SLE. Clin Rheumatol. 2007;26(11):1921–4.
69. Nakkuntod J, Wongsurawat T, Charoenwongse P, Snabboon T, Sridama V, Hirankarn N. Association of TNF-alpha, TNF-beta, IFN-gamma and IL-1Ra gene polymorphisms with Graves' disease in the Thai population. Asian Pac J Allergy Immunol. 2006;24(4):207–11.
70. Bouzgarrou N, Hassen E, Farhat K, Bahri O, Gabbouj S, Maamouri N, et al. Combined analysis of interferon-gamma and interleukin-10 gene polymorphisms and chronic hepatitis C severity. Hum Immunol. 2009;70(4):230–6.
71. Col-Araz N, Pehlivan S, Baspinar O, Oguzkan-Balci S, Sever T, Balat A. Role of cytokine gene (IFN-gamma, TNF-alpha, TGFbeta1, IL-6, and IL-10) polymorphisms in pathogenesis of acute rheumatic fever in Turkish children. Eur J Pediatr. 2012;171(7):1103–8.
72. Pehlivan M, Okan V, Sever T, Balci SO, Yilmaz M, Babacan T, et al. Investigation of TNF-alpha, TGF-beta 1, IL-10, IL-6, IFN gamma, MBL, GPIA, and IL1A gene polymorphisms in patients with idiopathic thrombocytopenic purpura. Platelets. 2011;22(8):588–95.
73. Korachi M, Ceran N, Adaleti R, Nigdelioglu A, Sokmen M. An association study of functional polymorphic genes IRF-1, IFNGR-1, and IFN-gamma with disease progression, aspartate aminotransferase, alanine aminotransferase, and viral load in chronic hepatitis B and C. Int J Infect Dis. 2013;17(1):e44–9.

Submit your next manuscript to BioMed Central and we will help you at every step:

- We accept pre-submission inquiries
- Our selector tool helps you to find the most relevant journal
- We provide round the clock customer support
- Convenient online submission
- Thorough peer review
- Inclusion in PubMed and all major indexing services
- Maximum visibility for your research

Submit your manuscript at
www.biomedcentral.com/submit