***Polymorhism of PD-1 gene in patients with oral lichen planus. A case control study***

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**Abstract**

**Aims:** The association between PD-1 gene variations and susceptibility to auto immune diseases has been recurrently reported. In this study, we aimed to investigate the association between two single nucleotide polymorphisms in PD-1 gene, +7146 G to A [PD-1.3, rs11568821, in intron 4] and +7785 C to T [PD-1.5, rs2227981, in exon 5], with genetic predisposition to oral lichen planus.

**Methods:** seventy three patients with confirmed oral lichen planus[Age mean 39±13] and 171 age / sex matched healthy volunteers[Age mean 43±14]with no history of autoimmune diseases or malignancy were enrolled in this case control study. DNA was extracted using salting out method andGenotypingwas determined using PCR-RFLP and NESTED-PCR RFLP assays. Data were analyzed by SPSS and Aarlequin software packages.

**Results:**

The frequensis of GG, GA and AA genotypes at position +7146 [PD1.3] G/A in PD1 gene were 59 [80.8%],10 [13.7%] and 4 [5.5%] in patients and 132[77%],34[20%] and 5[3%] in controls respectively. Distribution of the genotypes and alleles at this locus observed not to be significantly different between patients and controls [P =0.35 and 0.98 respectively]The percentage of CC, CT and TT genotypes at position +7785 [PD1.5] C/T in PD1 gene were 32[43.8%], 35[47.9%] and 6[8.2%] in patients and 99[58%], 66[39%] and 6[3%]in controls respectively. Genotypes and allele frequensis, howevwr, did not show any significant differences between patients and controls [p=0.0.7 and p=0.06 respectively].

**Conclusion:** Current data do not indicate the association of PD-1.3 [+7146] G/Aand PD-1.5 [+7785] C/T genetic markers with susceptibility of our study population to oral lichen planus.

**Key word**: Oral lichen planus, Gene variation, PD-1

**Introduction:**

Oral lichen planus (OLP) is a chronic inflammatory mucocutaneosdiseasewithunknownetiology.This oral lesion is the most common non-infectious soft tissue disease that exerts an impact on 1-2% of adult patients in oral medicine clinics. OLP has various forms include white striae, white papules, white plaques, erythema, erosions, or bullae. The most common site of the involvement is buccalmucosa[1,2,3].

 Female aged over 40 are affected more than males of all age ranges (1.4:1). Younger adults and children may experience it as well. OLPs are typically bilateral, and often appear as a combination of clinical subtypes. Approximately, all cases of OLP are considered as reticular keratotic streaks in the oral mucosa. Accordingly, all oral mucosal lesions should be carefully examined for fine keratotic lines proximal to atrophic and/or erosive areas in the buccal mucosa, ventral or lateral surface of the tongue, gingiva, or other sites. OLP gingival lesions often appear as fiery red erythema that involves the entire width of the attached gingiva. OLP lesions may occasionally be accompanied with melanin deposition[4].

Skin lesions in lichen planus are considered as pruritic flat-topped violaceous papules and plaques, mostly on the flexor sites of the wrists or ankles, or extensor aspects of the lower legs. Furthermore, some authors have reported the genital involvement similar to skin lesions.[5]

Programmed cell death protien 1, also known as PD1 and CD279 is a protien that encoded by PDCD1 gene in humans.Regulatory T cells and the PD-1: PD-ligand [PD-L] pathway are both critical to terminating immune responses.The PD-1 gene is a CD28 /CTLA-4familymember that is a memberof the immunoglobulin gene superfamily. Elimination of either can result in the breakdown of tolerance and the development of autoimmunity[6,7,8]

PD-1 is expressed in the thymus primarily on CD4-CD8- [double negative] T-cells late in the transition fromdouble negative to double positive cells. PD-L1 is expressed at high levels by activated CD4+T cells.As the main ligand for PD-1, PD-L1 induces a coinhibitory signal in activated T-cells and promotes T-cell

apoptosis, anergy and functional exhaustion[9]

Up regulation of these genes may lead to some immune diffeciency and cancers.

Tumor-associated immune suppression can lead to defectiveT-cell-mediated antitumor immunity. Based on the finding that PD-L1 is up-regulated on Hodjkin lymphoma cells, and PD-1 is markedly elevated in the tumor-infiltrating or peripheral T cells of HL patients, blockade of the PD-1 signaling pathway inhibits SHP-2 phosphorylation and restores the IFN-γ-producing function of HL-infiltrating T-cells[10]

Li Shi et al. reported that the upregulation of PD-1 and PD-L1 is a common

phenomenon in leukemia and lymphomas that leads to double T-cell immunodeficiency, low proliferation andactivation effects, and higher immune suppression in patients.[11].Accordingly, we investigated, in the present study, the PD1 gene SNPs at positions +7146 G/A [PD-1.3, dbSNPrs # cluster id: rs11568821, in intron 4] and +7785 C/T [PD-1.5 or +872, dbSNPrs # cluster id: rs2227981, in exon 5]to determine the association between PD1 gene polymorphism and genetic susceptibility to oral lichen planus in Iranian population.

***Methods***

73ethylenediaminetetraacetic acid [EDTA]-blood samples from known cases of OLP patient, including 14males and 59Females aged between 27-62 years old [ mean age39±13]and 171 sex- and age-matched healthy subjects, including 37 males and 134females aged between 19 and 65[mean age 43±14] were enrolled in this case-control study.

 In the case group ,OLP was clinically and histopathologically diagnosed in Oral Medicine Department of Shiraz University of Medical Sciences. Information on OLP patients suffering from any systemic disease, cancer, autoimmunity [themselves or their first-degree relatives] as well as on any medication-administering that might produce a lichenoid reaction in the last 3 months were collected from their profiles.

Control group consisted of sex- and age-matched healthy blood donors with no family history of canceror auto-immune diseaseswho were selected from blood transfusion center attendant.

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All participants were informed about the study and agreed to participate by signing a consent form. OLP lesions were divided into two forms according to their clinical pattern, reticular and/or plaque lesions[n=22]and erosive atrophic lesions[n=51]. Both patient group and healthy controls were living in south of Iran. The study was approved by the Ethics Committee of Shiraz University of Medical Sciences.

**DNA extraction and genotyping**

DNA was extracted from peripheral blood the white blood cells by salting out method[12].. After DNA extraction two different methods were used to determine genotypes in both patients and control groups. For PD-1.3 position of PDCD1 gene ,Polymerase Chain Reaction Restriction- Fragment Length Polymorphism (PCR-RFLP) methodwas performed to genotypes PD-1.3 using *PST* I [Fermentas, Lithuania] restriction enzyme and a sequence of primers designed by our group . For PD-1.5 position of PDCD1 gene ,Nested Polymerase Chain Reaction Restriction- Fragment Length Polymorphism [Nested PCR-RFLP] was done to genotypes PD-1.5 using *PVU* II [Fermentas, Lithuania] restriction enzyme[13]

The primers(Takapouzist, Iran), annealing temperatures and restriction enzyme used for genotyping each SNP are summarized in Table1.

**Statistical analysis**

Statistical analyses were performed using SPSS software [version19; SPSS Inc, Chicago, IL, USA] . Pearson’s Chi square test was applied to determine the differences in genotype and allele frequency between the two study groups. The level of significance was *P* < 0.05

**Results:**

Information on age, sex, frequency of different genotypes and alleles in [PD-1.3 ]+7146 G/A position in both patient and control group mentioned in Table2

 GG genotypes occurred in 79 patient [80.8%] ,GA genotype in ten [13/7%] and AA in four patients with OLP. In control group a frequency of 132 [77%] in GG genotype ,34 [20%] in GA and 5[3%] were detected respectively. As we seen in the table GG genotype were more prevalent in both group ,but the genotypes did not show any significant difference in the distribution at this locus between patients and the controls[*P* = 0.35].

About polymorphism of PD-1.5 ,32 patients[43.8%]had CC genotype ,35 patients [47.9%]had CT genotype and 6 patients[8.2%] had TT genotype. In control group 99persons [58%]had CC genotypes,66 one [39%] had CT genotypes and 6 of them[3%] had TT genotypes.

The most prevalentgenotypes in OLP group was CT heterozygot and in the controls was CC hemozygot. No relevant statistical differences were seen regarding mononeucleotid PD1 +7146 C/T polymorphism in both groups.[P=0.07].The most common founded allele in both group was C wild un mutated allele.[p=0.06]

The mean age [± standard deviation] of OLP patients and participants in the control group were39[±13] and43[±14], respectively. The most common OLP site was the buccalmucosa[39], followed by the tongue and buccal mucosa [16],tongue[11] and gingiva [7].

**Discussion**:

 Oral lichen planus is a T-cell mediated chronic inflammatory disease and many factors such as interaction between inflammatory cells, chemokines, cytokines, mast cell degranulation, and matrix metalloproteinase activation has a significant role in the disease development . [14]

In recent years, many studies focused on the role of genes which encodes immune regulatory proteins such as CTLA4 ,PD1 ligand ,P52 and other factors that influenced autoimmune and malignancy.[3,15,16]

CTLA-4 and PD-1 are receptors that negatively regulate T-cell activation. PD-1 is expressed on activated T lymphocytes but also on B cells, suggesting involvement in a broader spectrum of immune regulation than CTLA-4.Ligation of both CTLA-4 and PD-1 blocked CD3/CD28-mediated upregulationbyusing separate mechanisms[17 ,18]

Autoimmunity and PD-1 deficiency linkage was initiallyrevealed by the studies on PD-1-deficient mice on theC57BL/6 background, which exhibit hyperactivation of theimmune system such as splenomegaly and in vitro augmentedproliferation of B cells[19].

Astrong relationship between negative prognosis and PD-L expression on tumor cells has been estabilished on human cancer . Thompson [20]andco workers evaluated the expression of PD-L1 on clinical specimens of renal cell carcinoma and found that patients with high tumor and/or lymphocyte PD-L1 levels are 4.5 times more likely to die from their cancer than patients exhibiting low levels of PD-L1 expression.

Zamani and coworkers in Iran evaluated PD1 gene ligand polymorphism in infertile patients and healthy controls. He studied 145 Iranian subjects include 61 patients with antisperm antibody-related infertility and 84 healthy controls.he founded that frequencies of the G/A genotype were not significantly different in both group . However there was a significant difference in G/G and A/A genotype frequencies between cases and controls [P = 0.042, P = 0.00001, respectively]. Allele frequencies of PD1 also significantly differs in patients compared to healthy controls[P = 0.0012][21].

Topalian et al studied 296 patients with different body cancers[ advanced melanoma, non-small-cell lung cancer, castration-resistant prostate cancer, renal-cell or colorectal cancer]in order to evaluate the anti PD1antibody effect on the development of such malignancies . The safety and anti tumor activity of BMS-936558, a specific PD-1 activity blockers were assesed .All patients received

anti-PD-1 antibody at a dose of 0.1 to 10.0 mg per kilogram every 2 weeks. After each 8-week treatment cycle patients response was assessed. Complete or partial responses were seen in 236 patients complain of renal-cell cancer, melanoma, or non-small-cell lung cancer.

 They concluded that in approximately 20-25% of patients with non-small-cell lung cancer, melanoma, or renal-cell cancer anti-PD-1 antibody induced objective responses. There was no important adverse effects to prevent its use[22]

There is a dearth of research represent the association between oral lesions and PD1 ligand and or other genes polymorphism.

# Zandberg et al described the role of the PD-L1:PD-1 pathway in head and neck squamous cell carcinoma and paticullary considered how this pathway can be operated with therapeuticintention[23].

Shin et al studied 65 patients with oral submucousfibrosisandreported a relationship between the disease and CTLA-4 G allele polymorphism [24]

In current research we studied PD1 Ligand polymorhism and we did not find a relationship between oral lichen planus and this gene .

Ghapanchi and co workers examined the genomic DNA of 35 patients with OLP and compare it to 105 healthy controls . They freported that there was no significant relation between both groups in Polymorphisms of CTLA-4 genes in position +49 A/G in Shiraz, Iran.[3]

Another research in Iran also focused on the correlation between polymorphism of tumor protein p53 codon 72 and OLP. In this research also a significant relation ship was not founded.[15]Our findings was in accordance with both of these researches ,we also did not find a relevant correlation between PD-1.3 and PD-1. 5 polymorhism and OLP lesion.

Farzin et al collected 34 serum samples of OLP patients and compare it to the healthy controls .They founded that serum level of matrix metaloproteinase 3 in OLP group was significantly higher than controls and variations in clinical types of lichen planusare associated with significant differences in MMP-3 serum level.[25]

**Conclusion**

Current research showed that the polymorphism of PD-1.3 and PD-1.5 genes did not have a significant relationship with OLP patients in Shiraz. However, in order to generalize the obtained results, it is suggested that further studies be conducted on a larger population.

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**References**

1- Bowers KE, Sexton J, Sugerman PB. Commentary. *Clinical Dermatology* 2000;**18**:497-8.

### 2-Axéll T, Rundquist L. Oral lichen planus – A demographic study. [*Community Dentistry and Oral Epidemiology*](http://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=0CB0QFjAA&url=http%3A%2F%2Fonlinelibrary.wiley.com%2Fjournal%2F10.1111%2F%2528ISSN%25291600-0528&ei=OqdNVZfHMIG6ggTswYHgDw&usg=AFQjCNFFkSIGjzy29m2qiV6kiZym4pEfnQ&bvm=bv.92885102,d.eXY&cad=rja). 1987;15:52-6.

3-Ghapanchi J, Haghshenas M, Ghaderi H, Amanpour S, Nemati V, Kamali F.

###  CTLA-4 Gene Polymorphism in +49 A/G Position: A Case Control Study on Patients with Oral Lichen Planus. [*Journal of International Oral Health*](http://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&cad=rja&uact=8&ved=0CB4QFjAA&url=http%3A%2F%2Fwww.jioh.in%2F&ei=GKhNVfe0DKHIsQTX94G4Dg&usg=AFQjCNGR0KkD8wvvN2BvpxgCYrd-uhEHog&bvm=bv.92885102,d.cWc)

 *2014;* ***6****:17-21.*

4-Sugerman PB, Savage NW, Zhou X, Walsh LJ, Bigby M. Oral lichen planus. *Clinical Dermatolology* 2000;**18**:533-9.

5-Sugerman PB, Savage NW. Oral lichen planus: Causes, diagnosis andmanagement.*Australian Dental Journal*.2002;**47**:290-7.

6-Shinohara T, Taniwaki M, Ishida Y, Kawaichi M, HonjoT . Structure and chromosomal localization of the human PD-1 gene (PDCD1). *Genomics*. 1994;**23** 704–6.

### 7- Francisco LM, Sage PT, Sharpe AH. [The PD-1 pathway in tolerance and autoimmunity](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2919275). [[*Immunological Review*](http://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=0CCAQFjAA&url=http%3A%2F%2Fonlinelibrary.wiley.com%2Fjournal%2F10.1111%2F%2528ISSN%25291600-065X&ei=_qtNVYuVAYWbNsmXgOAI&usg=AFQjCNFPLEVa81pYo4xOVnASycpRH9Kf9w&bvm=bv.92885102,d.cWc&cad=rja).](http://www.ncbi.nlm.nih.gov/pubmed/?term=Francisco+LM%2C+Sage+PT%2C+Sharpe+AH.+%22The+PD-1+pathway+in+tolerance+and+autoimmunity%22.+Immunol.+Rev.+236%3A+219%E2%80%9342.) 2010;236:219-42.

8-Vibhakar R, Juan G, Traganos F, Darzynkiewicz Z, Finger LR. Activation-inducedexpression of human programmed death-1 gene in T-lymphocytes.[*Experimental Cell Research Journal*](http://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=2&sqi=2&ved=0CCkQFjAB&url=http%3A%2F%2Fwww.researchgate.net%2Fjournal%2F0014-4827_Experimental_Cell_Research&ei=WaxNVefRGLb7sASDtICACg&usg=AFQjCNFS5vZH2fJI3_CFWCfiKUdijN4YvA&bvm=bv.92885102,d.cWc&cad=rja) . 1997; **232**:25–28.

### 9- Chen L. Co-inhibitory molecules of the B7-CD28 family in the control of T-cell immunity. [*Nature Reviews Immunology*](http://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=4&cad=rja&uact=8&ved=0CDIQFjAD&url=http%3A%2F%2Fen.wikipedia.org%2Fwiki%2FNature_Reviews_Immunology&ei=Ia1NVcjPD4W1sATN2oDIDw&usg=AFQjCNFU-Zbb2aRQUXFmy1JeyOTXNpO26A&bvm=bv.92885102,d.cWc). 2004;4:336–347.

10- [Yamamoto R](http://www.ncbi.nlm.nih.gov/pubmed/?term=Yamamoto%20R%5BAuthor%5D&cauthor=true&cauthor_uid=18203952)1, [Nishikori M](http://www.ncbi.nlm.nih.gov/pubmed/?term=Nishikori%20M%5BAuthor%5D&cauthor=true&cauthor_uid=18203952), [Kitawaki T](http://www.ncbi.nlm.nih.gov/pubmed/?term=Kitawaki%20T%5BAuthor%5D&cauthor=true&cauthor_uid=18203952), [Sakai T](http://www.ncbi.nlm.nih.gov/pubmed/?term=Sakai%20T%5BAuthor%5D&cauthor=true&cauthor_uid=18203952), [Hishizawa M](http://www.ncbi.nlm.nih.gov/pubmed/?term=Hishizawa%20M%5BAuthor%5D&cauthor=true&cauthor_uid=18203952), [Tashima M](http://www.ncbi.nlm.nih.gov/pubmed/?term=Tashima%20M%5BAuthor%5D&cauthor=true&cauthor_uid=18203952), [Kondo T](http://www.ncbi.nlm.nih.gov/pubmed/?term=Kondo%20T%5BAuthor%5D&cauthor=true&cauthor_uid=18203952), [Ohmori K](http://www.ncbi.nlm.nih.gov/pubmed/?term=Ohmori%20K%5BAuthor%5D&cauthor=true&cauthor_uid=18203952), [Kurata M](http://www.ncbi.nlm.nih.gov/pubmed/?term=Kurata%20M%5BAuthor%5D&cauthor=true&cauthor_uid=18203952), [Hayashi T](http://www.ncbi.nlm.nih.gov/pubmed/?term=Hayashi%20T%5BAuthor%5D&cauthor=true&cauthor_uid=18203952), [Uchiyama T](http://www.ncbi.nlm.nih.gov/pubmed/?term=Uchiyama%20T%5BAuthor%5D&cauthor=true&cauthor_uid=18203952). PD-1-PD-1 ligand interaction contributes to immunosuppressive microenvironment of Hodgkinlymphoma. *Blood.* 2008;**111:**3220–3224.

11- Li Shi1, Shaohua Chen1, Lijian Yang1 and Yangqiu Li. The role of PD-1 and PD-L1 in T-cell immunesuppression in patients with hematological malignancies .*Journal of Hematology & Oncology.* 2013; **6**:74

12- Miller SA, Dykes DD, Polesky HF . A simple salting out procedure for extracting DNA from human nucleated cells.*Nucleic Acids Research* .1988;**16**1215

13- [Lin SC](http://www.ncbi.nlm.nih.gov/pubmed/?term=Lin%20SC%5BAuthor%5D&cauthor=true&cauthor_uid=15022318)1, [Yen JH](http://www.ncbi.nlm.nih.gov/pubmed/?term=Yen%20JH%5BAuthor%5D&cauthor=true&cauthor_uid=15022318), [Tsai JJ](http://www.ncbi.nlm.nih.gov/pubmed/?term=Tsai%20JJ%5BAuthor%5D&cauthor=true&cauthor_uid=15022318), [Tsai WC](http://www.ncbi.nlm.nih.gov/pubmed/?term=Tsai%20WC%5BAuthor%5D&cauthor=true&cauthor_uid=15022318), [Ou TT](http://www.ncbi.nlm.nih.gov/pubmed/?term=Ou%20TT%5BAuthor%5D&cauthor=true&cauthor_uid=15022318), [Liu HW](http://www.ncbi.nlm.nih.gov/pubmed/?term=Liu%20HW%5BAuthor%5D&cauthor=true&cauthor_uid=15022318), [Chen CJ](http://www.ncbi.nlm.nih.gov/pubmed/?term=Chen%20CJ%5BAuthor%5D&cauthor=true&cauthor_uid=15022318). Association of a programmed death 1 gene polymorphism with the development of rheumatoid arthritis, but not systemic lupus erythematosus. *Arthritis Rheumatology* .2004;**50**: 770–775.

### 14- [Sugerman PB](http://www.ncbi.nlm.nih.gov/pubmed/?term=Sugerman%20PB%5BAuthor%5D&cauthor=true&cauthor_uid=12191961)1, [Savage NW](http://www.ncbi.nlm.nih.gov/pubmed/?term=Savage%20NW%5BAuthor%5D&cauthor=true&cauthor_uid=12191961), [Walsh LJ](http://www.ncbi.nlm.nih.gov/pubmed/?term=Walsh%20LJ%5BAuthor%5D&cauthor=true&cauthor_uid=12191961), [Zhao ZZ](http://www.ncbi.nlm.nih.gov/pubmed/?term=Zhao%20ZZ%5BAuthor%5D&cauthor=true&cauthor_uid=12191961), [Zhou XJ](http://www.ncbi.nlm.nih.gov/pubmed/?term=Zhou%20XJ%5BAuthor%5D&cauthor=true&cauthor_uid=12191961), [Khan A](http://www.ncbi.nlm.nih.gov/pubmed/?term=Khan%20A%5BAuthor%5D&cauthor=true&cauthor_uid=12191961), [Seymour GJ](http://www.ncbi.nlm.nih.gov/pubmed/?term=Seymour%20GJ%5BAuthor%5D&cauthor=true&cauthor_uid=12191961), [Bigby M](http://www.ncbi.nlm.nih.gov/pubmed/?term=Bigby%20M%5BAuthor%5D&cauthor=true&cauthor_uid=12191961).The pathogenesis of oral lichen planus. [*Critical Reviews in Oral Biology & Medicine*](http://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=0CB0QFjAA&url=http%3A%2F%2Fcro.sagepub.com%2F&ei=ebBNVejKG4yqNsW1gGA&usg=AFQjCNFkH9jX3tMphMsNo_Jq-wsVcpLUQg&bvm=bv.92885102,d.eXY). 2002;13:350-65.

15- Ghabanchi J, Fattahi MJ, Mardani M, Tadbir AA, Paydar AA. Polymorphism of tumor protein p53 codon 72 showed no association with oral lichen planus in Shiraz, Iran. *Journal of Craniofacial Surgery*. 2009;**20**:2168-70.

16- [Okazaki T](http://www.ncbi.nlm.nih.gov/pubmed/?term=Okazaki%20T%5BAuthor%5D&cauthor=true&cauthor_uid=17606980), [Honjo T](http://www.ncbi.nlm.nih.gov/pubmed/?term=Honjo%20T%5BAuthor%5D&cauthor=true&cauthor_uid=17606980).PD-1 and PD-1 ligands: from discovery to clinical application. *International Immunology*, 2007 ;**19**:813-24.

17- Agata Y, Kawasaki A, Nishimura H, Ishida Y, Tsubata T, Yagita H,

Honjo T. Expression of the PD-1 antigen on the surface of stimulated

mouse T and B lymphocytes. *International Immunology*.1996; **8**:765–772.

# 18-[Parry](http://www.ncbi.nlm.nih.gov/pubmed/?term=Parry%20RV%5Bauth%5D) RV,  [Chemnitz](http://www.ncbi.nlm.nih.gov/pubmed/?term=Chemnitz%20JM%5Bauth%5D) JM, [Frauwirth](http://www.ncbi.nlm.nih.gov/pubmed/?term=Frauwirth%20KA%5Bauth%5D) KA, [Lanfranco](http://www.ncbi.nlm.nih.gov/pubmed/?term=Lanfranco%20AR%5Bauth%5D) AR, [Braunstein](http://www.ncbi.nlm.nih.gov/pubmed/?term=Braunstein%20I%5Bauth%5D) I,  [Kobayashi](http://www.ncbi.nlm.nih.gov/pubmed/?term=Kobayashi%20SV%5Bauth%5D), [Peter S. Linsley](http://www.ncbi.nlm.nih.gov/pubmed/?term=Linsley%20PS%5Bauth%5D)PS,[Thompson](http://www.ncbi.nlm.nih.gov/pubmed/?term=Thompson%20CB%5Bauth%5D) C B, [Riley](http://www.ncbi.nlm.nih.gov/pubmed/?term=Riley%20JL%5Bauth%5D) JL .CTLA-4 and PD-1 receptors inhibit T-Cell activation by distinct mechanisms .*Molecular and cellular Biology*. 2005 ; 25: 9543–9553.

19- Nishimura H, Nose M, Hiai H, Minato N, Honjo T. Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. *Immunity*.1999; **11**:141.

20- [Thompson RH](http://www.ncbi.nlm.nih.gov/pubmed/?term=Thompson%20RH%5BAuthor%5D&cauthor=true&cauthor_uid=15569934)1, [Gillett MD](http://www.ncbi.nlm.nih.gov/pubmed/?term=Gillett%20MD%5BAuthor%5D&cauthor=true&cauthor_uid=15569934), [Cheville JC](http://www.ncbi.nlm.nih.gov/pubmed/?term=Cheville%20JC%5BAuthor%5D&cauthor=true&cauthor_uid=15569934), [Lohse CM](http://www.ncbi.nlm.nih.gov/pubmed/?term=Lohse%20CM%5BAuthor%5D&cauthor=true&cauthor_uid=15569934), [Dong H](http://www.ncbi.nlm.nih.gov/pubmed/?term=Dong%20H%5BAuthor%5D&cauthor=true&cauthor_uid=15569934), [Webster WS](http://www.ncbi.nlm.nih.gov/pubmed/?term=Webster%20WS%5BAuthor%5D&cauthor=true&cauthor_uid=15569934), [Krejci KG](http://www.ncbi.nlm.nih.gov/pubmed/?term=Krejci%20KG%5BAuthor%5D&cauthor=true&cauthor_uid=15569934), [Lobo JR](http://www.ncbi.nlm.nih.gov/pubmed/?term=Lobo%20JR%5BAuthor%5D&cauthor=true&cauthor_uid=15569934), [Sengupta S](http://www.ncbi.nlm.nih.gov/pubmed/?term=Sengupta%20S%5BAuthor%5D&cauthor=true&cauthor_uid=15569934), [Chen L](http://www.ncbi.nlm.nih.gov/pubmed/?term=Chen%20L%5BAuthor%5D&cauthor=true&cauthor_uid=15569934), [Zincke H](http://www.ncbi.nlm.nih.gov/pubmed/?term=Zincke%20H%5BAuthor%5D&cauthor=true&cauthor_uid=15569934), [Blute ML](http://www.ncbi.nlm.nih.gov/pubmed/?term=Blute%20ML%5BAuthor%5D&cauthor=true&cauthor_uid=15569934), [Strome SE](http://www.ncbi.nlm.nih.gov/pubmed/?term=Strome%20SE%5BAuthor%5D&cauthor=true&cauthor_uid=15569934), [Leibovich BC](http://www.ncbi.nlm.nih.gov/pubmed/?term=Leibovich%20BC%5BAuthor%5D&cauthor=true&cauthor_uid=15569934), [Kwon ED](http://www.ncbi.nlm.nih.gov/pubmed/?term=Kwon%20ED%5BAuthor%5D&cauthor=true&cauthor_uid=15569934). Costimulatory B7-H1 in renal cell carcinoma patients: indicator of tumor aggressiveness and potential therapeutic target. [*Proceedings of the National Academy of Sciences*](http://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=2&cad=rja&uact=8&ved=0CCkQFjAB&url=http%3A%2F%2Fwww.ncbi.nlm.nih.gov%2Fnlmcatalog%2F7505876&ei=KslNVbP3IcjlUdXFgNAB&usg=AFQjCNEZUgu03QKukWophNL6ppjyMkErSA&bvm=bv.92885102,d.bGQ).2004 ;**101**:17174-9.

21-[Zamani MR](http://www.ncbi.nlm.nih.gov/pubmed/?term=Zamani%20MR%5BAuthor%5D&cauthor=true&cauthor_uid=25399062), [Asbagh FA](http://www.ncbi.nlm.nih.gov/pubmed/?term=Asbagh%20FA%5BAuthor%5D&cauthor=true&cauthor_uid=25399062), [MassoudAH](http://www.ncbi.nlm.nih.gov/pubmed/?term=Massoud%20AH%5BAuthor%5D&cauthor=true&cauthor_uid=25399062), [Salmaninejad A](http://www.ncbi.nlm.nih.gov/pubmed/?term=Salmaninejad%20A%5BAuthor%5D&cauthor=true&cauthor_uid=25399062), [Massoud A](http://www.ncbi.nlm.nih.gov/pubmed/?term=Massoud%20A%5BAuthor%5D&cauthor=true&cauthor_uid=25399062), [Rezaei N](http://www.ncbi.nlm.nih.gov/pubmed/?term=Rezaei%20N%5BAuthor%5D&cauthor=true&cauthor_uid=25399062). Association between a PD-1 gene polymorphism and antisperm antibody-related infertility in Iranian men. [*Journal of Assisted Reproduction and Genetics*](http://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&cad=rja&uact=8&ved=0CB0QFjAA&url=http%3A%2F%2Fwww.springer.com%2Fmedicine%2Fgynecology%2Fjournal%2F10815&ei=kslNVaSHB8z1Up7fgeAD&usg=AFQjCNFvB4LmcJaVenGqoTNBADrNcSmxYA&bvm=bv.92885102,d.bGQ). 2015 ;**32**:103-6.

### 22-[Topalian SL](http://www.ncbi.nlm.nih.gov/pubmed/?term=Topalian%20SL%5BAuthor%5D&cauthor=true&cauthor_uid=22658127), [Hodi FS](http://www.ncbi.nlm.nih.gov/pubmed/?term=Hodi%20FS%5BAuthor%5D&cauthor=true&cauthor_uid=22658127), [Brahmer JR](http://www.ncbi.nlm.nih.gov/pubmed/?term=Brahmer%20JR%5BAuthor%5D&cauthor=true&cauthor_uid=22658127), [Gettinger SN](http://www.ncbi.nlm.nih.gov/pubmed/?term=Gettinger%20SN%5BAuthor%5D&cauthor=true&cauthor_uid=22658127), [Smith DC](http://www.ncbi.nlm.nih.gov/pubmed/?term=Smith%20DC%5BAuthor%5D&cauthor=true&cauthor_uid=22658127), [McDermott DF](http://www.ncbi.nlm.nih.gov/pubmed/?term=McDermott%20DF%5BAuthor%5D&cauthor=true&cauthor_uid=22658127), [Powderly JD](http://www.ncbi.nlm.nih.gov/pubmed/?term=Powderly%20JD%5BAuthor%5D&cauthor=true&cauthor_uid=22658127), [Carvajal RD](http://www.ncbi.nlm.nih.gov/pubmed/?term=Carvajal%20RD%5BAuthor%5D&cauthor=true&cauthor_uid=22658127), [Sosman JA](http://www.ncbi.nlm.nih.gov/pubmed/?term=Sosman%20JA%5BAuthor%5D&cauthor=true&cauthor_uid=22658127), [Atkins MB](http://www.ncbi.nlm.nih.gov/pubmed/?term=Atkins%20MB%5BAuthor%5D&cauthor=true&cauthor_uid=22658127), [Leming PD](http://www.ncbi.nlm.nih.gov/pubmed/?term=Leming%20PD%5BAuthor%5D&cauthor=true&cauthor_uid=22658127), [Spigel DR](http://www.ncbi.nlm.nih.gov/pubmed/?term=Spigel%20DR%5BAuthor%5D&cauthor=true&cauthor_uid=22658127), [Antonia SJ](http://www.ncbi.nlm.nih.gov/pubmed/?term=Antonia%20SJ%5BAuthor%5D&cauthor=true&cauthor_uid=22658127), [Horn L](http://www.ncbi.nlm.nih.gov/pubmed/?term=Horn%20L%5BAuthor%5D&cauthor=true&cauthor_uid=22658127), [Drake CG](http://www.ncbi.nlm.nih.gov/pubmed/?term=Drake%20CG%5BAuthor%5D&cauthor=true&cauthor_uid=22658127), [Pardoll DM](http://www.ncbi.nlm.nih.gov/pubmed/?term=Pardoll%20DM%5BAuthor%5D&cauthor=true&cauthor_uid=22658127), [Chen L](http://www.ncbi.nlm.nih.gov/pubmed/?term=Chen%20L%5BAuthor%5D&cauthor=true&cauthor_uid=22658127), [Sharfman WH](http://www.ncbi.nlm.nih.gov/pubmed/?term=Sharfman%20WH%5BAuthor%5D&cauthor=true&cauthor_uid=22658127), [Anders RA](http://www.ncbi.nlm.nih.gov/pubmed/?term=Anders%20RA%5BAuthor%5D&cauthor=true&cauthor_uid=22658127), [Taube JM](http://www.ncbi.nlm.nih.gov/pubmed/?term=Taube%20JM%5BAuthor%5D&cauthor=true&cauthor_uid=22658127), [McMiller TL](http://www.ncbi.nlm.nih.gov/pubmed/?term=McMiller%20TL%5BAuthor%5D&cauthor=true&cauthor_uid=22658127), [Xu H](http://www.ncbi.nlm.nih.gov/pubmed/?term=Xu%20H%5BAuthor%5D&cauthor=true&cauthor_uid=22658127), [Korman AJ](http://www.ncbi.nlm.nih.gov/pubmed/?term=Korman%20AJ%5BAuthor%5D&cauthor=true&cauthor_uid=22658127), [Jure-Kunkel M](http://www.ncbi.nlm.nih.gov/pubmed/?term=Jure-Kunkel%20M%5BAuthor%5D&cauthor=true&cauthor_uid=22658127), [Agrawal S](http://www.ncbi.nlm.nih.gov/pubmed/?term=Agrawal%20S%5BAuthor%5D&cauthor=true&cauthor_uid=22658127), [McDonald D](http://www.ncbi.nlm.nih.gov/pubmed/?term=McDonald%20D%5BAuthor%5D&cauthor=true&cauthor_uid=22658127), [Kollia GD](http://www.ncbi.nlm.nih.gov/pubmed/?term=Kollia%20GD%5BAuthor%5D&cauthor=true&cauthor_uid=22658127), [Gupta A](http://www.ncbi.nlm.nih.gov/pubmed/?term=Gupta%20A%5BAuthor%5D&cauthor=true&cauthor_uid=22658127), [Wigginton JM](http://www.ncbi.nlm.nih.gov/pubmed/?term=Wigginton%20JM%5BAuthor%5D&cauthor=true&cauthor_uid=22658127), [Sznol M](http://www.ncbi.nlm.nih.gov/pubmed/?term=Sznol%20M%5BAuthor%5D&cauthor=true&cauthor_uid=22658127). Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. [[*New England Journal of Medicin*](http://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=0CB0QFjAA&url=http%3A%2F%2Fwww.nejm.org%2F&ei=6clNVbOvH4nXUey0geAE&usg=AFQjCNHrYSeMZeN0mO41BJVMHhzFiMYDkQ&bvm=bv.92885102,d.bGQ&cad=rja).](http://www.ncbi.nlm.nih.gov/pubmed/22658127) 2012 ;28;366.

# 23-[Zandberg DP](http://www.ncbi.nlm.nih.gov/pubmed/?term=Zandberg%20DP%5BAuthor%5D&cauthor=true&cauthor_uid=24819861), [Strome SE](http://www.ncbi.nlm.nih.gov/pubmed/?term=Strome%20SE%5BAuthor%5D&cauthor=true&cauthor_uid=24819861). The role of the PD-L1:PD-1 pathway in squamous cell carcinoma of the head and neck.[*Oral Oncolology*.](http://www.ncbi.nlm.nih.gov/pubmed/24819861) 2014;50:627-32.

# 24-Shin YN, Liu CJ, Chang KW, Lee YJ, Liu HF. Association of CTLA-4 gene polymorphism with oral submucous fibrosis in Taiwan. *Journal of Oral Pathology and Medicine.* 2004;33:200-3.

# 25-[Farzin M](http://www.ncbi.nlm.nih.gov/pubmed/?term=Farzin%20M%5BAuthor%5D&cauthor=true&cauthor_uid=22737547), [Mardani M](http://www.ncbi.nlm.nih.gov/pubmed/?term=Mardani%20M%5BAuthor%5D&cauthor=true&cauthor_uid=22737547), [Ghabanchi J](http://www.ncbi.nlm.nih.gov/pubmed/?term=Ghabanchi%20J%5BAuthor%5D&cauthor=true&cauthor_uid=22737547), [Fattahi MJ](http://www.ncbi.nlm.nih.gov/pubmed/?term=Fattahi%20MJ%5BAuthor%5D&cauthor=true&cauthor_uid=22737547), [Rezaee M](http://www.ncbi.nlm.nih.gov/pubmed/?term=Rezaee%20M%5BAuthor%5D&cauthor=true&cauthor_uid=22737547), [Heydari ST](http://www.ncbi.nlm.nih.gov/pubmed/?term=Heydari%20ST%5BAuthor%5D&cauthor=true&cauthor_uid=22737547), [AndishehTadbir A](http://www.ncbi.nlm.nih.gov/pubmed/?term=Andisheh%20Tadbir%20A%5BAuthor%5D&cauthor=true&cauthor_uid=22737547). Serum level of matrix metalloproteinase-3 in patients with oral lichen planus*.* [*Iran Red Crescent Medical Journal.*](http://www.ncbi.nlm.nih.gov/pubmed/22737547) 2012;14:10-3.

Table 1.specific primers and reaction conditions for PD1.3 and PD1.5 positions

| **Locus** | **Primer** | **Primer sequence** | **Annealing****temperature** | **Restriction****Enzyme** | **length of digested fragments** |
| --- | --- | --- | --- | --- | --- |
| PD-1.3 [+7146 G/A | forward primer | 5-GCCTGGAGGACTCACATTCT-3 | 58 °C | PST I | G: 381bp |
| A: 277bp, 104bp |
| reverse primer | 5-GTCCCCCTCTGAAATGTCC-3 |
| PD-1.5 [+7785 or +872 C/T] | Outer reaction forward primer | 5′-AGACGGAGTATGCCACCATTGTC-3′ | 58°C | PVUII | C: 89 bp |
| T: 48 bp, 41 bp |
| Outer reaction reverse primer | 5′-AAATGCGCTGACCCGGGCTCAT-3′ |
| Inner reaction forward primer | 5′-TAGCGGAATGGGCACCTCATC-3′ | 51°C |
| Inner reaction reverse primer | 5′-AGTGTCCATGCTCAGGCCTCA-3′ |

Table2. Genotypes and allele frequencies of PDCD-1 gene in positions of PD1.3 and PD1.5 in patients with oral lichen planus in comparison to controls

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | SNP | Oral lichen planus patients N=73 | ControlsN=171 | pv |
| +7146 G / A [PD-1.3] | GG | 59[80.8%] | 132[77%] | 0.35 |
| GA | 10[13.7%] | 34[20%] |
| AA | 4[5.5%] | 5[3%] |
| Missing | - | - |  |
| G | 128[87%] | 298[87%] | 0.98 |
| A | 18[13%] | 44[23%] |
| +7785 C / T [PD-1.5] | CC | 32[43.8%] | 99[58%] | 0.07 |
| CT | 35[47.9%] | 66[39%] |
| TT | 6[8.2%] | 6[3%] |
| Missing | - | - |
| C | 99[68%] | 254[76.5%] | 0.06 |
| T | 47[32%] | 78[23.5%] |