Vitamin D receptor genetic polymorphisms were associated with oral lichen planus susceptibility in Chinese Han population

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Abstract

Vitamin D receptor (VDR) is involved in multiple immune-mediated disorders including Oral lichen planus (OLP). This study was aimed to investigate the association between VDR gene polymorphisms and the risk of OLP. 177 OLP patients and 207 healthy participants were recruited from Affiliated Hospital of Stomatology, Nanjing Medical University. Eight single nucleotide polymorphisms (SNPs: rs731236, rs739837, rs757343, rs2107301, rs2239185, rs7975232, rs11574129 and rs11568820) on the VDR gene were selected and genotyped. The results showed that the OLP risk was increased in subjects with the rs2239185 TT genotype (Recessive model: adjusted OR = 2.68, 95% CI = 1.28-5.62, P = 0.009) and rs7975232 CC genotype (Recessive model: adjusted OR = 2.25, 95% CI = 1.10-4.58, P = 0.026). And the significant cumulative effects on OLP risk were found in rs2239185 and rs7975232 (P < 0.01). The haplotype analysis showed that haplotype CC (rs2239185-rs7975232) was associated with increased OLP risk (OR =3.11, 95% CI = 1.42-6.83, P = 0.005), compared with haplotype AC. In conclusion, the variants of VDR rs2239185 and rs7975232 may influence the OLP susceptibility and VDR gene polymorphisms may be the candidate susceptibility region of OLP in Chinese Han population.

Background

Oral lichen planus (OLP) is a chronic inflammatory disease of oral mucosal mediated by T cells, whose etiology remains unknown. It is characterized as dense lymphocyte infiltration and basal keratinocyte degeneration under microscope [1]. OLP, the typical clinical feature of whom is white stripes, could manifest as reticular, papular, plaque-like, erosive, atrophic and bullous [2,3]. Erosive-like lesions are considered to be the most threatening condition characterized by pain, ranging from mild discomfort to severe onset [4]. And pain seriously affects the patient's eating experience and food digestion, reducing the quality of life of patients.

Previous studies have suggested that vitamin D deficiency may be associated with an increased risk of some inflammatory diseases, such as OLP and inflammatory bowel disease[5,6]. OLP patients presented a nearly 50% reduction in mucosal VD levels, which may be caused by immunoreaction[6]. As a ligand-induced transcription factor, VDR(chromosome location 12q12-14) encoded by VDR gene plays an important role in regulating the role of vitamin D [7.8]. Increasing evidence suggests that single nucleotide polymorphisms (SNPs) of vitamin D-related genes could affect the properties of vitamin D, such as its anti-carcinogenic effects[9]. Thus, we speculated that the polymorphisms of the VDR gene may be related to OLP. And since OLP is considered as a potential precancerous lesion, specific SNPs of the VDR or vitamin D pathway genes may also play an important role in oral cancer.

Based on the above, we conducted this study in Chinese Han Population to investigate the association between the key polymorphisms in VDR genes and OLP susceptibility.

Material And Methods

Study groups and samples

A total of 177 patients with OLP were enrolled from the Affiliated Hospital of Stomatology, Nanjing Medical University, Jiangsu Province, China between January 2017 and June 2018 in this study. The inclusion criteria of OLP patients were as follows: (1) > 18 years old; (2) diagnosed as OLP by the oral pathologist via the biopsy specimen; (3) treatment-naive. Pregnant women, patients who have received systemic or topical steroids in the past three months, and who have had autoimmune diseases were excluded. And the control group included 207 healthy subjects who underwent physical examination at the physical examination center and had no oral mucosal lesions, inflammation, infection, and autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). The diagnostic criteria used in this study was a diagnostic guideline developed by van der Meij et al. according to the World Health Organization (WHO) definition of OLP [10].

The oral mucosa of all participants was assessed by two experienced oral clinicians. If there was a disagreement between two examiners, a third clinically experienced mucosal dentist would make the judgment. Main clinical features, including clinical subtype, affected sites, the number of sites, the presence of cutaneous lesions, the type of mouth lesions and symptoms, were collected for further analyses. All subjects were informed of the purpose of the study and signed the informed consent. Information, such as demographic data, alcohol consumption habits and oral hygiene, were collected by one-to-one survey using a questionnaire designed according to our research content. Prior to OLP diagnosis, Participants who drank more than 20 alcohol drinks per week were classified as heavy drinkers[11]. The periodontal status of all subjects including gingival index (GI), periodontal index (PI) and bleeding on probing (BOP) were evaluated in both groups. Oral hygiene of subjects was defined as poor when GI and PI both ≥2, and BOP score was 1. In addition, 10 ml venous blood was collected from each subject for biochemical test and SNPs determination.

DNA isolation and genotyping

Genomic DNA was extracted from peripheral blood samples using protease K digestion and phenol/chloroform purification according to standard protocol. The TaqMan allelic discrimination technology through ABI 7900HT Sequence Detection system (Applied Biosystems, San Diego, California, USA) was used to explore polymorphisms at the chosen SNPs. Polymerase chain reaction (PCR) was executed by the following thermal profile: 50 twarer 2 min to preheat, 95 °C for 10 min to preincubate, then 40 cycles at 95 e following thermal profile: 50 twarer 2 mito anneal. The genotyping results were detected by LightCycler LC480 real-time PCR (roche Diagnostics, mannheim, Germany), with a 100% success rate. Two blank controls were specified to a 384-well format for quality control with a randomly selected 10% of samples as repeat samples, producing 100% concordance.

Information regarding SNPs in VDR was acquired from the NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/SNP) and the Chinese Han population database of HapMap (http://www.hapmap.org). All SNPs were selected according to the following criteria: (1) minor allele frequency (MAF) ≥0.05 in the Chinese population and (2) the *P*-value of the Hardy-Weinberg equilibrium

test was ≥0.05. Tag SNPs were chosen to represent a set of variants with strong linkage disequilibrium (LD) [11]. A total of eight SNPs in VDR gene (rs731236, rs739837, rs757343, rs2107301, rs2239185, rs7975232, rs11574129 and rs11568820) were picked according to the above steps.

Statistical analysis

All analyses were operated in Stata/SE (V.12.0 for Windows). The difference of individual demographic characteristics was analyzed by Student t test or the chi-square (χ^2) test (for categorical variables). The relationship between candidate SNP and OLP risk was estimated by multivariate logistic regression analysis, and the results were expressed as odds ratio (ORs) and its 95% confidence intervals (CIs). The heterogeneity between the corresponding subgroups was examined by *Q* test. The Cochran-Armitage test was used for trend analysis. And haplotype analysis was performed to explore the relationship between two significant SNPS and OLP risks. PHASE software (v2.1) was used to estimate haplotype frequency based on observed genotypes. Single-fold view software (version 4.2) was used to analyze linkage disequilibrium (LD) parameters (i.e., D and r2) [12], and Thesias software (version 3.1) was used to analyze associations of identified haplotypes in the VDR gene with OLP [13].

Results

The demographic information of 177 OLP patients and 207 healthy subjects were shown in Table 1. There was similar distribution of age and gender between the two groups (*P*=0.155 and 0.091, respectively). However, compared with the control group, OLP patients had more alcohol consumption and better oral hygiene (*P* <0.05).

The genotype distribution of these eight SNPs in two groups is described using dominant, recessive and additive genetic models in Table 2. The recessive genetic model computed by logistic regression analyses showed that rs2239185 and rs7975232 were significantly associated with OLP susceptibility. Patients carrying the rs2239185-TT genotype (adjusted OR=2.39, 95%CI=1.10 -5.18, *P* = 0.027) and those with rs7975232-CC genotype (adjusted OR=2.65, 95%CI=1.24 -5.66, *P* = 0.012) tend to have a higher risk of OLP.

Cumulative effects of the two SNPs on OLP were evaluated by comparing the effects among genotypes with different degrees of mutation. The results showed that as the number of mutation increased, the risk of OLP increased (Table 3). The Cochran-Armitage trend test also showed that the OLP susceptibility increased in subjects carrying one or two alleles of rs2239185 and rs7975232 (OR= 2.33, 95% CI=1.22-4.43). In stratified analyses on the combined variant alleles (rs2239185 and rs7975232) and OLP risk susceptibility, no heterogeneity was observed (Table 4).

LD information of the two SNPs is shown in Table 5. We performed haplotype analysis to assess the effect of the haplotype rs2239185 and rs7975232 variant alleles on OLP risk (Table 5). When compared

with the most frequent AC haplotype, CC haplotype was significantly associated with OLP susceptibility (OR= 3.11, 95%CI=1.42-6.83), which was consistent with the single SNP analysis.

To further explore the biological significance of VDR rs2239185 and rs7975232, we also searched for expression quantitative trait loci (eQTL) evidence based on the public GTEx database (https://gtexportal.org/). It is found that VDR rs2239185 and rs7975232 genotype were significantly associated with the expression of VDR in whole blood. Mutations in VDR rs7975232 and rs2239185 would down-regulate of VDR gene expression in whole blood (P = 0.002 and 0.006, respectively, Fig. 1).

Discussion

Though the etiology of OLP is unknown, immunodeficiency, heritable variation, stress, trauma, virus, diabetes, hypertension and Vitamin D deficiency can be considered as one of etiological factors, and

meanwhile which may interact with each other. A family study further emphasized the importance of genetic tendency to OLP susceptibility[14].0.5% to 2.0% of OLP patients can develop a frequent malignant transformation[15].Finding new molecular biomarkers could effectively contribute in the identification of OLP patients with a higher tendency to frequent malignant transformation. This retrospective study aimed to discuss the relationship of environment, clinical features, genetic variation and susceptibility of OLP. In our study, adult females could increase the susceptibility of OLP, which was the same as other studies [16,17].

The effects of Vitamin D and its receptor on the regulation of the immune system as well as calcium phosphorous homeostasis and bone metabolism have obviously been recognized. The function of *VDR* could be affected by *VDR* genetic variants (rs1544410, rs7975232 and rs731236) on modulating the biological effects of vitamin D[18]. Many immune-mediated diseases related to genetic variations in *VDR*, such as tuberculosis [19], systemic lupus erythematosus[20], hepatocellular carcinoma[21]. Recent studies have linked vitamin D deficiency with some immune disorder diseases. Accumulative evidence suggests that vitamin D defificiency is highly prevalent among the general population in China[22]. Several studies have revealed the association between SNPs in the *VDR* gene and prostate cancer (P-Ca) risk in European and Asian populations. Therefore, this studies further revealed the relationships between *VDR* genetic mutations and OLP susceptibility.

Through the recessive model, *VDR* rs2239185 and rs7975232 polymorphism of significant differences in allele and genotype distribution between OLP patients and control group suggested the possible importance in OLP susceptibility. The cumulative effects of *VDR* rs2239185 and rs7975232 in OLP indicated that carrying two unfavorable alleles offered the highest risk effect (P<0.05). One finding in African-American (AA) men showed that, nine *VDR* SNPs were analyzed in a case-control study .The tendency for the number of risk alleles to increase in the haplotype was more associated with prostate cancer risk, which was consisted with our study[23].The heterogeneity test suggested no interaction between different genotypes in different population (P>0.05), which was consistent with previous studies

[24,25]. Cumulative analysis showed the importance of considering the SNPs-SNPs combined with *VDR* gene analysis for OLP susceptibility.

In the current study, TT genotype of *VDR* gene rs2239185 might be one of the potential genetic risk factors for community-acquired pneumonia(CAP), to increase the susceptibility and severity [24]. In our study, LD was found between *VDR* rs7975232 and rs2239185 polymorphisms in both OLP and control groups. Compared with the most frequent haplotype AC, carrying the haplotype CC showed an increased risk of OLP, which was consistent with the single SNP effects. However, the haplotype carrying rs2239185 C expressed a significant risk effect, although the single SNP analysis did not show obvious associations. Thus, effects of rs2239185 and rs7975232 may not be independent, and further fine mapping studies are needed. Recent study found that the distribution of AT and CC haplotypes were significantly different between patients and controls, indicating that *VDR* haplotype had an effect on OLP susceptibility [11]. However, the effect of AT on OLP risk reduction was not found in our study, which might be attributed to the racial difference. But it also underscores again the necessity of further research. Moreover, our findings highlighted the importance of haplotype blocks analysis over individual SNPs approach for complex diseases.

The gene encoding *VDR*, located on chromosome 12q13, consists of nine exons and eight introns, which is about 75 kb in length [26]. Both rs2239185 and rs7975232 are located in an intron of *VDR* gene, which may change the expression and function of *VDR* by regulating gene transcription, messenger RNA (m-RNA) output, and protein translational efficiency [27]. Therefore, the two polymorphisms may alter the expression and function of *VDR*. Rs11574129 (C/T) is located in

the 3' untranslated region (3'UTR) of *VDR* gene. The recent study speculated that the influence of rs11574129 on the secondary structure of *VDR* 3'-UTR mRNA using the RNA fold Web Server, and then found the mutations in the 3'-UTR might affect *VDR* transcription and *VDR* protein level through various pathways including translational control[28].

Various analyses had been used in our study to reveal the potential effect of *VDR* gene polymorphism on OLP susceptibility. But epidemiological evidence here of only two SNPs data (rs2239185 and rs7975232) were insufficient to speculate on the role of VDR gene, indicating that relevant functional studies were needed to confirm our findings and identify the real-acting SNPs. Apart from the ethnic variations, geographical differences and the interaction between *VDR* gene variants and environmental conditions may also differ between populations[29].

Conclusion

This study was the first time to discover that genetic mutations at *VDR* rs2239185 and rs7975232 were associated with OLP susceptibility, which might be the candidate susceptibility region of OLP in Chinese Han population. But larger prospective studies of OLP patients are also necessary, including other markers, serum levels of vitamin D, and comparative studies with other oral inflammatory diseases.

Declarations

Author Contributions

HS, PH, RY and JY participated in the design of the study. HS, QL and GW carried out the surveys and experiments. HS, FZ and LZ performed the statistical analysis.ML, LZ, JW and HF contributed materials and analysis tools. HS wrote the paper. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Informed consent:

All subjects were provided the voluntary informed consent to participate in the study.

Ethical approval:

The name of IRB: Epidemiological study on genetic variation of RLRs family and HCV chronic infection and prognosis

The date of approval:2017,2,23

The approval number: Nanjing Medical University ethical review2017(445)

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Tables

Table 1. Characteristics of clinical data between OLP and control group

Variables	OLP	Control	*P*-value
	(n = 177)	(n = 207)	
Mean age, year	49.20 ± 14.14	48.47 ± 10.88	0.569
< 45	61(34.46)	86(41.55)	0.155
≥ 45	116(65.54)	121(58.45)	
Gender			
Male (%)	49(27.68)	74(35.75)	0.091
Female (%)	128(72.32)	133(64.25)	
Alcohol			
Heavy	11(6.21)	2(0.97)	0.005
Non Heavy	166(93.79)	205(99.03)	
Oral hygiene			
Good	131(74.01)	119(57.49)	0.001
Poor	46(25.99)	88(42.51)	

Abbreviation: OLP, oral lichen planus.

Table 2. Distribution of VDR genotypes between OLP and control group

Genotype	OLP	Control	OR (95% CI)	*P*-value
rs731236				
CC	154(87.01)	180(86.96)	1.00	–
CT	21(11.86)	24(11.59)	0.99(0.51-1.91)	0.979
TT	2(1.13)	3(1.45)	0.93(0.15-6.06)	0.946
Dominant			0.99(0.52-1.85)	0.965
Recessive			0.94(0.15-6.05)	0.947
Additive			0.98(0.57-1.71)	0.954
rs739837				
CC	98(55.37)	112(54.11)	1.00	–
CA	61(34.46)	82(39.61)	0.73(0.45-1.20)	0.218
AA	18(10.17)	13(6.28)	1.36(0.60-3.09)	0.468
Dominant			0.82(0.51-1.31)	0.404
Recessive			1.60(0.73-3.50)	0.242
Additive			0.98(0.69-1.41)	0.927
rs757343				
AA	113(63.84)	128(61.84)	1.00	–
AG	53(29.94)	73(35.27)	0.70(0.43-1.15)	0.163
GG	11(6.21)	6(2.9)	2.06(0.70-6.13)	0.180
Dominant			0.81(0.50-1.29)	0.375
Recessive			2.36(0.81-6.89)	0.117
Additive			0.97(0.66-1.44)	0.893
rs2107301				
CC	83(46.89)	96(46.38)	1.00	–
CT	79(44.63)	93(44.93)	1.03(0.67-1.60)	0.883
TT	15 (8.47)	18 (8.7)	1.19(0.54-2.58)	0.668
Dominant			1.06(0.69-1.61)	0.800
Recessive			1.17(0.55-2.45)	0.686
Additive			1.07(0.77-1.48)	0.706

rs2239185				
CC	90 (50.85)	111(53.62)	1.00	–
CT	62(35.03)	83 (40.10)	0.78(0.47-1.27)	0.315
TT	25(14.12)	13 (6.28)	2.39(1.10-5.18)	0.027
Dominant			1.00(0.63-1.59)	0.995
Recessive			2.68(1.28-5.62)	0.009
Additive			1.24(0.88-1.74)	0.206
rs7975232				
AA	81(45.76)	118(57.00)	1.00	–
AC	70(39.55)	74(35.75)	1.36(0.83-2.23)	0.225
CC	26(14.69)	15(7.25)	2.65(1.24-5.66)	0.012
Dominant			1.57(0.97-2.50)	0.061
Recessive			2.25(1.10-4.58)	0.026
Additive			1.54(1.09-2.18)	0.014
rs11574129				
CC	119(67.23)	146(70.53)	1.00	–
CT	50(28.25)	57(27.54)	0.97(0.59-1.61)	0.921
TT	8(4.52)	4(1.93)	2.49(0.70-8.84)	0.159
Dominant			1.08(0.67-1.75)	0.740
Recessive			2.50(0.71-8.84)	0.154
Additive			1.17(0.78-1.76)	0.439
rs11568820				
AA	61(34.46)	74(35.75)	1.00	–
AG	81(45.76)	96(46.38)	1.10(0.69-1.76)	0.695
GG	35(19.77)	37(17.87)	1.26(0.69-2.28)	0.454
Dominant			1.14(0.74-1.78)	0.551
Recessive			1.19(0.70-2.03)	0.520
Additive			1.12(0.83-1.50)	0.455

Logistic regression analyses adjusted for age, gender, alcohol, oral hygiene.

Abbreviation:VDR,vitamin D receptor ;OLP, oral lichen planus; OR, odds ratio; CI, confident interval.

Table 3. Cumulative Effects of rs2239185 and rs7975232 on OLP risk

Variables	OLP n(%)	Control n(%)	OR(95%CI)	*P*-value
0	145 (81.92)	188 (90.82)	1.00	-
1	13 (7.34)	10 (4.83)	1.82 (0.74-4.48)	0.190
2	19 (10.73)	9 (4.35)	2.87 (1.21-6.81)	0.017*
Trend				*P* a=0.007
0	145 (81.92)	188 (90.82)	1.00	-
1-2	32 (18.08)	19 (9.18)	2.33 (1.22-4.43)	0.010

Variables are numbers of combined unfavorable alleles (rs2239185-TT and rs7975232-CC). Logistic regression analyses adjusted for age, gender, alcohol consumption and oral hygiene. *Pa*-value was analyzed by Cochran-Armitage trend test.

Abbreviation: OLP, oral lichen planus; OR, odds ratio; CI, confident interval.

Table 4. Stratified analyses on combined variant alleles (rs2239185 and rs7975232) and OLP risk

Variables	OLP risk (0 vs.1-2)		OR (95%CI)	*P a*	*P b*
	OLP	**Control**			
Age, year					
≥ 45	50/11	75/11	1.69 (0.60-4.71)	0.318	0.457
< 45	95/21	133/8	3.13 (1.28-7.65)	0.012	
Gender					
Male	40/9	58/16	0.61 (0.21-1.72)	0.347	0.267
Female	105/23	130/3	10.11 (2.82-36.30)	<0.001	
Alcohol					
Heavy	8/3	1/1	–	–	–
Non Heavy	137/29	187/18	2.42 (1.25-4.67)	0.008	
Oral hygiene					
Good	109/22	103/16	1.59 (0.75-3.37)	0.231	0.453
Poor	36/10	85/3	6.47 (1.56-26.90)	0.010	

Logistic regression was used in the implicit model to determine the adjusted *P a* value according to age, gender, alcohol consumption and oral hygiene, heterogeneity was used to test

P b-value.

Abbreviation: OLP, oral lichen planus; OR, odds ratio; CI, confident interval.

Table 5. Haplotypes analysis of rs2239185 and rs7975232 with OLP risk

Haplotype	**OLP n(%)**	**Control n(%)**	OR	*P*
AC	218 (61.59)	295 (71.26)	1.00	1.00
CT	98 (27.68)	94 (22.70)	1.38 (0.96-1.97)	0.084
CC	24 (6.78)	10 (2.42)	3.11 (1.42-6.83)	0.005
AT	14 (3.95)	15 (3.62)	1.21 (0.55-2.66)	0.633

Logistic regression analyses adjusted for age, gender,alcohol consumption and oral hygiene

SNPs order: rs2239185 and rs797523

Figures

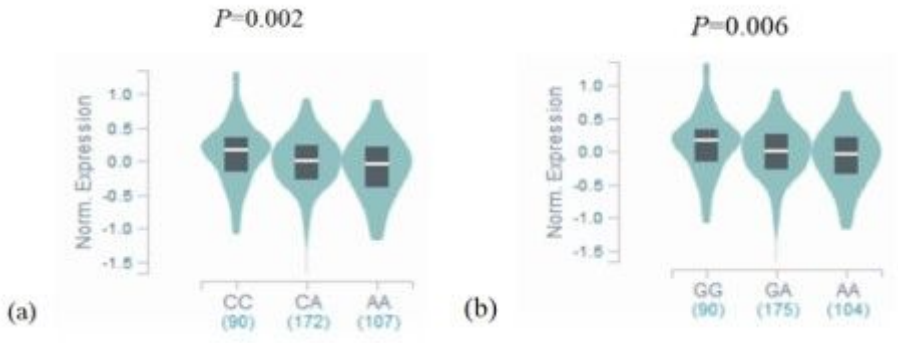

Figure 1

(a). Results of eQTL analysis on VDR rs7975232 loci. in whole blood. (b). Results of eQTL analysis on VDR rs2239185 loci. in whole blood.