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Association Between Single Nucleotide Polymorphisms in the Vitamin D Receptor and Incidence of Dry Eye Disease in Chinese Han Population

Authors' Contribution:

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Data Interpretation D
Manuscript Preparation E
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Background: Dry eye disease (DED) is a chronic dysfunction of the ocular surface and has become an important public problem. Vitamin D receptor (*VDR*) gene polymorphism has been found to be associated with different kinds of diseases. The relationship between single nucleotide polymorphisms (SNPs) in the *VDR* gene should be studied.





Material/Methods: In the present case-control study, we investigated the association of *VDR* gene polymorphism with DED risk. Clinical data including age, gender, body mass index (BMI, kg/m²), smoking history, diabetes, and blood pressure were recorded. Serum 25-hydroxy vitamin D (25[OH]D) was chosen as the main parameter that reflected the level of vitamin D. We identified SNPs of *VDR* gene *Apa-1*, *Bsm-1*, *Fok-1*, and *Taq-1* in both DED cases and healthy controls.

Results: A total of 124 DED cases and 135 healthy controls were included in this study. It was reported that aa in *Apa-1* (OR=2.803, 95% CI, 1.350–5.820) and tt in *Taq-1* (OR=0.362, 95% CI, 0.141–0.930) were associated with increased the risk of DED. Analysis of the allele frequencies of *VDR* gene polymorphisms among DED patients and healthy controls showed that allele differences in *Apa-1* were significantly associated with higher risk.

Conclusions: SNPs of *VDR* gene (*Apa-1* and *Taq-1*) were associated with the risk of DED. No significant association of *Bsm-1* and *Fok-1* in *VDR* gene demonstrated significant effect in the incidence of DED. Thus, we found that several SNPs of *VDR* gene could provide significant pathogenic effects in the risk of DED.

MeSH Keywords: **Case-Control Studies • Dry Eye Syndromes • Mediator Complex Subunit 1 • Polymorphism, Single Nucleotide**

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Background

Tears with sufficient quality, a tear film of normal composition, and regular blinking are prerequisites for moisturizing the ocular surface, thereby maintaining normal visual function and protecting the ocular surface from dryness. The tear film and ocular surface tissue constitute a complex and stable system, but it can be destroyed by a variety of factors (including ocular surface inflammation, sexual dysfunction imbalance, and neurological disorders), leading to dry eye syndrome (DED). DED is a common and complex condition with reduced ocular comfort and impaired visual performance, and has been reported to be a one of the most common ocular disorders throughout the world. The incidence rate of DED is around 25% and varies among different age groups [1,2]. Although there is a high incidence of DED, the detailed pathogenesis remains unclear. The generally accepted viewpoint is that DED is a multifactorial disease with the presentation of chronic immunologic processes, ocular surface damage, and neurosensory abnormalities [3,4]. Combined genetic, metabolic, and environmental factors all contribute to the incidence and development of DED, and the knowledge of the genetic contributions in the DED could help in the management of DED.

A study showed that Spdef is required for conjunctival goblet cell differentiation and Spdef^{-/-} mice, which lack conjunctival goblet cells distribution, demonstrated a phenotype consistent with dry eye with significantly increasing corneal surface fluorescein staining and tear volume [4]. Single nucleotide polymorphisms (SNPs) demonstrate important regulation effect of incidence of different diseases [5,6]. A study based on the tissue samples from dry eye patients and normal controls showed that *MUC1* SNP genotype frequency of the normal control group was statistically different from non-Sjögren's aqueous-deficient dry eye group using ocular surface staining [7]. There is growing evidence showing the effect of vitamin D in the development of DED. Result from the Study Group for Environmental Eye Disease report showed that higher serum vitamin D levels were not associated with a significantly reduced risk of DED in the crude analysis or in the adjusted analysis, and the findings support previous reports that serum vitamin D levels were not associated with DED [8]. While in a study based on 24 DED cases and 21 healthy controls, it was found that vitamin D deficiency might be associated with DED in non-Sjögren's syndrome cases [9]. In our previous case control study that included 70 DED cases and 70 healthy controls, it was found that there was a significant association between serum 25(OH)D level and DED incidence [10]. So, it is reasonable to conjecture that vitamin D processing and vitamin D receptor might be associated with the development of DED. There are different SNP locations in the *VDR* gene, and an association between *VDR* SNPs and risk of different diseases. Currently, the evidence for the association between *VDR* SNPs

and DED cases remains limited. In our study, we selected 4 of the most commonly reported SNP sites (rs7975232, rs1544410, rs2228570, and rs731236) and detected their mutations and the associated risk of DED.

In this study, we aimed to investigate the genetic variations in the *VDR* SNPs and their effect on the development of DED. In addition, the frequencies of these variants are described in Chinese Han ethnic groups.

Material and Methods

Study participants and ethical approval

This case-control perspective study was conducted in Department of Ophthalmology, Changshu No 2 People's Hospital. A total of 124 DED patients and 135 matched controls were included in this study. All the DED patients were diagnosed from January 2017 to August 2018 at the outpatient clinic of the Department of Ophthalmology, Changshu No. 2 People's Hospital. All the DED cases included in this study met the diagnostic criteria defined according to the 2016 Diagnostic Criteria from the Asia Dry Eye Society [11]. In general, the diagnosis of DED is based on only 2 positive items, subjective symptoms (ocular surface disease index [OSDI] score was used in this study) and decreased tear breakup time (TBUT) of less than 5 seconds. Also, the Schirmer test was conducted and the results recorded for the data analyses. The control group was patients without DED in the same time period.

The inclusion criteria of the DED case group were as follows: 1) diagnosed as DED in our hospital; and 2) signed the written informed consent. The exclusion criteria were as follows: 1) previous ocular surgical history within the year; 2) mental disorder or serious systemic disease, such as cancer, hematological system disease, or hyperthyroidism; 3) immune diseases, including Sjogren syndrome, rheumatoid arthritis, lupus erythematosus, or any other immune diseases; 4) use of any ophthalmic eye drops or contact lenses within recent 1 month, eyelid or eyelash abnormalities, nasolacrimal apparatus abnormalities glaucoma, uveitis, retinal hemorrhage, or optic neuritis; and 5) unable to co-operate in the required study procedures. The participants would be excluded when they were found to meet any of the aforementioned exclusion criteria during the study.

Written informed consent was obtained from all study participants. Ethical approval of this current study was obtained from the Human Research Ethics Committee of Changshu No. 2 People's Hospital. All experiments were carried out in compliance with the Declaration of Helsinki.

Table 1. Clinical characteristics in dry eye disease patients and normal controls.

	DED group (n=124)	Control group (n=135)	P value
Age (years)	51.4±12.8	52.3±13.6	0.585
Gender			
Male	59	66	0.466
Female	65	69	
BMI (kg/m ²)	22.9±5.1	23.1±5.6	0.765
Smoking	41 (33.1%)	52 (38.5%)	0.217
Diabetes	39 (31.5%)	33 (24.4%)	0.132
SBP (mmHg)	129.4±24.2	127.9±19.8	0.584
DBP (mmHg)	76.6±12.1	79.7±11.6	0.036
Fluorescein TBUT (s)	3.3±1.9	10.4±3.1	<0.001
Schirmer test (mm/5 min)	9.2±4.1	14.3±5.7	<0.001
OSDI (points)	45.3±16.8	12.4±5.5	<0.001

DED – dry eye disease; BMI – body mass index; SBP – systolic blood pressure; DBP – diastolic blood pressure; TBUT – tear breakup time; OSDI – ocular surface disease index.

DNA extraction and genotyping

The blood samples were obtained from all the DED cases and the healthy controls. The DNA extraction of whole blood was performed using DNeasy blood and tissue kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. The extracted DNA samples were quantitated by ultraviolet (UV) absorption at 260 and 280 nm and stored at –20°C until analyzed.

Polymerase chain reactions (PCRs) were conducted with 25 ng of genomic DNA as a template in a mixture of PCR buffer, 0.4 pmol of each primer, 200 nM dNTPs, 2.5 mM MgCl₂, and 0.75 units of Taq polymerase (Solgent, Daejeon, Korea). PCR products were then restricted with 10 units of TaqI enzyme through incubating at 65°C for 30 minutes and resolved in 2% agarose gels to visualize restrictions fragments. After that the genotyped SNP was observed in Hardy-Weinberg equilibrium in both DED cases and in healthy controls.

Clinical data collection

We collected clinical data, including body mass index (BMI, kg/m²), tobacco smoking history, diabetes, and blood pressure. The BMI and blood pressure were detected by a trained staff, while tobacco smoking history and diabetes status were obtained through the questioning of the participants or reviewing their medical records. An investigator was trained to measure the OSDI quantitation. The OSDI scale included 12 questions, which covered 3 major aspects (ocular symptoms, visual function, and environmental stimulation). Detailed explanations of each major point were used to reach a relative

accurate conclusion. The DED-related examinations were evaluated after a full ophthalmologic examination was performed. All the ocular surface examinations were performed by 2 investigators (MYF and LJ). For quality control, 5% of samples were re-genotyped in a blinded fashion.

Statistical analysis

All the SNPs indicated in this study were assessed for Hardy-Weinberg equilibrium (HWE) by a hi-square (χ^2) test in both groups (cases and controls) with HaploView 4.0 software. Allelic and genotypic frequencies were estimated for all the indicated VDR polymorphisms (rs7975232, rs1544410, rs2228570, and rs731236) and inheritance models were constructed and assessed for the polymorphisms. The quantitative variables were present as mean \pm standard deviation (SD). Data analyses were conducted using SPSS 18.0 (SPSS, Inc., Chicago, IL, USA). Comparisons of the means between the 2 groups were determined by grouped *t*-test. The chi-square (χ^2) test or Fisher's exact test was employed for categorical variables. A *P* value <0.05 was considered statistically significant.

Results

Characteristics of the study population

The clinical characteristics of all the included DED cases and healthy controls are presented in Table 1. In this current case-control study, a total of 124 DED cases (59 males and 65 females) and 135 matched controls (66 males and 69 females)

Table 2. Primer sequences with annealing temperature and restriction enzymes used for genotyping.

VDR polymorphism	Enzyme for RFLP	Primer sequence, 5'-3'	PCR products (bp)
rs7975232	<i>Apa-1</i>	F: TGGGCACGGGATAGAGAAG R: ACGGAGAAGTCACTGGAGGG	630
rs1544410	<i>Bsm-1</i>	F: AACCTGAAGGGAGACGTAGCA R: TTGTACCCTGCCCGCAAGAAA	348
rs2228570	<i>Fok-1</i>	F: ACCAAGGATGCCAGCTGG R: GCTTCTTCCCTCCCTTTC	267
rs731236	<i>Taq-1</i>	F: TGGTGGGATTGAGCAGTGAG R: GTACTGCTTGGAGTGCTCCT	630

were included. There was no difference between BMI or blood pressure in the DED group and the control group ($P>0.05$). When smoking history and diabetes status was considered, there were no statistically significant differences between the DED group and the control group ($P>0.05$). As TBUT is part of the diagnoses of DED, a significantly reduced TBUT was detected in the DED cases (DED group, 3.3 ± 1.9 seconds compared to the control group, 10.4 ± 3.1 seconds, $P<0.001$). The subjective symptoms were also an important part in the diagnoses of DED, and the OSDI score was used in this study. Compared with the control group, the mean OSDI value was significantly higher in the DED group ($P<0.001$). The Schirmer I test was also considered an important index for the DED, and there was a significant difference between the DED group (9.2 ± 4.1 mm/5 minutes) and the control group (14.3 ± 5.7 mm/5 minutes, $P<0.001$) when the Schirmer I test was considered.

Genotyping of VDR SNPs

We reported on 4 SNP sites for VDR: rs7975232 (*Apa-1*), rs1544410 (*Bsm-1*), rs2228570 (*Fok-1*) and rs731236 (*Taq-1*). The genotyping data are present in Table 2. In the present study, the VDR genotype frequencies were in agreement with the Hardy-Weinberg equilibrium (all $P>0.05$). The frequency distribution of genotypes, different models, and association analysis are described in Table 3. A significant difference in the distribution of *Apa-1* aa (OR=2.803, 95% CI, [1.350–5.820], $P=0.004$) and *Taq-1* position tt (OR=0.362, 95% CI, [0.141–0.930], $P=0.025$) between DED patients and healthy controls. While it was found that there was no significant association between *Bsm-1* and *Fok-1* genotype with the incidence of DED comparing with the normal controls. When the mode of inheritance was recessive, the OR (95% CI) for aa versus AA+Aa in *Apa-1* was 2.456 (1.303–4.629, $P=0.004$) and OR (95% CI) for tt versus TT+Tt in *Taq-1* was 0.365 (0.148–0.902, $P=0.019$). When the mode of inheritance was dominant, no significant association was detected in neither *Apa-1* (OR=0.645, 95% CI, [0.376–1.107], $P=0.072$) or *Taq-1* (OR=1.196, 95% CI,

[0.730–1.960], $P=0.280$) locates. No significant difference was observed in the any inheritance mode of *Bsm-1* and *Fok-1* SNP genotypes between DED and healthy controls.

Allelic frequencies of VDR SNPs

We also conducted advanced studies about the allelic frequencies of the 4 VDR gene polymorphism in 124 DED patients and 135 healthy controls (Table 4). For the VDR gene, the allelic distribution of *Apa-1* (rs7975232) was different between the DED patients and the controls ($P=0.005$). No significant association between other allelic frequencies of VDR SNPs (*Apa-1*, *Bsm-1*, and *Fok-1*) was detected in this study.

Discussion

DED is a chronic dysfunction of the ocular surface marked by persistent discomfort symptoms, including pain, irritation, and burning. Age, female sex, low humidity environments, systemic medications, and gland dysfunction were reported to be common risk factors for DED [12,13]. Recent reports have focused on SNPs and their genetic effect on the incidence of different diseases [14], including DED. In this case-control study, we identified multiple SNPs in VDR genes (rs7975232[*Apa-1*], rs1544410[*Bsm-1*], rs2228570[*Fok-1*] and rs731236[*Taq-1*]) and detect their roles in the incidence of DED. We found that aa in *Apa-1* and tt in *Taq-1* were associated with increased with the risk of DED. Analysis of the allele frequencies of VDR gene polymorphism among DED patients and healthy controls showed that allele differences in *Apa-1* was significantly associated with higher risk. It was an important finding of this study that the genetic variation in VDR gene was a potential risk factor for DED cases.

Vitamin D is known to be important in the regulation of different biological progresses such as oxidative stress, metabolic regulation, and organ development [15,16]. It was also reported

Table 3. Genotype frequencies of VDR gene polymorphisms in the studied groups and the incidence with DED.

Genotype	DED n (%)	Control n (%)	OR (95% CI) DED vs. control	P value
<i>Apa-1</i> (rs7975232)				
AA (TT)	31 (25.0)	46 (34.1)	1.00 (reference)	
Aa (TG)	59 (47.6)	71 (52.6)	1.233 (0.696 to 2.184)	0.283
aa (GG)	34 (27.4)	18 (13.3)	2.803 (1.350 to 5.820)	0.004
AA vs. Aa+aa			0.645 (0.376 to 1.107)	0.072
aa vs. AA+Aa			2.456 (1.303 to 4.629)	0.004
<i>Bsm-1</i> (rs1544410)				
BB (GG)	58 (46.8)	66 (48.9)	1.00 (reference)	
Bb (GA)	61 (49.2)	64 (47.4)	1.085 (0.660 to 1.783)	0.423
bb (AA)	5 (4.0)	5 (3.7)	1.138 (0.314 to 4.132)	0.550
BB vs. Bb+bb			0.919 (0.564 to 1.497)	0.415
bb vs. BB+Bb			1.092 (0.309 to 3.869)	0.571
<i>Fok-1</i> (rs2228570)				
FF (CC)	60 (48.4)	62 (45.9)	1.00 (reference)	
Ff (CT)	56 (45.2)	59 (43.7)	0.980 (0.589 to 1.634)	0.522
ff (TT)	8 (6.4)	14 (10.4)	0.590 (0.231 to 1.508)	0.191
FF vs. Ff+ff			0.953 (0.580 to 1.566)	0.474
ff vs. FF+Ff			0.596 (0.241 to 1.474)	0.183
<i>Taq-1</i> (rs731236)				
TT (TT)	55 (44.4)	54 (40.0)	1.00 (reference)	
Tt (TC)	62 (50.0)	62 (45.9)	0.981 (0.587 to 1.642)	0.524
tt (CC)	7 (5.6)	19 (14.1)	0.362 (0.141 to 0.930)	0.025
TT vs. Tt+tt			1.196 (0.730 to 1.960)	0.280
tt vs. TT+Tt			0.365 (0.148 to 0.902)	0.019

DED – dry eye disease.

Table 4. Allele frequencies of VDR gene polymorphism among dry eye disease patients and healthy controls.

VDR polymorphism	Allele	DED (n=124)	Control (n=135)	P value
<i>Apa-1</i> (rs7975232)	A(T)	121	163	0.005
	a(G)	127	107	
<i>Bsm-1</i> (rs1544410)	B(G)	177	196	0.416
	b(A)	71	74	
<i>Fok-1</i> (rs2228570)	F(C)	176	183	0.245
	f(T)	72	87	
<i>Taq-1</i> (rs731236)	T(T)	172	170	0.075
	t(C)	76	100	

DED – dry eye disease.

that vitamin D deficiency was associated with the risk of different kinds of diseases, including different cancers, postpartum depression, and Parkinson's disease [15,17,18]. As a common related factor, vitamin D was reported to be associated with the risk of DED. The results reported from the Study Group for Environmental Eye Disease showed that higher serum vitamin D levels were associated with a non-significantly reduced risk of DED [8]. Another study showed that the effects of topical eye drops were dependent on serum 25(OH)D levels [19]. Cholecalciferol supplementation enhanced the efficacy of topical treatment and may be a useful adjuvant therapy for patients with DED refractory to topical lubricants. Our group conducted a previous case-control study that found that vitamin D deficiency was more common in the DED cases. In another study, it was found that there were statistically significant associations between serum 25(OH)D levels and the Schimer test, TBUT, and OSDI scales [10]. In our study, we tried to explore the association between *VDR* SNPs and the risks of DED incidence. As reported in previous studies, vitamin D is quite important in immune function [20], and immune dysfunction plays a key role in the incidence of DED, therefore, abnormal expression of vitamin D might regulate the incidence of DED. DED was also found to be secondary to some autoimmune disease, including primary Sjögren's syndrome. In our current study, we excluded the DED cases secondary to primary Sjögren's syndrome to avoid the influence of immune system disorders.

VDR is a nuclear hormone receptor and transcription factor that is expressed in a variety of tissues including the intestines, adipose tissue, and liver, as well as modulates metabolic and immune system processes [21,22]. There have also been reports on SNP in *VDR* gene associated with the incidence of different disorders. In a case-control study with acute pancreatitis patients (n=129) and an alcohol-abuse control group (n=110), it was found that polymorphism *Taq-1* occurred in the vitamin D receptor [23] and had an impact on the development of acute pancreatitis due to the lack of the protective role of vitamin D. There was also a synonymous SNP rs731236 in the *VDR* gene that has been reported in another study to be associated with a higher risk of Crohn's disease [24]. Other studies have detected the effect of SNPs in *VDR* gene on the risk of DED. One case-control study with 64 DED cases and 51 controls, found

that 2 SNPs (*Fok-1* and *Apa-1*) in the *VDR* gene also varied between DED cases and controls [25]. In an advanced stratified study, it was reported that among patients with depression, DED cases were 3.93 times more likely to have the minor allele A of the *Val66Met* SNP compared to controls. The site *Apa-1* (rs7975232) was also detected in our study, while it should be noted that *Fok1rs* was not associated with risk of DED. The differences between these 2 studies might be caused by racial difference in the study populations. Importantly, we choose the 2016 Diagnostic Criteria from the Asia Dry Eye Society to use as our study diagnostic criteria. The difference in the diagnostic criteria might be associated the differences in outcomes comparing previous studies.

The most important finding of this current study was the significant association of the *Apa-1* and the *Taq-1* SNP in the *VDR* gene with the incidence of DED. Additionally, the results of allelic frequencies of *VDR* SNPs showed that allelic difference of *Apa-1* (rs7975232) were significantly associated with the risk of DED cases comparing with the controls. More samples (a total of 124 DED cases and 135 healthy controls) were included in this study, which might be helpful in the detection of potential significantly important targets. It is also important to detect the expression pattern of vitamin D in cases with different *VDR* genotypes in future studies, as was done in previous studies by Jia et al. [26] and Rivera-Parivera-Paredes et al. [27].

Conclusions

Our present study demonstrated that SNPs of *VDR* gene (*Apa-1* and *Taq-1*) were associated with the risk of DED. No significant association of *Bsm-1* and *Fok-1* in *VDR* gene demonstrated a significant effect in the incidence of DED. Thus, SNPs of *VDR* gene could provide significant pathogenic effects in the risk of DED. In future studies, the levels of vitamin D and *VDR* mRNA expression in the DED cases and their relationship with DED patient's clinical pathology characteristics should be explored.

Conflict of interest

None.

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