# Single Nucleotide Polymorphisms in the BDNF, VDR, and DNASE 1 Genes in Dry Eye Disease Patients: A Case-Control Study

Joelle A. Hallak,[1,2] Sapna Tibrewal,[1] Neil Mohindra,[1] Xiaoyi Gao,[3] and Sandeep Jain[1]

[1]Corneal Neurobiology Laboratory, Department of Ophthalmology and Visual Sciences, University of Illinois at Chicago, College of Medicine, Chicago, Illinois, United States
[2]Quantitative Scientific Solutions, LLC, Arlington, Virginia, United States
[3]Quantitative Ocular Genomics Laboratory, Department of Ophthalmology and Visual Sciences, University of Illinois at Chicago, College of Medicine, Chicago, Illinois, United States

Correspondence: Joelle A. Hallak, Department of Ophthalmology and Visual Sciences, University of Illinois at Chicago, 1855 W. Taylor Street, Chicago, IL 60612, USA; joelle@uic.edu.

Submitted: April 7, 2015
Accepted: August 13, 2015

Citation: Hallak JA, Tibrewal S, Mohindra N, Gao X, Jain S. Single nucleotide polymorphisms in the BDNF, VDR, and DNASE 1 genes in dry eye disease patients: a case-control study. *Invest Ophthalmol Vis Sci*. 2015;56:5990–5996. DOI:10.1167/iovs.15-17036

**Purpose.** To identify single nucleotide polymorphisms (SNPs) in the brain-derived neurotrophic factor (*BDNF*), vitamin D receptor (*VDR*), and *DNASE1* genes that may be associated with dry eye disease (DED), and determine whether this association varies by the presence of depression.

**Methods.** A case-control study was performed with 64 DED cases and 51 controls. We collected 2 mL of saliva following a routine eye exam. Genotyping was performed using both custom and predesigned TaqMan SNP genotyping assays for 12 hypothesized SNPs. Genotype and allele frequencies of cases and controls were evaluated. Odds ratios were calculated for allele frequencies. Stratified analysis was performed to determine if the association between SNPs and DED varied by depression status.

**Results.** A total of 18% of cases had the minor allele A of Val66Met (rs6265) SNP in the *BDNF* gene compared with 9% of the controls ($P = 0.05$). Odds ratio was 2.22. Two SNPs (FokI-rs2228570 and ApaI-rs7975232) in the *VDR* genes also varied between DED cases and controls. Cases were 1.72 and 1.66 times more likely to have the minor allele A in rs2228570 and rs7975232, respectively, than controls ($P = 0.06$ for both). While not statistically significant, 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.

**Conclusions.** This pilot study showed that Val66Met in the *BDNF* gene and two SNPs, FokI and ApaI, in the *VDR* gene may potentially be associated with DED. Additionally, the association between DED and Val66Met may vary by depression status.

Keywords: dry eye, polymorphisms, genetics, depression

Dry eye disease (DED) is a complex multifactorial common phenotype resulting from interactions of genetic and nongenetic factors, with prevalence in adult populations ranging from 5% to over 35% at various ages.[1] Despite this high prevalence, the causes of DED are not understood. Common symptoms of DED patients include pain, irritation, itching, burning, and grittiness. The clinical research is complicated by the lack of correlation between symptoms and clinical signs. Epidemiologic studies have identified numerous exposures—including medication use, hormonal changes, environmental exposures, and neural alterations—to be associated with DED and its symptoms. Additionally, recent studies have reported an association between depression and DED,[2–7] post-traumatic stress disorder and dry eye,[4] and anxiety and dry eye.[5,7] Contrary to the identification of lifestyle factors, genetic factors contributing to the pathogenesis of DED have yet to be elucidated.

Studies have shown that genes have a contributory role in DED. Vehof et al.[8] have demonstrated that there is a heritability of approximately 30% for DED symptoms and 40% for a DED diagnosis, and a varying heritability of 25% to 80% for DED in a cohort of British middle-aged and elderly female twins. Gene studies with DED have included some small candidate gene studies and some genome wide association studies (GWAS) on Sjogren syndrome.[9,10] The candidate gene studies have identified polymorphisms in interleukin proinflammatory cytokine genes,[11] and killer cell immunoglobulin-like receptor and human leukocyte antigen-C.[12] However, results have not been replicated. Burbelo et al.[13] in a review summarized the genetic findings from GWAS associated with Sjogren and described the disease relevance of newly identified genes and their corresponding pathways. More than 15 robust susceptibility loci have now been associated with Sjogren. Many of these risk genes play important roles in immune activity and many are often shared with systemic lupus erythematosus (SLE).[3] Despite these findings, the associations have been weak. One explanation is that possible interaction with other factors may be important for triggering DED. Studies are needed to evaluate hypothesized genes, especially between the association of DED and mental health as this association has a major effect on treatment decisions. The mechanism by which DED and psychiatric disorders, such as depression, are correlated

has yet to be determined. Looking at specific polymorphisms may shed light on identifying biological underpinnings between DED and depression. This hypothesis-based gene study looked at the brain-derived neurotrophic factor (*BDNF*), vitamin D receptor (*VDR*), and *DNASE1* genes. The hypothesis was generated to cover the three main gland/tissues of DED: trigeminal ganglion (BDNF), main accessory and lacrimal gland (DNASE1), and meibomian gland (VDR).

Brain-derived neurotrophic factor is a member of the neurotrophin family and is widely expressed throughout the central nervous system. Serum levels of BDNF have been shown to be higher in patients with primary Sjogren syndrome as compared with controls.[14] Additionally, findings from studies support a complex and functional role of BDNF in depression and antidepressant action.[15,16] The genes *DNASE1* and *VDR* are also included in our study as they are hypothesized to play roles in the pathogenesis of diseases that are associated with DED and depression. Studies have shown that DNASE 1 is associated with SLE.[17,18] We have shown that extracellular DNA (eDNA) production and clearance mechanisms are dysregulated in DED.[19] In patients with severe DED, tear fluid nuclease deficiency allows eDNA, neutrophils, and neutrophil extracellular traps to accumulate in the precorneal tear film and cause ocular surface inflammation. Therefore, it is reasonable to explore single nucleotide polymorphisms (SNPs) in the *DNASE1* gene that may be involved in the pathogenesis of DED and depression. Also vitamin D receptor gene SNPs have been investigated in the risk of SLE,[20] and as with similar autoimmune diseases, SLE has been associated with both depression and DED.[21,22]

The purpose of this case-control study was to identify SNPs in the *BDNF*, *VDR*, and *DNASE1* genes that may be associated with DED. We also determined the association and interaction between SNPs and depression. Identifying these SNPs will allow us to examine a common biological mechanism between DED and depression and will move us a step closer to making more informed treatment decisions.

## Methods

### Study Overview

Study approval was obtained from the institutional review board of the University of Illinois at Chicago. Subjects were enrolled and written informed consent was obtained from all patients after the nature and possible consequences of research were explained. Research was conducted in accordance with the requirements of the Health Insurance Portability and Accountability Act and tenets of the Declaration of Helsinki. Saliva was collected from eligible DED patients (cases) and non-DED patients (controls). Sociodemographic data and psychological and medication history were obtained by chart review. All subjects included in our study were aged >18 years.

### Study Population

Sixty-four patients were recruited from our dry eye clinic at the Department of Ophthalmology and Visual Sciences, University of Illinois at Chicago. The sample of DED included established patients of DED who visited our clinic between November 2012 and June 2014. The diagnostic criteria were: (1) a reporting of symptoms: burning sensation, irritation, grittiness or foreign body sensation, light sensitivity, pain, dryness, soreness, or discomfort in the eye; (2) a Schirmer value of <10 mm/5 minute in either eye using Whatman filter strips #41 (Haag-Streit, Essex, UK); or (3) positive corneal staining and/or Rose Bengal corneal and conjunctival staining of >1.

Fifty-one control patients who visited our general eye clinic with refraction-related complaints were recruited to the study. The inclusion criteria included no significant symptoms of DED, a Schirmer value of >10 mm/5 min, and no corneal staining. None of the control subjects enrolled were using tear supplements.

### Selection of Polymorphisms

We selected SNPs from the *BDNF*, *VDR*, and *DNASE*1 genes. The brain-derived neurotrophic factor gene is located on human chromosome 11 (11p13) and contains 11 exons. This gene may play a role in the regulation of stress response and in the biology of mood disorders.[23] Multiple transcript variants encoding distinct isoforms have been described for this gene. Decreased BDNF levels in humans have been associated with the met allele of *BDNF* Val66Met polymorphism (rs6265). Identified in codon 66 of the *BDNF* gene, this SNP causes the substitution of methionine (Met) for valine (Val) (Val66Met). This specific SNP (Rs6265) was included in our study.

*Vitamin D receptor* gene is located on human chromosome 12 (12q13.11) and contains 11 exons. It encodes the nuclear hormone receptor for vitamin D3. The receptor belongs to the family of trans-acting transcriptional regulatory factors and shows sequence similarity to the steroid and thyroid hormone receptors. Downstream targets of this nuclear hormone receptor are principally involved in mineral metabolism though the receptor regulates a variety of other metabolic pathways, such as those involved in the immune response and cancer.[24] Several studies have demonstrated the role of VDR SNPs in the development of SLE and its clinical manifestations, which includes DED. The presence of the VDR FokI (rs2228570), BsmI (rs1544410), ApaI (rs7975232), and TaqI (rs731236) SNPs have been investigated in the association with SLE and other autoimmune diseases.[20] These same 4 SNPs were included in our study.

The gene *DNASE1* is located on human chromosome 16 (16p13.3) and contains 14 exons. This gene encodes a member of the DNase family. At least six autosomal codominant alleles have been characterized. Dry eye disease frequently accompanies autoimmune diseases. Data from our laboratory has shown that qualitative or quantitative deficiency of DNASE1 may be associated with DED inflammation.[19] Mutations in this gene have been associated with autoimmune diseases.[25] A biochemical and genetic study by Yasuda et al.[26] on all non-synonymous SNPs of the *DNASE1* gene relevant identified some of these SNPs. As such, the following SNPs from the *DNASE1* gene were included for analysis: rs8176927, rs34923865, rs8176919, rs1799891, rs1053874, rs8176920, and rs34186031.[26]

### Saliva and Genotyping

We collected 2 mL of saliva, following a routine eye exam, in a saliva collection kit (Oragene Sample DNA collection kit; DNA Genotek, Inc., Ontario, Canada) and transported to the laboratory. The kit is pretreated for DNA stabilization, extraction, and purification. In brief, saliva samples were incubated overnight at 50°C to release cellular DNA and to inactivate nucleases. Samples were then incubated with a purifier (Oragene-Purifier; DNA Genotek, Inc.) and centrifuged to precipitate and pellet various impurities from the sample. The aqueous phase was then transferred to fresh tubes. We precipitated DNA present in the aqueous phase using 95% to 100% ethanol and pelleted. Supernatant was removed and the DNA pellet was washed using 70% ethanol. After ethanol wash, the DNA pellet was air-dried to remove residual ethanol and then resuspended in TE Buffer and stored long term at −20°C. Nucleic acid concentration was determined using a commer-

Investigative Ophthalmology & Visual Science

**Table 1.** SNPs and Their Primers Used for Genotyping

| Gene | RS No. | Alleles | Primers for PCR Amplification (5′–3′) |
|---|---|---|---|
| *BDNF* | rs6265 | G/A | F: ACTCTGGAGAGCGTGAATGG<br>R: ACTACTGAGCATCACCCTGGA |
| *DNASEI* | R-21S (rs8176927) | G/T | F: GCCAGCTGTTTGGCTTTCTGGA<br>R: CAGCGCCCCCAGCAGCTTCAT |
| *DNASEI* | Y95S (rs34923865) | A/C | F: AGGTGTCTGCGGTGGACAGGT<br>R: GTGTGTGACACAGGCATTCCA |
| *DNASEI* | R105G (rs8176919) | G/A | F: CAGGTGTCTGCGGTGGACAGC<br>R: GTGTGTGACACAGGCATTCCA |
| *DNASEI* | P132A (rs1799891) | C/G | F: GCTGACATGGTGACTGAACCT<br>R: ATAGGCACAGTGCGTGGGTGT |
| *DNASEI* | Q222R (rs1053874) | A/G | F: CATCTGGGGATAAGAGGAGAG<br>R: AGTCGGGAACAACGGCGACT |
| *DNASEI* | L186L (rs8176920) | A/G | F: TCCCAGTGGTCATCCATCCGCAT<br>R: CTTTGAGGCTTCTGAAGCCCG |
| *DNASEI* | P197S (rs34186031) | C/T | F: GACGTCATGTTGATGGGCGA<br>R: ATAGGCACAGTGCGTGGGTGT |
| *VDR* | rs2228570 | C/T | F: GCACTGACTCTGGCTCTGAC<br>R: ACCCTCCTGCTCCTGTGGCT |
| *VDR* | rs1544410 | A/G | F: GGAGACACAGATAAGGAAATAC<br>R: CCGCAAGAAACCTCAAATAACA |
| *VDR* | rs7975232 | A/C | F: TGGGCACGGGGATAGAGAAG<br>R: ACGGAGAAGTCACTGGAGGG |
| *VDR* | rs731236 | T/C | F: TCCTGTGCCTTCTTCTCTATC<br>R: CTAGCTTCTGGATCATCTTGG |

cial product (NanoDrop; Thermo Fisher Scientific, Inc., Wilmington, DE, USA).

Following DNA extraction, the samples were sent to the Duke Molecular Physiology Institute. They were diluted to 1 ng/μL using distilled water (UltraPure DNase/RNase-Free; Life Technologies, Grand Island, NY, USA). We transferred 3 ng of each sample to a 384-well plate using an automated pipetting system (epMotion 5075 TMX; Eppendorf North America, Hauppauge, NY, USA).

Genotyping was performed using both custom and predesigned assays (TaqMan SNP Genotyping Assays; Life Technologies) for the 12 SNPs. Table 1 lists the SNPs and their primers. We performed PCR according to manufacturer protocols on 3 ng of genomic DNA in 5 μL reaction volumes, using a PCR system (GeneAmp 9700 Dual 384-Well; Life Technologies) and subsequently scanned on a real-time PCR System (ViiA 7; Life Technologies). Data were assessed on, and exported from commercial software (ViiA 7 RUO Software v1.2.1; Life Technologies). Samples of CEPH (NIGMS Repository, Coriell Institute for Medical Research, Camden, NJ, USA), study sample replicates, and no template controls (NTCs) were used for quality control (QC). Replicates of QC were required to match 100%, and NTCs were required to have no amplification.

### Sociodemographic Variables and Assessment of Depression

Demographic data (date of birth, sex, race) were collected from medical records of cases and controls. Depression (yes/no) was assessed through medical chart review of any diagnosed history of clinical depression and ever being prescribed anti-depressants.

### Statistical Analysis

We tested the SNPs for Hardy–Weinberg equilibrium (HWE) using $\chi^2$ test. Any SNPs that deviated from HWE ($P < 0.05$) were excluded from further analysis. Genotype and allele frequencies of cases and controls were evaluated with $\chi^2$ test. The odds ratios of associations were calculated with 95% confidence intervals (CI). Logistic regression was also performed to determine the association between SNPs, DED, and depression. Stratified analysis by depression status was also performed between SNPs and DED. All analyses were performed using PLINK 1.07 (available in the public domain at http://pngu.mgh.harvard.edu/~purcell/plink/index.shtml), and statistical software (STATA SE 12.0; StataCorp LP., College Station, TX, USA) software.

## Results

### Demographics and Patient Characteristics

Table 2 shows the demographic distribution for patients and control subjects. Mean age was 54.6 in cases compared with 46.8 in controls and was statistically significantly different using independent *t*-test ($P = 0.01$). The proportion of females as compared to males was much higher among the DED cases than the controls (78.1% females versus 21.9% males among cases and 54.9% vs. 45.1% among controls, $P = 0.01$). Among DED, 42.2% were diagnosed with depression and 57.8% did not have a depression diagnosis. Among controls, only 11.8% were diagnosed with depression and 88.2% did not have a diagnosis of depression ($P < 0.001$).

The symptom and clinical characteristics were assessed for DED patients. Twenty-percent reported mild dryness symptoms, 39.7% reported moderate symptoms, and 38.1% reported severe symptoms. The mean Schirmer score for the worst eye among all patients was 6.6 ± 6.41. For Rose Bengal corneal staining: 30.2% had 1+, 23.8% had 2+, 15.9% had 3+, and 6.3% had 4+. A total of 9% of patients scored 0+ on corneal staining, but 4.8% of those patients had conjunctival staining of 1+, and 4.8% had pain without staining. A total of 20% had a score of 0 for lid margin disease, 27% had 1+, 23.8% had 2+, 15.9% had 3+, and 6.3% had 4+. With regard to diagnosis,

**Table 2.** Demographic Characteristics Between Cases and Controls

| Variables | Dry Eye Cases, $N = 64$, $n$ (%) | Controls, $N = 51$, $n$ (%) | $P$ Value* |
|---|---|---|---|
| Mean Age, y | 54.56 ± 15.48 | 46.75 ± 15.48 | 0.01 |
| Sex | | | 0.01 |
| Male | 14 (21.9) | 23 (45.1) | |
| Female | 50 (78.1) | 28 (54.9) | |
| Race | | | 0.11 |
| White | 28 (43.8) | 15 (29.4) | |
| Non-White | 36 (56.3) | 36 (70.6) | |
| Depression | | | <0.001 |
| Yes | 27 (42.2) | 6 (11.8) | |
| No | 37 (57.8) | 45 (88.2) | |

* $\chi^2$ test for categorical variables, Fisher's exact test $P$ value when cell size < 50, and Student's $t$-test for continuous variables.

68.3% had dry eye syndrome, 50.8% had meibomian gland dysfunction (MGD; some dry eye syndrome patients had MGD as well), 15.9% had Sjogren, and 17.5% had graft-versus-host disease.

## Association of Polymorphisms With DED

Following genotyping, 6 SNPs (rs8176927, rs34923865, rs8176919, rs1799891, rs8176920, and rs34186031) in the *DNASE1* gene were excluded from the analysis as they did not show any variability (results were either all homogeneous or all heterogeneous) in cases or controls. The remaining six SNPs were analyzed. Allelic frequencies were calculated and their genotypic distributions tested for Hardy-Weinberg equilibrium. No significant deviations were detected at 0.05. Table 3 shows the allelic and genotypic distributions between cases and controls. The most significant was Rs6265 in the *BDNF* gene, where the number of the minor allele A was higher in cases compared with controls (22 vs. 9). Cases were 2.22 times more likely to have the minor allele A in SNP rs6265 as compared to the controls ($P = 0.05$; 95% CI 0.97–5.08; Table 3). Genotypes GA and AA were higher in cases compared with controls, 30% and 3% among the cases, and 18% and 0% among controls, respectively. The trend $P$ value was 0.05; Rs2228570 and rs7975232 in the *VDR* gene also showed different distributions between cases and controls but this difference were also marginally significant. Genotype AA for rs2228570 was higher in cases as compared to controls, 25% among cases and 12% among controls. The trend $P$ value was 0.08. Cases were 1.72 times more likely to have the minor allele A for rs2228570 compared with controls ($P = 0.06$; 95% CI 0.98–3.01; Table 3). Genotype CC for rs7975232 was higher in cases compared with controls, 28% among cases and 14% among controls. The trend $P$ value was 0.06. Cases were 1.66 times more likely to have the minor allele A for rs7975232 compared with controls ($P = 0.06$; 95% CI 0.97–2.84).

Multivariable logistic regression was performed controlling for age, sex, and race for rs6265, rs2228570, and rs7975232 (Table 4). Adjusted ORs were 1.99 ($P = 0.16$), 1.61 ($P = 0.27$), and 2.16 ($P = 0.10$) for each of the 3 SNPs, respectively.

**Table 3.** Genotype and Allele Distribution Between Cases and Controls

| SNP | Chr | Gene | Allele | Cases, $n$ (%) | Controls, $n$ (%) | $P$ Value | OR | 95% CI |
|---|---|---|---|---|---|---|---|---|
| rs6265 | 11 | BDNF | GG | 41 (67) | 41 (82) | 0.05* | | |
| | | | GA | 18 (30) | 9 (18) | | | |
| Position 27658369 | | | AA | 2 (3) | 0 (0) | | | |
| | | | A | 22 (18) | 9 (9) | 0.05** | 2.22 | 0.97–5.08 |
| | | | G | 100 (82) | 91 (91) | | | |
| rs2228570 | 12 | VDR | GG | 20 (35) | 24 (48) | 0.08* | | |
| | | | GA | 23 (40) | 20 (40) | | | |
| | | | AA | 14 (25) | 6 (12) | | | |
| Position 47879112 | | | A | 51 (45) | 32 (32) | 0.06** | 1.72 | 0.98–3.01 |
| | | | G | 63 (55) | 68 (68) | | | |
| rs1544410 | 12 | VDR | GG | 28 (49) | 20 (40) | 0.30* | | |
| | | | GA | 27 (47) | 27 (54) | | | |
| | | | AA | 2 (4) | 3 (6) | | | |
| Position 47846052 | | | A | 31 (27) | 33 (33) | 0.35** | 0.76 | 0.42–1.36 |
| | | | G | 83 (73) | 67 (67) | | | |
| rs7975232 | 12 | VDR | AA | 14 (23) | 17 (34) | 0.06* | | |
| | | | AC | 29 (48) | 26 (52) | | | |
| | | | CC | 17 (28) | 7 (14) | | | |
| Position 47845054 | | | C | 63 (53) | 40 (40) | 0.06** | 1.66 | 0.97–2.84 |
| | | | A | 57 (48) | 60 (60) | | | |
| rs731236 | 12 | VDR | AA | 31 (51) | 20 (40) | 0.15* | | |
| | | | AG | 25 (41) | 22 (44) | | | |
| | | | GG | 5 (8) | 8 (16) | | | |
| Position 47844974 | | | G | 35 (29) | 38 (38) | 0.14** | 0.66 | 0.37–1.15 |
| | | | A | 87 (71) | 62 (62) | | | |
| rs1053874 | 16 | DNASE1 | GG | 23 (37) | 17 (34) | 0.29* | | |
| | | | GA | 30 (48) | 20 (40) | | | |
| | | | AA | 9 (15) | 13 (26) | | | |
| Position 3657746 | | | A | 48 (39) | 46 (46) | 0.27** | 0.74 | 0.43–1.27 |
| | | | G | 76 (61) | 54 (54) | | | |

* $P$ value: trend.
** $P$ value: Allelic distribution; OR and 95% CI is for the allelic distribution.

Investigative Ophthalmology & Visual Science

**Table 4.** Multivariable Logistic Regression of the Association Between SNPs and DED

| Models DED as Outcome | OR | *P* Value | 95% CI |
|---|---|---|---|
| BDNF rs6265 (AA+GA vs. GG) | 1.99 | 0.16 | 0.76–5.18 |
| Age | 1.03 | 0.04 | 1.00–1.06 |
| Sex (Female vs. Male) | 2.63 | 0.03 | 1.10–6.29 |
| Race (Non-White vs. White) | 0.63 | 0.3 | 0.27–1.49 |
| VDR rs2228570 (AA+GA vs. GG) | 1.61 | 0.27 | 0.69–3.76 |
| Age | 1.04 | 0.01 | 1.01–1.07 |
| Sex (Female vs. Male) | 2.67 | 0.03 | 1.09–6.55 |
| Race (Non-White vs. White) | 0.55 | 0.19 | 0.23–1.34 |
| VDR rs7975232 (CC+AC vs. AA) | 2.16 | 0.1 | 0.87–5.35 |
| Age | 1.03 | 0.04 | 1.00–1.06 |
| Sex (Female vs. Male) | 2.88 | 0.02 | 1.19–6.97 |
| Race (Non-White vs. White) | 0.57 | 0.2 | 0.24–1.34 |

## Association of Polymorphisms With Depression and Stratified Analysis

The distribution of SNPs with depression is shown in Table 5.

Depression and its interaction with the SNPs was mainly apparent for rs6265, where rs6265 (Val66Met) in the *BDNF* gene varied by depression status. Among patients diagnosed with depression, 38.7% had the GA genotype, whereas among patients with no depression 18.8% had the GA genotype. Logistic regression between depression and Rs6265 revealed an OR of 2.34 ($P = 0.06$ and 95% CI 0.95–5.75). This means that patients diagnosed with depression were 2.34 times more likely to have the GA or AA genotype compared with controls. Stratified analysis of the association between DED and rs6265 by depression showed that among the depressed group the OR was 3.93 compared to 1.45 among the nondepressed group (Tables 6, 7).

## Discussion

This pilot study revealed that Val66Met (rs6265) in the *BDNF* gene and two SNPs, FokI (rs2228570) and ApaI (rs7975232), in the *VDR* gene may potentially be associated with DED. Adjusting for age, sex, and race disparity revealed associations of similar magnitude to the unadjusted, but that are not statistically significant. Additionally, the association between DED and Val66Met increases with the presence of depression. Despite the limited sample size, the results in this pilot study point to SNPs in *BDNF* and *VDR* genes as being associated with DED and a possible common biological link through a SNP in the *BDNF* gene that may explain the association between DED and depression. The most significant practical finding from this study is the observed effect sizes, which can be used to plan larger studies. Furthermore, it is important to understand these associations along the duration and prognosis of DED and the effects of other confounding factors such as age, gender, and race disparity.

Identifying SNPs that are common to both DED and depression may form the basis of management strategies that take into account and target these common biological links. We found that Val66Met, a SNP in the *BDNF* gene, is associated with DED and with depression. The proposed mechanism for BDNF in DED is that chronic DED causes ocular discomfort sensations and corneal inflammation which induce expression of BDNF in the trigeminal ganglion and a phenotypic shift in the expression of BDNF from small diameter C-type nociceptor neurons to large diameter A-alpha/A-beta type non-nociceptive neurons. This phenotypic shift may be the "injury switch" that leads to corneal allodynia and hyperalgesia. Additionally, Val66Met has been shown to be associated with depressive disorder and depression-related phenotypes.[27–30]

Our study also revealed two SNPs potentially associated with DED in the *VDR* gene. We hypothesize that the active form of vitamin D may play a role in evaporative DED through two potential mechanisms. The first relates to cathelicidin. Cathelicidin micropeptides are overexpressed in patients with rosacea which presents as ocular rosacea and evaporative DED.[31] The identification of the cationic antimicrobial peptide cathelicidin as a vitamin D target gene[32] created a previously unknown and unexpected link between innate immunity and the vitamin D system. The second mechanism relates to androgen. Androgen receptors are located in the lacrimal and meibomian glands. It has been shown that in MGD, a deficiency in androgens results in loss of the lipid layer, specifically triglycerides, cholesterol, monounsaturated essential fatty acids exacerbating DED.[33] Vitamin D receptor polymorphisms, BsmI, ApaI and TaqI wild variants of the *VDR* gene were associated with lower vitamin D levels,[34] which in turn affect levels of androgen.[35] There is also evidence that VDR is also expressed in the brains of several species during development.[34] We have found that VDR FokI and ApaI SNPs are more common among patients with DED.

**Table 5.** Distribution of SNPs With Depression

| SNPs | Depression, *n* (%) | No Depression, *n* (%) | *P* Value |
|---|---|---|---|
| BDNF rs6265 | | | 0.07 |
| AA | 0 (0) | 2 (3) | |
| GA | 12 (39) | 15 (19) | |
| GG | 19 (61) | 63 (79) | |
| VDR rs2228570 | | | 0.55 |
| AA | 7 (23) | 13 (17) | |
| GA | 13 (43) | 30 (39) | |
| GG | 10 (33) | 34 (44) | |
| VDR rs7975232 | | | 0.65 |
| CC | 8 (28) | 16 (20) | |
| AC | 14 (48) | 41 (51) | |
| AA | 7 (24) | 24 (30) | |

**Table 6.** Stratified Analysis of DED and BDNF Val66Met (rs6265) in Patients With Depression

| Depression, Yes | DED Cases, *n* (%) | Controls, *n* (%) | Total, *n* (%) |
|---|---|---|---|
| rs6265 A | 11 (44) | 1 (17) | 12 (39) |
| rs6265 G | 14 (56) | 5 (83) | 19 (61) |
| Total | 25 (100) | 6 (100) | 31 (100) |

OR, 3.93; 95% CI: 0.35–202.4.

**Table 7.** Stratified Analysis of DED and BDNF Val66Met (rs6265) in Patients Without Depression

| Depression, No | DED Cases, *n* (%) | Controls, *n* (%) | Total, *n* (%) |
|---|---|---|---|
| rs6265 A | 9 (25) | 8 (18) | 17 (21) |
| rs6265 G | 27 (75) | 36 (81) | 63 (79) |
| Total | 36 (100) | 44 (100) | 80 (100) |

OR, 1.5; 95% CI, 0.45–5.1.

Investigative Ophthalmology & Visual Science

While statistical significance and borderline significance was mainly observed in the unadjusted associations, the magnitude of the adjusted associations to age, sex, and race was comparable. This may be due to our limited sample size. Age and sex have long been shown to be associated with DED. Based on data from large studies with representative population based sampling, such as the Women's Health Study and the Physicians' Health Study, it has been estimated that about 3.23 million women and 1.68 million men, for a total of 4.91 million Americans aged $\geq$50 years, have dry eye.[36,37] Galor et al.[3] evaluated the prevalence of dry eye syndrome and its associated risk factors in a US Veterans Affairs population receiving ocular care services.[3] Female sex showed a 2.40 increased risk of DES over male sex. Additionally, they showed that female patients had a higher percentage of dry eye in each age group compared with male patients and the overall risk of dry eye increased by 1.06 for each increasing decade.[3] In our study, age and sex differences were observed between cases and controls, it therefore was important to perform an association analysis adjusting for age and sex. In addition, we also observed disparity in race (white versus non-white) which was not statistically significant. However, ancestry markers need to be analyzed to make sure that there is no confounding by population stratification. It will be possible to perform such an analysis in a larger study.

This study has several limitations. First of which is the small sample size which limited our ability to detect differences with statistical certainty. For this pilot study, we enrolled the maximum and feasible number of patients from one center. Second, the case-control design of this study limited our interpretation regarding temporality between DED and depression. Additionally, the duration of DED was difficult to delineate. While our inclusion criteria included new and established patients, most of our patients are established and the patients who were new to the clinic were either previously diagnosed with DED at an alternative clinic, or have been suffering from symptoms for some time. Further, results from this study cannot be generalized. This was not a population-based study. Most of our patients were referral patients who are characterized as having the most difficult and complicated prognosis. This limits our ability to apply some of our findings to the entire spectrum of DED patients. Despite these limitations, the findings from our study lay the foundation for larger genetic case-control and prospective cohort studies studying the biological link between DED and depression. These studies will help identify the interplay of all factors involved in the pathogenesis of DED, ultimately improving treatment decisions.

## *Acknowledgments*

Supported by the Illinois Society to Prevent Blindness (JAH); an unrestricted grant from Research to Prevent Blindness (departmental grant: applies to all authors); Grants R01EY024966 (SJ) and R01EY022651 (XG); and an RPB Physician-Scientist Award (SJ). DNA sequencing support was provided by the Molecular Genomics Core at the Duke Molecular Physiology Institute.

Disclosure: **J.A. Hallak**, Quantitative Scientific Solutions, LLC (E, C); **S. Tibrewal**, None; **N. Mohindra**, None; **X. Gao**, None; **S. Jain**, None

## *References*

1. The epidemiology of dry eye disease: report of the Epidemiology Subcommittee of the International Dry Eye WorkShop. *Ocul Surf*. 2007;5:93–107.
2. Kim KW, Han SB, Han ER, et al. Association between depression and dry eye disease in an elderly population. *Invest Ophthalmol Vis Sci*. 2011;52:7954–7958.
3. Galor A, Feuer W, Lee DJ, et al. Prevalence and risk factors of dry eye syndrome in a United States veterans affairs population. *Am J Ophthalmol*. 2011;152:377–384.
4. Galor A, Feuer W, Lee DJ, et al. Depression, post-traumatic stress disorder, and dry eye syndrome: a study utilizing the national United States Veterans Affairs administrative database. *Am J Ophthalmol*. 2012;154:340–346.
5. Li M, Gong L, Sun X, Chapin WJ. Anxiety and depression in patients with dry eye syndrome. *Curr Eye Res*. 2011;36:1–7.
6. Labbé A, Wang YX, Jie Y, Baudouin C, Jonas JB, Xu L. Dry eye disease, dry eye symptoms and depression: the Beijing Eye Study. *Br J Ophthalmol*. 2013;97:1399–1403.
7. van der Vaart R, Weaver MA, Lefebvre C, Davis RM. The association between dry eye disease and depression and anxiety in a large population-based study. *Am J Ophthalmol*. 2015;159:470–474.
8. Vehof J, Wang B, Kozareva D, Hysi PG, Snieder H, Hammond CJ. The heritability of dry eye disease in a female twin cohort. *Invest Ophthalmol Vis Sci*. 2014;55:7278–7283.
9. Burbelo PD, Ambatipudi K, Alevizos I. Genome-wide association studies in Sjögren's syndrome: what do the genes tell us about disease pathogenesis? *Autoimmun Rev*. 2014;13:756–761.
10. Ice JA, Li H, Adrianto I, et al. Genetics of Sjögren's syndrome in the genome-wide association era. *J Autoimmun*. 2012;39:57–63.
11. Na KS, Mok JW, Kim JY, Joo CK. Proinflammatory gene polymorphisms are potentially associated with Korean non-Sjogren dry eye patients. *Mol Vis*. 2011;17:2818–2823.
12. Lowe DP1, Cook MA, Bowman SJ, Briggs DC; UK Sjögren's Interest Group. Association of killer cell immunoglobulin-like receptors with primary Sjogren's syndrome. *Rheumatology (Oxford)*. 2009;48:359–362.
13. Burbelo PD, Ambatipudi K, Alevizos I. Genome-wide association studies in Sjögren's syndrome: what do the genes tell us about disease pathogenesis? *Autoimmun Rev*. 2014;13:756–761.
14. Fauchais AL, Boumediene A, Lalloue F, et al. Brain-derived neurotrophic factor and nerve growth factor correlate with T-cell activation in primary Sjogren's syndrome. *Scand J Rheumatol*. 2009;38:50–57.
15. Sairanen M, Lucas G, Ernfors P, Castrén M, Castrén E. Brain-derived neurotrophic factor and antidepressant drugs have different but coordinated effects on neuronal turnover, proliferation, and survival in the adult dentate gyrus. *J Neurosci*. 2005;25:1089–1094.
16. Polyakova M, Stuke K, Schuemberg K, Mueller K, Schoenknecht P, Schroeter ML. BDNF as a biomarker for successful treatment of mood disorders: a systematic & quantitative meta-analysis. *J Affect Disord*. 2014;174:432–440.
17. Tinazzi E, Puccetti A, Gerli R, et al. Serum DNase I, soluble Fas/FasL levels and cell surface Fas expression in patients with SLE: a possible explanation for the lack of efficacy of hrDNase I treatment. *Int Immunol*. 2009;21:237–243.
18. Skiljevic D, Jeremic I, Nikolic M, et al. Serum DNase I activity in systemic lupus erythematosus: correlation with immunoserological markers, the disease activity and organ involvement. *Clin Chem Lab Med*. 2013;51:1083–1091.
19. Sonawane S, Khanolkar V, Namavari A, et al. Ocular surface extracellular DNA and nuclease activity imbalance: a new paradigm for inflammation in dry eye disease. *Invest Ophthalmol Vis Sci*. 2012;53:8253–8263.
20. Mostowska A1, Lianeri M, Wudarski M, Olesińska M, Jagodziński PP. Vitamin D receptor gene BsmI, FokI, ApaI and TaqI polymorphisms and the risk of systemic lupus erythematosus. *Mol Biol Rep*. 2013;40:803–810.
21. Mak A, Tang CS, Ho RC. Serum tumour necrosis factor-alpha is associated with poor health-related quality of life and

depressive symptoms in patients with systemic lupus erythematosus. *Lupus*. 2013;22:254–261.

22. Palejwala NV, Walia HS, Yeh S. Ocular manifestations of systemic lupus erythematosus: a review of the literature. *Autoimmune Dis*. 2012;2012:290898.
23. NCBI Gene Summary. BDNF brain-derived neurotrophic factor [Homo sapiens (human)].Gene ID: 627, updated on January 1, 2015. Available at: http://www.ncbi.nlm.nih.gov/gene/627. Accessed January 4, 2014.
24. NCBI Gene Summary. VDR vitamin D (1, 25- dihydroxyvitamin D3) receptor [Homo sapiens (human)] Gene ID:7241, updated on January 1, 2015. Available at: http://www.ncbi.nlm.nih.gov/gene/7421. Accessed January 4, 2014.
25. NCBI Gene Summary. DNASE1 deoxyribonuclease I [Homo sapiens (human)] Gene ID:1773, updated on January 1, 2015. http://www.ncbi.nlm.nih.gov/gene/1773. Accessed January 4, 2014.
26. Yasuda T, Ueki M, Takeshita H, et al. A biochemical and genetic study on all non-synonymous single nucleotide polymorphisms of the gene encoding human deoxyribonuclease I potentially relevant to autoimmunity. *Int J Biochem Cell Biol*. 2010;42:1216–1225.
27. Cardoner N, Soria V, Gratacòs M, et al. Val66Met BDNF genotypes in melancholic depression: effects on brain structure and treatment outcome. *Depress Anxiety*. 2013;30:225–233.
28. Kanellopoulos D, Gunning FM, Morimoto SS, et al. Hippocampal volumes and the brain-derived neurotrophic factor val66met polymorphism in geriatric major depression. *Am J Geriatr Psychiatry*. 2011;19:13–22.
29. Frodl T, Schüle C, Schmitt G, et al. Association of the brain-derived neurotrophic factor Val66Met polymorphism with reduced hippocampal volumes in major depression. *Arch Gen Psychiatry*. 2007;64:410–416.
30. Montag C, Weber B, Fliessbach K, Elger C, Reuter M. The BDNF Val66Met polymorphism impacts parahippocampal and amygdala volume in healthy humans: incremental support for a genetic risk factor for depression. *Psychol Med*. 2009;39:1831–1839.
31. Yamasaki K, Gallo RL. Rosacea as a disease of cathelicidins and skin innate immunity. *J Investig Dermatol Symp Proc*. 2011;15:12–15.
32. Gombart AF, Borregaard N, Koeffler HP. Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1, 25-dihydroxyvitamin D3. *FASEB J*. 2005;19:1067–1077.
33. Krenzer KL, Dana MR, Ullman MD, et al. Effect of androgen deficiency on the human meibomian gland and ocular surface. *J Clin Endocrinol Metab*. 2000;85:4874–4882.
34. Santos BR, Mascarenhas LP, Satler F, Boguszewski MC, Spritzer PM. Vitamin D deficiency in girls from South Brazil: a cross-sectional study on prevalence and association with vitamin D receptor gene variants. *BMC Pediatr*. 2012;12:62.
35. Harms LR, Burne TH, Eyles DW, McGrath JJ. Vitamin D and the brain. *Best Pract Res Clin Endocrinol Metab*. 2011;25:657–669.
36. Schaumberg DA, Sullivan DA, Buring JE, Dana MR. Prevalence of dry eye syndrome among US women. *Am J Ophthalmol*. 2003;136:318–326.
37. Schaumberg DA, Dana R, Buring JE, Sullivan DA. Prevalence of dry eye disease among US men: estimates from the Physicians' Health Studies. *Arch Ophthalmol*. 2009;127:763–768.

Investigative Ophthalmology & Visual Science