Dry Eye Disease and Depression: Epidemiological and Biological Links

BY

JOELLE HALLAK
BSc University of Balamand, Beirut, Lebanon 2005
MS University of Illinois at Chicago, Chicago 2010

THESIS
Submitted as partial fulfillment of the requirements
for the degree of Doctor of Philosophy in Public Health Sciences
in the Graduate College of the
University of Illinois at Chicago, 2015

Chicago, Illinois

Defense Committee:

Supriya Mehta, Chair and Advisor
Sandeep Jain, Ophthalmology
Xiaoyi "Raymond" Gao, Ophthalmology
Ronald Hershow, Epidemiology
Mark Stein, University of Washington, Psychiatry

This thesis is dedicated to my parents, Georges and Joe.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BDI	Beck Depression Inventory
BDNF	Brain-Derived Neurotrophic Factor
CI	Confidence Interval
DED	Dry Eye Disease
DEWS	Dry Eye Workshop
DNASE1	Deoxyribonuclease 1
DSM	Diagnostic and Statistical Manual of Mental Disorders
eDNA	Extracellular DNA
GVHD	Graft-versus-Host Disease
HWE	Hardy-Weinberg Equilibrium
IDEEL	Impact of Dry Eye on Everyday Life
MGD	Meibomian Gland Dysfunction
NEI-VFQ	National Eye Institute-Visual Function Questionnaire
NHRI	National Health Research Institute
NTC	No Template Controls
OR	Odds Ratio
OSDI	Ocular Surface Disease Index
QC	Quality Control
SD	Standard Deviation
Se	Sensitivity
SLE	Systemic Lupus Erythematosus
SNP	Single Nucleotide Polymorphism
Sp	Specificity
VDR	Vitamin-D receptor

SUMMARY

Dry Eye Disease (DED) is a symptomatic multifactorial phenotype reportedly associated with depression. There is a lack of studies concentrating on the examination of compelling hypotheses for DED and depression. This thesis focused on understanding the potential epidemiological and biological links between DED and depression using tools that measure and correlate the symptoms (Specific Aim A and Specific Aim B), and through examining biological links using hypothesized single nucleotide polymorphisms (SNPs) (Specific Aim C).

To address specific aims A and B, we developed a new tool to assess the symptom burden for DED and used the Beck Depression Index (BDI) to assess depressive symptoms in two studies. The first study was a cross-sectional study to measure DED symptoms (Specific Aim A). A symptom burden tool was developed from well-established and validated tools and included two dimensions and four domains: The sensory dimension, which included the domains of symptom persistence and symptom intensity; and the reactive dimension, which included domains for activity and affective interference. This tool was administered to DED patients and showed that persistence of DED symptoms correlates with affective interference more than activity interference, and that intensity of symptoms may be important for treatment decisions.

The second study was a case-control study designed to measure depressive symptoms and DED symptoms to determine the symptom correlation between the two diseases (Specific Aim B). The BDI questionnaire, Ocular Surface Disease Index Questionnaire (OSDI), and the symptom burden tool (developed in Specific Aim A) were administered to DED patients and controls. This study showed that patients with DED exhibit more depressive symptoms than controls. After adjusting for age, gender, race, and psychiatric medication, the regression coefficient was 1.71 (95% CI 1.02, 2.40) between DED symptoms and depressive symptoms.

SUMMARY (continued)

Logistic regression revealed an OR of 2.86 (95% CI 1.04, 7.87) for the association between DED and diagnosis of depression after controlling for age, gender, and race.

To complement the clinical epidemiological association between DED and depression, this thesis also included a study on the biological links between DED and depression (Specific Aim C). Twelve SNPs in three main genes were investigated: Brain-Derived Neurotrophic Factor (BDNF), Vitamin-D Receptor (VDR), and Deoxyribonuclease 1 (DNASE1) in DED patients and controls. Val66Met (rs6265), an SNP in the BDNF gene and two SNPs Fokl (rs2228570) and Apal (rs7975232) in the VDR gene were found to be potentially associated with DED (Specific Aim C1). Results, while not significant, seem to show that the association between DED and Val66Met (rs6265) varies by depression status. Additionally, the role of Val66Met in treatment response was investigated through DED symptom measurement over time (Specific Aim C2). A pilot study was performed where patients were followed up for a minimum of six months. The study revealed that symptoms like dryness and pain persist in patients with the minor allele a (Met carriers) of Val66Met despite treatment; whereas symptoms are significantly reduced in patients without the minor allele.

1. INTRODUCTION

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 more than 35% at various ages (1). Numerous exposures—including medication use, hormonal changes, environmental exposures, and neural alterations—are involved in the pathogenesis of dry eye. Common symptoms of dry eye patients include pain, irritation, itching, burning, and grittiness (1). Recent studies have reported an association between depression and dry eye (2,3), post-traumatic stress disorder and dry eye (4), and anxiety and dry eye (5). The mechanism by which DED and psychiatric disorders, such as depression, are correlated has yet to be determined.

This thesis was built upon preliminary research findings generated in our clinic and laboratory. In our clinic, we have observed that 53.5% of patients reporting symptoms of dry eye have either been clinically diagnosed with depression and anxiety disorders, are on antidepressant medications, or have visited a psychiatric clinic in the past year. Pain was the most common symptom reported by this patient population. This is consistent with recent studies. Vehof J et al., for example, studied the risk factors of DED in a female cohort. They showed that depression and widespread pain syndrome had the highest effect sizes with DED (6). Both DED and depression rely heavily on symptoms reported by patients. In this thesis, we hypothesized that the presence of depression in DED may cause patients to perceive symptoms in an anomalous fashion compared to patients without depression.

While investigating the molecular basis of symptoms in DED in our laboratory, we discovered that BDNF and extracellular DNA (eDNA) may be important molecular mediators or modifiers of the association between DED and depression (7,8). Polymorphisms in the BDNF gene, such as VAL66MET, have been shown to play a significant role in

1

susceptibility of an individual to stress disorders. Additionally, patients with systemic lupus erythematosus (SLE) (9) and Rosacea (10) are also more susceptible to psychiatric disorders. As such, we hypothesized that SNPs in the BDNF, DNASE I, and VDR genes may play a role on susceptibility to depression and dry eye. This role may also be present in treatment response.

1.1. **Problem Statement**

Dry eye disease is a significant public health problem. It is one of the leading causes of patient visits to ophthalmologists and optometrists in the United States due to its debilitating symptoms (11). Studies have shown that at least 14% of the US population older than 50 years has DED (12,13). The symptoms and signs of DED do not correlate. Patients with DED suffer from inexplicable pain, and are either clinically diagnosed with depression or report depressive symptoms. This is supported by studies showing that depression is a common comorbid condition with DED (14). The order of occurrence between DED and depression remains unclear. One possibility is that the symptoms of DED, such as pain, could induce the occurrence of depressive symptoms. Another possibility is that antidepressants are suggested to be risk factors for DED, however some studies have shown that depression itself is involved in the pathophysiology of DED and not just its treatments (4).

There are common risk factors for DED and depression such as female gender and age. Biological studies have showed a dysregulation of neuropeptides and an increased production of inflammatory cytokines in depression, which are also involved in the mechanism of DED. Given the potential epidemiological and biological overlap between DED and depression, this thesis sought to study the relationship of symptoms and the common genetic links between the two diseases. Identifying a common biological mechanism between these two diseases will allow us to make more informed treatment decisions.

1.2.   **Objective and Specific Aims**

The objective was to investigate epidemiological and biological links between DED and depression. Our line of investigation is supported by epidemiologic observations (frequent co-occurrence of DED and depression; shared risks for DED and depression) and laboratory studies providing a plausible biological mechanism (increased BDNF and eDNA expression in DED, BDNF polymorphisms in depression, and epigenetic regulation).

Three specific aims were proposed to achieve this objective:

1.2.1. **Specific Aim A**

Develop a DED symptom burden tool consisting of a sensory domain (symptom persistence and intensity) and reactive domain (activity and affective interference) to measure symptoms in DED patients in a cross-sectional study. Several clinical tests are available to measure aspects of DED. However, there is no gold standard for diagnosis, and clinicians rely on patient reported symptoms of ocular discomfort to make treatment decisions. Additionally, a four-domain tool that comprehensively measures the symptom burden of DED without increasing respondent burden is lacking and is needed for daily clinical use and diagnosis. This aim entailed developing a DED symptom burden tool and administering it to DED patients in a cross-sectional pilot study.

1.2.2. **Specific Aim B**

Measure depressive symptoms in DED patients and controls using the BDI questionnaire and determine the correlation between these symptoms and DED symptoms. Both DED and depression are symptomatic diseases. Their diagnosis, prognosis, and treatment are heavily based on symptoms. Given that descriptive studies are showing an association between the diagnosis of both diseases, this aim focused on measuring the symptoms of DED and depression using a case-control study.

1.2.3. **Specific Aim C**

Investigate whether DED and depression are linked through common genetic polymorphisms using a case-control study (C1), and determine whether the response to DED treatment is related to the presence of specific SNPs in a subset of DED patients (C2). Gene polymorphisms have been shown to be associated with depression, (such as VAL66MET BDNF polymorphism), and with conditions such as SLE which have high prevalence of DED as well as of depression (DNASE I polymorphism). In this aim, we investigated whether genetic polymorphisms within molecular targets like BDNF, DNASE I, and VDR have higher prevalence in DED as well as depression as this will provide a biological basis that independently links the two conditions. Using a case-control design, salivary DNA was analyzed for SNPs and then correlated with the presence of DED and depression.

Additionally, genes play an important role in response to disease treatment. In a subset of patients, we investigated whether treatment response to DED varied by SNP status. Data on symptoms and signs of DED were collected for at least 6 months follow-up following initial treatment assignment and were stratified by SNP status.

2. BACKGROUND

2.1.   **Dry Eye Disease Burden and Risk Factors**

Dry eye disease is a symptomatic disease caused by either decreased tear production or increased tear film evaporation. It is prevalent in more than 50% of the American population aged 50 years and older (15). Based on data from large studies with representative population based sampling, such as the Women's Health Study and the Physicians' Health Study, where it was calculated that 3.23 million women and 1.68 million men, out of 4.91 million Americans aged ≥ 50 years, have dry eye (16,17). The prevalence of DED reported from cross-sectional population-based epidemiological studies in the United States, Australia, and Asia (with sample sizes ranging from 926 to 36,995) ranged from 5.5% to over 33% at various ages (1,18,19). However, different definitions of dry eye were employed in these studies making comparisons difficult.

The National Eye Institute workshop on clinical trials in dry eye defined DED as "a disorder of the tear film due to tear deficiency or excessive tear evaporation which causes damage to the interpalpebral ocular surface and is associated with symptoms of ocular discomfort" (13). The 2007 report of the Dry Eye Workshop (DEWS) defined DED as a "multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability, with potential damage to the ocular surface. It is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface" (1). Dry eye disease encompasses many clinical conditions which are due to either tear deficiency (such as Sjögren's syndrome) or eyelid inflammation (such as meibomian gland disease) or are postoperative complications of surgical procedure (such as LASIK-induced neurotrophic epitheliopathy).

The public health significance of DED is evident from the reported irritative symptoms responsible for care and therapy-seeking behavior and for hindered daily activities. The

economic impact of DED comes from utilization of healthcare systems: direct costs of office visits, surgical interventions, medications, alternative therapeutics, and specialized eye wear. Indirectly, DED affects productive work time, and symptoms of DED have intangible costs such as decreased leisure time, impaired physical functioning and quality of life, and a large impact on social interactions and mental health (20).

Yu et al., in a prevalence-based cost-of-illness analysis using an Internet-based survey, estimated the direct and indirect annual cost of DED in the United States (21). They reported the average annual direct cost for patients seeking medical care to be $783 per patient. Taking into account the US DED prevalence among adults aged 50 years or older, the overall burden of DED for the healthcaresystem is $3.84 billion (21). The average annual cost to society was $11,302 per subject with DED. The overall burden to the US society was $55.4 billion (21). The loss because of diminished productivity was more than the direct costs of DED treatment, where Yu et al. reported that the total productivity loss per person ranged from $12,569 to $18,168 per year and that the loss because of presenteeism was substantially higher than the loss because of absenteeism (21). Yamada et al. investigated the impact of dry eye on work productivity of office workers, in presenteeism, in Japan (22). They reported that the degree of work performance loss was 5.65% in the definite dry eye group (those who have both symptoms and a diagnosis), 4.37% in the marginal dry eye group (those who have no symptoms but a diagnosis), 6.06% in the self-reported dry eye group (those who have symptoms but no diagnosis), and 4.27% in the control group (those who have neither symptoms nor a diagnosis). Results were only significant between self-reported dry eye group and the control group. They also estimated the annual cost of work productivity loss associated with dry eye as USD 741 per person (22).

Epidemiological studies began to study potential lifestyle, dietary, and other risk factors for DED in 1995 (1). The Epidemiology Subcommittee at DEWS noted that most associations between risk factors and DED, generated from population-based studies, may attenuate the true

effect potentially due to the grouping of different types of DED. In addition, some studies included objective examination, but many did not. Additionally, some studies used a non-hypothesis-driven approach. Findings, therefore, may be purely statistical, and important associations could have easily been overlooked (1).

As summarized in the DEWS report (1), the consistent risk factors for DED across these population-based studies are: female gender, older age, contact lens use, postmenopausal estrogen therapy, a diet that is low in omega 3 essential fatty acids or has a high ratio of omega 6 to omega 3 fatty acids, refractive surgery, vitamin A deficiency, radiation therapy, bone marrow transplantation, hepatitis C, and systemic and ocular medications, including antihistamines (1,23–26). Conflicting results have been reported on the associations between DED and alcohol, cigarette smoking, caffeine, acne, and menopausal status (1). Other risk factors may also include diabetes, HIV, human T-cell lymphtropic virus type 1 infections, connective tissue diseases, systemic cancer chemotherapy, and other medications such as isotretinoin, antidepressants, anxiolytics, beta-blockers, and diuretics (1). However, there is a lack of studies investigating these factors.

Mental health problems, such as depression and anxiety, are additional risk factors that have been reported (2,4). Dry eye disease has been shown to negatively impact quality of life, including general quality of life and vision-related quality of life (27). Furthermore, DED has been shown to be correlated with anxiety and depression (5). The negative impact on the quality of life is mainly due to the progression of dry eye symptoms creating a complex situation that interferes with daily activities and the emotional state of DED patients (1).

In this thesis, we investigated the epidemiological and biological links between DED and depression as we hypothesized that there may be common mechanisms linking the both conditions.

2.2.   **Depression and Dry Eye Disease**

Studies reporting on the association between depression and DED were case-control studies and cross-sectional surveys (2–5,28). They examined risk factors for DED or assessed quality of life. None of these studies evaluated the biological link.

Examination of the association between depression and DED started when some researchers revealed that depressive mood is one of the underlying causes of subjective dry mouth (29,30). Others suggested that dry eye symptoms and mood status may influence each other (31). The 2007 DEWS report included a discussion on the debilitating symptoms of dry eye and their result in both psychological and physical effects that impact the quality of life. Subsequently, Li et al. assessed vision-related quality of life and psychosocial impacts and found correlations with depression and anxiety (27).

The mechanisms that underlie the association between depression and dry eye symptoms are unknown; however, hypothesized reasons for the association include: a common pathophysiology and common risk factors between the two diseases (female sex, age, and hormonal influence).

Two population-based retrospective studies in the United States Veterans Affairs population in Miami reported high prevalence of depression in subjects with DED, 17% versus 10% in the first study and 24% versus 18% in the second study (3,4). The first study was a non-hypothesis-driven retrospective case-control study evaluating the prevalence of DED and its associated factors. The study included a total of 16,862 male and female patients between 21 and 90 years of age (2,056 patients who received some form of dry eye therapy diagnosed as cases and 14,806 controls) seen in the Miami and Broward Veterans Affairs eye clinics between 2005 and 2010 (3). The reported odds ratio (OR) for depression, after adjusting for age and gender, was 1.91 (95% CI 1.73–2.10). The second case-control study included a total of 2,454,458 male and female patients between 21 and 100 years of age (462,641 patients who

received some form of dry eye therapy diagnosed as cases and 1,991,817 controls) seen in a Veterans Affairs eye clinic between 2006 and 2011 to study the prevalence of DED and its associated risk factors in veterans on a national level and to evaluate the relationship between psychiatric diagnoses and DED (4). The reported OR for depression after adjusting for age and gender was comparable to the OR reported in the 2011 study (1.92, 95% CI 1.91–1.94). A major strength of both studies is their large sample size. However, both studies relied on ICD-9 codes and medication use to define and exclude DED, which may have led to misclassification between cases and controls when documentation was not accurate. Additionally, neither study used a hypothesis-based approach. There could have also been misclassification on exposure status especially given the subjectivity in measuring depression. It was also not possible for them to assess severity of DED or depression. Including a mix of diagnoses could have influenced the strength and direction of the association (4).

Other studies reporting on the association between depression and/or DED have been performed internationally. In a recent population-based cross-sectional study of 657 Korean elders $\geq$65 years of age (mean age was 71.9) randomly selected from an official household registration database in Yongin, Korea, Kim et al. investigated the association between DED and depression (2). The symptoms of DED were assessed using the 6-item dry eye questionnaire and signs were assessed using the Schirmer test, fluorescein staining, and tear film break-up time. They assessed depression using the Korean version of the Short Geriatric Depression Scale. Depression was more prevalent in patients with DED (33.3% versus 18.1%) and their sex-and-region (urban versus rural) adjusted analyses revealed depression as an independent risk factor for DED (OR 3.08; 95% CI 1.93–4.93). The authors of this study listed several limitations including the lack of assessment of medication, the cross-sectional nature of the study, and the severity of DED, which was assessed using the dry eye questionnaire. Subjective symptoms may be better quantified using tools including the visual analog scale and

the ocular surface disease index score, especially when using symptom-based diagnostic criteria for DED (2,32,33).

In another population-based case-control study in Taiwan, Wang et al. investigated the comorbidities of DED. They used a nationwide subset database released by the Taiwan National Health Research Institute (NHRI) in 2006. The program to create the database covered 22 million enrollees, representing more than 98% of the island's population. The NHRI used a systematic, random sampling method to extract 5% of the enrollees (n=1,073,891). The DED cases consisted of 12,007 patients (after excluding patients under 18 years of age) who sought ambulatory care with a principal diagnosis of DED and 36,021 randomly selected controls. The prevalence of psychiatrically diagnosed depression was higher in patients with DED (7.20% versus 3.55%) and the reported adjusted OR was 2.11 (95% CI 1.93–2.31) (34). Heart diseases, systemic lupus, asthma, pulmonary circulation disorders, diabetes, liver diseases, and solid tumors and metastasis were also reported to be associated with patients who have DED compared to those who do not have DED. The limitations of this study included the lack of assessment of medications in the association between depression and DED, the authors reported to only have controlled for gender, age, monthly income, and level of urbanization. Additionally, the authors looked at several comorbodities and did not include appropriate statistical analysis such as controlling for multiple comparisons. The severity of DED was also not assessed, only ICD-9-CM codes were used.

The strength of these studies lies in their representative samples and large sample sizes. However, the assessment of DED and depression and their severity is a limitation. The different methods of assessment of DED between both studies may have contributed to the differences in prevalence distribution and magnitude of association. The study by Wang et al. only used ICD-9 codes to assess DED and the Elixhauser comorbidity index to assess comorbidities, while the study by Kim et al. used clinical signs and symptoms (dry eye

questionnaire) to assess DED disease and used the Geriatric Depression Scale to assess depression.

The assessment of DED in epidemiological studies is challenging given the long-standing lack of a uniform set of criteria for the diagnosis of DED (lack of a gold standard). In this thesis, we developed and used a symptom-based diagnostic approach using more than one method along with clinical signs to assess DED and used the BDI tool to assess depressive symptoms.

2.3.   **Clinical Assessment of Dry Eye Disease**

A variety of diagnostic tests are commonly used in the clinic for the diagnosis of DED. These tests include: Schirmer test, tear film breakup time, Rose Bengal staining of the cornea and conjunctiva, tear film index, tear turnover rate, osmolarity, and meibomian gland assessment. The diagnostic validity of these tests (sensitivity, specificity, false-positive rates, and positive predictive values) have been evaluated. Goren et al. reported a sensitivity of 25% and a specificity of 90% for Rose Bengal staining at any positive cutoff value (35). At least four studies have evaluated the performance of the Schirmer test at different cut-off points. Van Bijsterveld et al. reported a sensitivity of 85% and a specificity of 83% with a cut-off point of <5.5 mm/5 mins of the Schirmer test when compared to diagnosis (36). Farris et al. reported a sensitivity of 10% and specificity of 100% for a cut-off value of <3mm/5 mins of the Schirmer test compared to diagnosis (37). Clearly, the choice of cutoff and comparison standard can have significant impact on the classification of disease. Two main factors have influenced the DEWS recommendations for diagnostic tests (1): (i) many candidate tests derive from studies that were subject to various forms of bias due to unreliable cut-off points and (ii) several tests are not available outside the clinic. It is, therefore, recommended to use a combination of tests either in parallel or in a series (simultaneous or sequential), along with symptom assessment questionnaires.

The diagnosis of DED cannot be done with clinical tests alone. Dry eye disease is a symptomatic disease, and, currently, symptom questionnaires are among the most repeatable used diagnostic tests, as they may provide a more integrated view of the clinical condition over time.

2.4.   **Assessment of Dry Eye Disease Symptoms**

A positive diagnosis of DED is heavily based on symptom assessment because symptoms are an essential component of the disease (1). The reported symptoms of DED include: pain, dryness, grittiness, itchiness, redness, burning or stinging, foreign body sensation, and light sensitivity. Symptom questionnaires should be used in combination with objective clinical measures of DED. The most commonly used symptom questionnaires of DED are listed in Table I. These questionnaires have been validated to differing extents. For example, measures of sensitivity and specificity are available for some questionnaires but not all. The questionnaires vary in length, intended use, and population tested (1).

In addition to developing a symptom burden tool for DED symptoms in this thesis, we used the OSDI questionnaire.

TABLE I
SYMPTOM QUESTIONNAIRES MOST COMMONLY IN CURRENT USE IN THE UNITED STATES

Instrument/ Questionnaire	Description/Use	Questions Administered	Sensitivity	Specificity
McMonnies	Screening questionnaire—used in dry eye clinic Population	12 items—most dichotomous yes/no, weighted scoring	87%–98% compared to clinical diagnosis (39)	87%–97% compared to clinical diagnosis (39)
Ocular Surface Disease Index (OSDI)	Measures the **severity of DED**; end points in clinical trials, **symptoms**, functional problems, and environmental triggers queried for the past week. Validated in dry eye population and used as outcome measure in RCT	12-item questionnaire: Visual function (6); ocular symptoms (3); environmental triggers (3)	Done by Physician Rating Normal versus all DED: 60% Normal versus severe DED: 92% Based on Composite Score Normal versus all DED: 80% Normal versus severe DED: 87% (32)	Done by Physician Rating Normal versus all DED: 83% Normal versus severe DED: 83% Based on Composite Score Normal versus all DED: 79% Normal versus severe DED:96% (32)
IDEEL	Epidemiologic and clinical studies for impact of dry eyes on patients' lives. Validated in dry eye population of 210 subjected with range of dry eye severity.(38)	3 modules (57 questions) 1. Daily Activities 2. Treatment Satisfaction 3. Symptom Bother	NA: Item convergent validity (greater than 0.4), item discriminant validity ($\geq$ 90%), and test retest reliability (Cronbach's alpha exceeded the standard of 0.7) (40)	NA: Item convergent validity (greater than 0.4), item discriminant validity ($\geq$ 90%), and test retest reliability (Cronbach's alpha exceeded the standard of 0.7) (40)
Dry Eye Questionnaire	Characterize the frequency of ocular surface symptoms and their diurnal intensity	21 items: Frequency and intensity of symptoms	NA	NA
Short Dry Eye Questionnaire	Epidemiologic and clinical studies: includes 2 questions on symptoms	3 questions	Compared with the clinical exam in diagnosing DED: 76% With Schirmer $\leq$10mm or Rose Bengal staining of $\geq$3 unit: 82% (41) 77% with Schirmer 1 value $\leq$/=10 mm or tear breakup time <10 seconds (41)	Compared with the clinical exam in diagnosing DED: 83% With Schirmer $\leq$10mm or Rose Bengal staining of $\geq$3: 69% (41) 84% with Schirmer 1 value $\leq$/=10 mm or tear breakup time <10 seconds (41)
Women's Health Study Questionnaire	Women's Health Study/Epidemiologic Studies	3 items from 14-item original questionnaire	NA: similar to all 14-items included in questionnaire. Concurrent validity, internal reliability, and test-retest reliability were reported (42)	NA: similar to all 14-items included in questionnaire. Concurrent validity, internal reliability, and test-retest reliability were reported (42)

Information in this table was adapted from reference 1

2.5.   **Ocular Surface Disease Index**

The OSDI) is the most frequently used survey instrument for the assessment of ocular surface disease and its severity in DED research (43). The advantage of the OSDI is that it also measures severity of DED. It was developed by the Outcomes Research Group at Allergan Inc (Irvine, California), as a 12-item questionnaire designed to assess symptoms of ocular irritation and the impact on vision-related activity. The OSDI has three subscales: vision-related function, ocular symptoms, and environmental triggers. Each of the subscales has its own questions and each question has the same five-category Likert-type response. Schiffman et al. evaluated the reliability and validity of the OSDI in 109 patients with DED and 30 normal controls (32). The reliability as measured by the Cronbach's alpha ranged from good to excellent for the overall instrument (internal consistency 0.92 and test retest ICC 0.82) and each subscale (internal consistency range 0.78–0.92; test retest ICC 0.70–0.82). Discriminant and concurrent validity of the OSDI were assessed by evaluating OSDI scores by disease severity, and by correlating OSDI scores with other questionnaires, such as McMonnies, respectively (32). Sensitivity and specificity of the OSDI results are shown in Table I. Correlations of diagnosis with other questionnaires ranged from 0.24 to 0.77. The reliability and validity of the OSDI have also been tested using Rasch analysis (43). Dougherty et al. reported that the person separation index for the OSDI was at 2.16, demonstrating that the OSDI is an instrument that can be used for DED patients with varying levels of severity (43). Numerous studies have used the OSDI and several studies have used the OSDI to assess the severity of DED with certain conditions, such as Graves' disease and glaucoma (44,45,46) and to investigate the efficacy of dry eye treatments (47,48,49).

Unlike other symptom and pain inventories in chronic diseases, the OSDI only measures two domains of symptom burden: Persistence and activity interference. Given that DED is a chronic progressive disease and that discomfort and pain are the most widely reported

symptoms, additional symptom domains that are assessed in other chronic diseases, such as cancer, need to be included to improve the symptom assessment of DED. We therefore developed a tool from validated pain questionnaires, such as the Brief Pain Inventory, the Brief Fatigue Inventory, and the MD Anderson Symptom Inventory, to comprehensively evaluate the symptom burden of DED.

2.6.   **Assessment of Depression and the Beck Depression Inventory**

Depression is a subjective state of mind that is typically described and quantified using self-report measures. The BDI, the Center for Epidemiological Studies Depression Scale, the Hamilton Rating Scale for Depression, and the Zung Self-Rating Depression Scale are the widely used depression tests (50). The BDI is the best known and most widely used tool for measuring the severity of depression (50). The tool is a 21-question multiple-choice self-report inventory that was created by Aaron T. Beck and first published in 1961(51). The items for the BDI cover emotional, behavioral, and somatic symptoms. Beck, Steer, and Grabin concluded that reviews of factors analyses have identified three factors: Negative attitude toward self, performance impairment, and somatic disturbances (52). Table II shows two studies reporting the sensitivity and specificity of the BDI-21 compared to the Diagnostic and Statistical Manual of Mental DisordersDSM, ranging from 79% to 87.7% for sensitivity and 83.9 to 91% for specificity.

TABLE II
PSYCHOMETRIC PROPERTIES OF THE BECK DEPRESSION INVENTORY COMPARED TO
DSM

Study	Comparison	Population	Cut-off point	Sensitivity	Specificity	PPV	NPV
Yeung 2002 (53)	DSM-III-R	503 Chinese Americans, USA	≥16	79%	91%	79%	91%
Dutton 2004 (54)	DSM-IV	220 African American Primary Care Patients, USA	>14	87.7%	83.9%	70%	94%

The BDI has also been used to assess depression in patients with chronic debilitating

diseases (55,56) and with patients experiencing chronic pain. Geisser et al, examined the ability

of the BDI to discriminate between chronic patients with and without major depression, and

reported 68.2% for sensitivity (Se) and 78.4% for specificity (Sp) (57). Lustman et al. in 1997,

evaluated the BDI-21 as compared to the DSM-III in 172 diabetic patients at cut-off points of ≥8

(Se 99%, Sp 52%), 10 (Se 98%, Sp 70%), 12 (Se 90%, Sp 84%), 14 (Se 82%, Sp 89%), and 16

(Se 73%, Sp 93%). They reported the optimal cut-off point at ≥13 (58). Watnick et al. reported a

sensitivity of 91% and specificity of 86% as compared to the DSM-IV in dialysis patients at a

cut-off of 16 (59). Poole et al. showed that the BDI is an excellent tool for screening depression

in chronic pain patients when compared to the Structural Clinical Diagnostic Interview with a

large area under the ROC curve (0.97, 95% CI 0.93–1.02) (58). The optimal cut-off point was

reported to be at 22.0 with sensitivity being 89% and specificity 90% (60). The BDI has also

been shown as a valuable tool to assess depression in older adult patients (61). The scaling

and cut-off points used for depression varied between studies. The standard cut-off points have

been reported as follows: (0–9 minimal depression; 10–18 mild depression; 19–29 moderate

depression; 30–63 severe depression) (52). Suija K et al. evaluated the validity of the BDI in older adults with depression and chronic health problems and reported a sensitivity of 88.0% and a specificity of 81.7% for a cut-off value of 11 (62). Moreover, Anderson et al. reported that the self-report BDI is an accurate and reliable tool to use in studies with more than one time point (63). In this thesis, we measured depressive symptoms using the BDI.

As noted previously, a focus of this thesis is that DED and depression have common biological underpinnings through the mechanistic action of mutual SNPs. The SNPs used in this thesis are hypothesis-based and belong to the following genes: BDNF), eDNA, and VDR. The selection of these genes is based on a priori knowledge (gathered from the literature and from experiments in our laboratory) and assumptions of the potential involvement of these genes in the pathogenesis of DED, through the trigeminal ganglion (BDNF), the ocular surface (DNASE 1) and the eyelid (meibomian gland disfunction, VDR).

2.7.   **Hypothesized Single-Nucleotide Polymorphisms**

The BDNF is a member of the neurotrophin family and is widely expressed throughout the central nervous system. Findings from studies support a complex and functional role of BDNF in depression and antidepressant action (64,65). While investigating the molecular basis of symptoms in dry eye disease in our laboratory, we found that: (i) BDNF is expressed in inflamed corneas in the vicinity of nerves; (ii) BDNF expression is enhanced in the ipsilateral trigeminal ganglion of mice with corneal inflammation; (iii) mice with inflamed corneas have audible vocalization signifying pain perception; and (iv) corneal stromal fibroblasts robustly express BDNF, which can be detected in the conditioned media. Figure 1 shows the expression of BDNF (red) along the corneal nerves (red). 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 is the "injury switch" that leads to corneal allodynia and hyperalgesia. We further propose that BDNF expressed by the stromal fibroblasts in the cornea contributes to trigeminal/central sensitization.
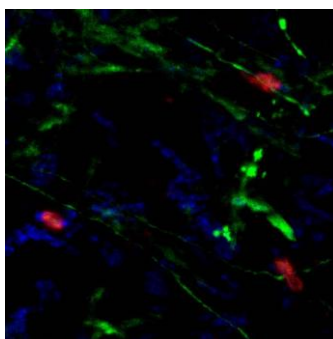
Figure 1: BDNF expression along corneal nerves.

The role of BDNF in depression has also been investigated by determining the association between gene-encoding variants. Single nucleotide polymorphism VAL66MET is a BDNF prodomain SNP resulting in a valine-to-methionine substitution that has been studied extensively and has been shown to be associated with depression-related phenotypes and brain alterations involving regions consistently association with depressive disorder (66–69). However, this association has been inconsistent as some studies reported no effect of VAL66MET on depression or on antidepressant treatment response (70,71). Recent meta-analysis have also been inconsistent, where some conclude that there is an association (72,73) and some conclude that there is no association (74,75). Potential reasons for this inconsistency include: presence of genetic heterogeneity, different populations, population stratification (one study found the VAL66MET to be associated with depression in men and not women; [76]),

gene-gene interactions, and gene-environment interactions. Interestingly and more recently, Jiang et al. showed that depression levels increased significantly more as a function of adulthood chronic stress among Val/Val genotype carriers than Met Carriers (77). Chen et al. also showed that the Val allele enhanced the correlation between stress life events frequency and adolescent depressive symptoms (78).

We investigated additional SNPs in the DNASE1 gene (ocular surface) and the VDR gene (meibomian gland). These genes have been shown 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 (79). We have shown that eDNA production and clearance mechanisms are dysregulated in DED (8). 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. The practical implication of our findings is the suggestion of new therapeutic interventions based on clearing eDNA, neurotrophic extracellular traps, and their molecular components from the ocular surface, as well as inhibiting eDNA signaling pathway gene expression. It therefore seems reasonable to explore SNPs in the DNASE1 gene that may be involved in the pathogenesis of DED and depression. Also VDR gene BSMl, Fokl, Apal and Taql polymorphisms have been investigated in the risk of SLE (80), which is also associated with depression (81).

3. MATERIAL AND METHODS

This thesis included more than one study design to address each specific aim: cross-sectional (Specific Aim A), case-control (Specific Aims B and C), and a follow-up cohort study of a subset of patients included in the case-control (section of Specific Aim C). This laddered approach for each aim allowed us to address the main goal of further understanding the links between DED and depression.

The sections below elaborate on the designs and samples for each aim, along with the statistical methods used. All analyses were performed using SAS software (SAS Institute Inc., Cary, North Carolina), and STATA SE 12.0 (StataCorp LP., College Station, Texas) software. PLINK 1.07 (http://pngu.mgh.harvard.edu/~purcell/plink/index.shtml) was additionally used for the genetics data in Specific Aim C.

3.1 Specific Aim A: Develop A Dry Eye Disease Symptom Burden Tool Consisting of a Sensory Domain (Symptom Persistence and Intensity) and Reactive Domain (Activity and Affective Interference) to Measure Symptoms in Dry Eye Disease Patients in a Cross-Sectional Study

3.1.1 Key Questions that were Explored

Which sensory (symptom persistence and intensity) and reactive (activity and affective interference) domains of symptom analysis are essential for assessing symptom burden in DED patients? What are the roles of symptom persistence and symptom intensity of DED, and their impact on activity and affective interference? Do they have roles in treatment decisions? Do the domain scores of persistence and activity interference from this new symptom burden tool correlate with the similar domains present in the OSDI questionnaire, which does not contain a mood/affect domain?

3.1.2 **Study Design and Study Sample**:

A cross-sectional pilot study was performed with 48 patients visiting our Dry Eye Clinic of the Department of Ophthalmology and Visual Sciences at the University of Illinois at Chicago over a period of six months from October 2012 through March 2013. New and established patients were included. This sample is best described as a convenience sample given our limited budget and that patients had the choice of participating or not participating in the study.

3.1.3. **Tools/Questionnaires and Clinical Assessment of Dry Eye Disease**

A four-domain DED symptom burden tool was developed adapting methods from well-established and validated symptom burden tools. For example, the affective interference domain included the same questions from the MD Anderson Symptom Questionnaire (mood, enjoyment of life, and social relations with others). The symptom burden tool along with the already established OSDI questionnaire (which had demonstrated discriminant and concurrent validity) were administered to the sample of 48 DED patients at one time point in an interview during one visit. Subjects were asked about the persistence of their symptoms over the past week to minimize recall limitation.

Additionally, DED was confirmed through clinical examination that included measuring tear production by the Schirmer test (without anesthesia) at five minutes, using Whatman filter strips #41 (Haag-Streit, Essex, UK). Ocular surface disease was also assessed using Rose Bengal dye, where saline moistened (1%) Rose Bengal-impregnated strips were used to instill the dye on the inferior palpebral conjunctiva, and scoring of corneal and conjunctival staining was performed by a slit lamp examination after 15 seconds.

The inclusion criteria for the DED patients were Schirmer test results of <10 mm in either eye and Rose Bengal corneal and conjunctival staining of ≥1. Patients who were less than 18 years and women who were pregnant were excluded from the study.

To determine whether intensity of symptoms correlated with treatment decision, out of the 48 subjects included in the study, we randomly selected 9 pairs (18 patients), where each pair had equal symptom persistence scores but varying intensity scores. Prescribed treatments for each patient pair were collected. We scored each treatment option as either 1 point or 2 points as follows: artificial tears (1 point); Restasis (1 point); doxycycline/erythromycin eye ointment (1 point); steroids (2 points); therapeutic contact lens use (2 points); serum/DNase/other (2 points). Total treatment scores were then computed for each patient.

To determine the total score for the symptom burden tool, a weighted item response analysis was performed: items from the persistence domain were summed and multiplied with the intensity, and the sum of activity and affective scores was then added to compute a total symptom burden score. Intensity scores were computed by multiplying the overall intensity with the number of times a patient reported waking up at night due to symptoms. The OSDI (index) score was calculated from OSDI item responses following standard procedures.

3.1.4 **Statistical Analysis**

Items in each domain were summed to generate domain scores. Domain scores were then standardized by subtracting the mean from each individual score in each domain and dividing by the standard deviation (SD) to generate normalized comparative scores. Q-Q plots and Shapiro Wilk tests were run to determine whether the data are normally distributed. Inter-domain correlations were performed using the nonparametric Spearman test for each of the symptom burden and OSDI questionnaires, to determine whether persistence of symptoms with or without intensity correlated with activity and affective interference. Pearson correlation was not utilized because the data were not normally distributed; however, fitted lines with scatter plots were generated for data representation. Cross-domain and subscale correlations were also performed. Subscales A and B in the OSDI were considered to represent persistence of symptoms and activity interference, respectively.

To determine whether intensity of symptoms correlated with treatment decision, the total treatment score assigned to subjects in each pair were compared using a matched paired t-test.

Bland-Altman analyses were also performed to determine agreement between normalized symptom burden scores and normalized OSDI scores. A range of agreement was defined as mean ± 2 SD. Confidence intervals (CIs) at the 95% level were computed, and significance was determined if the interval did not include 0.

3.2 Specific Aim B: Measure Depressive Symptoms in DED Patients and Controls Using the BDI Questionnaire and Determine the Correlation between these Symptoms and DED Symptoms.

3.2.1 Key Questions that were Explored

Do DED patients exhibit more depressive symptoms than controls? Are there differences in BDI scores in DED patients diagnosed with depression compared to DED patients without clinical diagnosis of depression?

3.2.2 Study Design and Study Sample

A case-control study was performed with 53 cases and 41 controls. The cases included DED patients who visited our clinic between November 2012 and June 2014. The diagnostic criteria were as follows: (i) a reporting of symptoms: burning sensation, irritation, grittiness or foreign body sensation, light sensitivity, pain, dryness, soreness, or discomfort in the eye; (ii) a Schirmer value of <10mm/5min in either eye using Whatman filter strips #41 (Haag-Streit, Essex, UK); or (iii) positive corneal staining and/or Rose Bengal corneal and conjunctival staining of >1 (1). As mentioned in Specific Aim A, the sampling method intended a sample of all DED patients attending the clinic between the dates mentioned above. However, given that

subjects are asked whether they would like to participate in this study, the final sample is characterized as a convenience sample.

The controls were recruited from our general eye clinic with refraction-related complaints. The inclusion criteria included no clinical diagnosis of DED, a Schirmer value of >10mm/5min, and no corneal staining. None of the control subjects enrolled were using tear supplements. Sampling was similar to cases, where an "all sample" technique was intended, however taking into consideration the willingness to participate characterizes this as more of a convenient sample.

3.2.3 <u>**Assessment of Depressive Symptoms and History of Depression**</u>

Depressive symptoms were measured using the BDI. The BDI is a widely used tool for measuring the severity of depressive symptoms. The standard cut-off points for the BDI are 0–9: indicating minimal depression; 10–18: indicating mild depression; 19–29: indicating moderate depression; 30–63: indicating severe depression. In this study, the BDI score was divided into $\leq$9 or >9. Depression status was determined as a composite variable through chart review as "ever having depression" through medical and psychological history and/or through any history of prescribed medications specific to depression. This composite variable was determined because for some patients a diagnosis of clinical depression in their charts was not indicated; however antidepression medication was listed among their medications. Psychiatric medication was also determined as a separate variable.

3.2.4 <u>**Assessment of Dry Eye Disease Symptoms**</u>

Similar to Specific Aim A, DED symptoms were assessed using two tools: The Symptom Burden Tool and the OSDI tool. The Symptom Burden Tool assesses four domains: persistence, intensity, activity, and affective interference. The OSDI tool measures persistence, activity interference, and environmental triggers. Scores on the OSDI range from 0 to 100 and

from 0 to 520 for the Symptom Burden Tool. The use of the two tools is complementary,

because the more used and validated tool OSDI does not contain an affective domain.

3.2.5 **Statistical Analysis**

Histograms with the normal curve, Q-Q plots, and the statistical tests for normality were

used to determine if the data is normally distributed. Demographic data were summarized as

means $\pm$ SD and percent distribution. For data representation and clinical interpretation, OSDI

scores, DED symptom burden questionnaire scores, and BDI scores were summarized as mean

$\pm$ SD. Regression diagnostics (studentized residuals and leverage) were performed to detect

any outliers and unusual influential data. Scatter plots were generated with fitted lines between

DED symptoms and depression symptoms. Linear statistical models and polynomial regression

were run to determine the type of relationship between depressive symptoms and DED

symptoms.

Independent t-test was performed to determine differences of BDI scores between cases

and controls. Similarly, independent t-test comparison of DED symptom scores among cases

diagnosed with depression and those without depression was also performed. Linear regression

was used to estimate the association between DED symptom continuous scores and BDI

depression scores. Logistic regression was used for the DED dichotomous outcome and

depression status as exposure. Unadjusted and adjusted regression analysis was performed.

Chi-square and Fisher's exact test were used for categorical variables. Medians were also

calculated for each scoring variable. Statistical significance was set at 0.05. Data were analyzed

using STATA/SE v12 software.

3.3   **Specific Aim C. Investigate Whether DED and Depression Are Linked through Common Genetic Polymorphisms Using a Case-Control Study (C1), and Determine Whether the Response to DED Treatment Is Related to the Presence of Specific SNPs in a Subset of DED Patients (C2).**

3.3.1   **Key Questions that Were Explored**

Are SNPs in the BDNF, DNASEI, or VDR genes associated with DED? Are SNPs in the BDNF, DNASEI, or VDR genes associated with depression? Do any of these SNPs play a role in treatment response of DED?

3.3.2   **Study Design and Study Sample**

This was a case-control study for Specific Aim C1 and follow-up cohort for Specific Aim C2. For Specific Aim C1, 64 cases and 51 controls were recruited from our Dry Eye Clinic at the Department of Ophthalmology and Visual Sciences, University of Illinois at Chicago. The DED sample included patients who visited our clinic from 2012 till 2014. Similar to Specific Aim B, the diagnostic criteria were: (i) a reporting of symptoms: burning sensation, irritation, grittiness or foreign body sensation, light sensitivity, pain, dryness, soreness, or discomfort in the eye; (ii) a Schirmer value of <10mm/5min in either eye using Whatman filter strips #41 (Haag-Streit, Essex, UK); or (iii) positive corneal staining and/or Rose Bengal corneal and conjunctival staining of >1 (1). Similar to Specific Aims A and B, the sampling method intended a sample of all DED patients attending the clinic between the dates mentioned above. However, given that subjects are asked whether they would like to participate in this study, the final sample is characterized as a convenience sample.

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 >10mm/5min, and no corneal staining. None of the control subjects enrolled were using tear supplements.

For Specific Aim C2, a subset of the cases in Specific Aim C1 was randomly selected. The intent was to select 50% of the cases. Taking into consideration that some of these patients may be lost to follow-up; a simple random sample of 36 patients was performed. Patients were then followed up over time between November 2012 and June 2014 for clinical and symptom assessment. Patients were asked to rate their symptoms on a scale of 0 to 4 related to: dryness, irritation, light sensitivity, and pain at baseline and at their follow-up. Additionally, during each follow-up, patients were asked about their symptom experience as follows: (i) no change; (ii) 25% better; (iii) 50% better; (iv) 75% better; or (v) worse. Prescribed DED treatments for each patient were also collected. The last follow-up visit for each patient was selected because a minimum of six months follow-up was required if a patient were to show significant improvements in symptoms.

3.3.3 <u>**Assessment of Gene Polymorphisms**</u>

A saliva sample of 2ml was collected, following a routine eye exam, in a saliva collection kit (Oragene Sample DNA collection kit) and transported to the laboratory. The kit is pretreated for DNA stabilization, extraction, and purification. Following DNA extraction, the samples were sent to the Duke Molecular Physiology Institute. They were diluted to 1ng/ul using UltraPure™ DNase/RNase-Free Distilled Water (Life Technologies, Grand Island, New York). A total of 3ng of each sample was transferred to a 384 well plate using an epMotion 5075 TMX automated pipetting system (Eppendorf North America, Hauppauge, New York).

Genotyping was performed using both custom and predesigned TaqMan® SNP Genotyping Assays (Life Technologies, Grand Island, New York) for the 12 SNPs. Table III lists the SNPs and their primers. The PCR was performed according to manufacturer protocols on 3 ng of genomic DNA in 5 µl reaction volumes, using the GeneAmp® 9700 Dual 384-Well PCR

system (Life Technologies, Grand Island, New York) and subsequently scanned on a ViiA™ 7

Real-Time PCR System (Life Technologies, Grand Island, New York). Data were assessed on,

and exported from, ViiA 7 RUO Software v1.2.1. CEPH samples (NIGMS Repository, Coriell

Institute for Medical Research, Camden, New Jersey), study sample replicates, and no template

controls (NTCs) were used for quality control (QC). The QC replicates were required to match

100%, and NTCs were required to have no amplification.

For example, BDNF genotyping was performed as described in previous studies (82).

The following primers 5-ACT CTG GAG AGC GTG AAT GG-3 and 5-ACT ACT GAG CAT CAC

CCT GGA-3, a 171 base-pair product will be amplified, followed by digestion with PmaCI

restriction enzyme and agarose gel electrophoresis. The following genotypes will be assigned:

GG two bands 99 bp and 72 bp; GA two bands 171 and 99 bp; AA one band 171 bp.

TABLE III
SNPS AND THEIR PRIMERS USED FOR GENOTYPING

Gene	rs no.	Alleles	Primers for PCR amplification (5' – 3')
BDNF	Rs6265	G/A	F:ACTCTGGAGAGCGTGAATGG
			R: ACTACTGAGCATCACCCTGGA
DNASEI	R-21S (rs8176927)	G/T	F: GCCAGCTGTTTGGCTTTCTGGA
			R: CAGCGCCCCCAGCAGCTTCAT
DNASEI	Y95S (rs34923865)	A/C	F: AGGTGTCTGCGGTGGACAGGT
			R: GTGTGTGACACAGGCATTCCA
DNASEI	R105G (rs8176919)	G/A	F: CAGGTGTCTGCGGTGGACAGC
			R: GTGTGTGACACAGGCATTCCA
DNASEI	P132A (rs1799891)	C/G	F: GCTGACATGGTGACTGAACCT
			R: ATAGGCACAGTGCGTGGGTGT
DNASEI	Q222R (rs1053874)	A/G	F: CATCTGGGGATAAGAGGAGAG
			R: AGTCGGGAACAACGGCGACT
DNASEI	L186L (rs8176920)	A/G	F: TCCCAGTGGTCATCCATCCGCAT
			R: CTTTGAGGCTTCTGAAGCCCG
DNASEI	P197S (rs34186031)	C/T	F: GACGTCATGTTGATGGGCGA
			R: ATAGGCACAGTGCGTGGGTGT
VDR	rs2228570	C/T	F: GCACTGACTCTGGCTCTGAC
			R: ACCCTCCTGCTCCTGTGGCT
VDR	rs1544410	A/G	F: GGAGACACAGATAAGGAAATAC
			R: CCGCAAGAAACCTCAAATAACA
VDR	rs7975232	A/C	F: TGGGCACGGGGATAGAGAAG
			R: ACGGAGAAGTCACTGGAGGG
VDR	rs731236	T/C	F: TCCTGTGCCTTCTTCTCTATC
			R: CTAGCTTCTGGATCATCTTGG

3.3.4 <u>**Sociodemographic Variables and Assessment of Depression**</u>

Demographic data (date of birth, gender, race) were collected from medical records of cases and controls. As in Specific Aim B, depression (yes/no) was assessed through medical chart review of any diagnosed history of clinical depression and ever being prescribed antidepressants. Depression status was determined as a composite variable through chart review as "ever having depression" through medical and psychological history and/or through any history of prescribed medications specific to depression. This composite variable was determined because for some patients a diagnosis of clinical depression in their charts was not indicated; however antidepressant medication was listed among their medications.

Additionally for Specific Aim C2, prescribed DED medication was extracted from medical records. Each treatment option was scored as either 1 point or 2 points as follows: artificial tears (1 point); Restasis (1 point); doxycycline/erythromycin eye ointment (1 point); steroids (2 points); therapeutic contact lens use (2 points). Points were summed, ranging from 0 to 7.

3.3.5 <u>**Statistical Analysis**</u>

For Specific Aim C1: The SNPs were tested for Hardy-Weinberg equilibrium (HWE) using χ2-test. Any SNPs that deviated from HWE (P<0.01) were excluded from further analysis. Genotype and allele frequencies of cases and controls were evaluated. The trend P-Value for additive effects was determined for genotypes, and the allele frequencies were evaluated using chi-square. Odds ratios and 95% CIs were also calculated for allele frequencies. Logistic regression was also performed to determine the association between SNPs and DED. Stratified analysis was performed to determine if the association between SNPs and DED varied by depression status.

For Specific Aim C2, histograms with the normal curve, Q-Q plots, and the statistical Shapiro-Wilk test were used to determine if the data were normally distributed. Demographic data were summarized as means $\pm$ SD and percent distribution. Baseline symptom scores were

calculated and compared with scores from the last follow-up time point for each patient using a paired t-test. To test our hypothesis, we stratified change in symptom score by genotype. Given that data from some of our patients were incomplete and that some were lost to follow-up, we compared baseline characteristics of those who were included in the follow-up analysis and those who were not (missing).

4. STUDY 1
SYMPTOM BURDEN OF PATIENTS WITH DRY EYE DISEASE: A FOUR DOMAIN ANALYSIS[1]

Dry eye disease is a complex symptomatic disease with inexplicable clinical variations. With a prevalence ranging from 5% to more than 35% at various ages (1), DED is one of the leading causes of patient visits to ophthalmologists and optometrists in the United States due to its debilitating symptoms (11,13). Several clinical tests are available to measure the aspects of DED. However, there is no gold standard for diagnosis, and clinicians rely on patient reported symptoms of ocular discomfort to make treatment decisions.

The reported symptoms of DED include pain, dryness, grittiness, itchiness, redness, burning or stinging, foreign body sensation, and light sensitivity. These symptoms have been reported to negatively impact the quality of life, with a greater risk of depression and anxiety for those with more symptoms (4,5). Given the variability of clinical tests, assessing DED symptoms in their entirety becomes fundamentally important to guide treatment decisions. In other chronic diseases, symptoms are thought of as a "burden," and are measured in domains to encompass both the persistence and intensity of the symptoms and the patient's perception of the impact of the symptoms (83,84). The total assessment of symptoms in similar domains is not often used in DED. While there are tools that measure the entire scope of DED, their utility is limited to clinical research. Developing a brief tool that comprehensively measures the symptom burden of DED without increasing respondent burden is needed for daily clinical use and diagnosis. A starting point is to adopt concepts used to measure symptoms in other diseases, and tailor them to symptoms of DED.

The method of domain assessment of symptoms is used in chronic diseases such as symptom control of cancer, especially when cure or remission is no longer possible (84). Pain

---

[1] Published in Plos One: Hallak, J.A., Jassim, S., Khanolkar, V., Jain, S.. Symptom burden of patients with dry eye disease: a four domain analysis. *PLoS One.* 2013 Dec 13;8(12).

questionnaires, such as the Brief Pain Inventory, the Brief Fatigue Inventory, and the MD Anderson Symptom Inventory were developed to measure pain and discomfort. These tools are designed to assess symptoms in multiple dimensions and domains (84). The domains include intensity and severity (sensory dimension), and affective and activity interference (reactive dimension) (83,84). The rationale for use of a four-domain tool is that it is specifically tailored to measuring multiple patient-reported symptoms and their impact. This applies very well to DED, given the underlying neurophysiological mechanisms of pain, and that DED is a chronic progressive disease. We therefore hypothesize that a more complete symptom assessment using four domains that characterize the "symptom burden" of DED will be more reflective of the disease and will better indicate optimal treatment.

In this study, we developed a tool to investigate the four-domain symptom burden of DED for ease of use in clinical settings, to determine the roles of symptom persistence and symptom intensity of DED, and their impact on activity and affective interference. We also performed a cross-sectional pilot study administering both the DED symptom burden tool and the OSDI questionnaire for cross-comparison.

4.1   **Methods**

Study approval was obtained from the Institutional Review Board of the University of

Illinois at Chicago. Symptomatic patients with DED 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.

4.1.1   **Developing a Four-Domain Symptom Burden Tool**

Based on our findings from the literature regarding DED symptoms, a four-domain DED

symptom burden tool was developed adapting methods from well-established and validated

symptom burden tools. For example, the affective interference domain included the same

questions from the MD Anderson Symptom Questionnaire (mood, enjoyment of life, and social

relations with others).

Classification into dimensions and domains: The two main dimensions assessed were

sensory and reactive dimensions. Based on these dimensions the symptom burden was

classified into four main domains (Figure 2): (i) Sensory Dimensions—Symptom Persistence

and Symptom Intensity, and (ii) Reactive Dimensions—Activity Interference and Affective

Interference. Symptom persistence can be defined as the continuous occurrence of symptoms,

whereas symptom intensity is the severity of symptoms. Activity interference is the effect of dry

eye symptoms on day-to-day activities of an individual. Affective interference is the effect on the

emotional and social well-being of an individual due to dry eye symptoms.

Scales used in domains: Various scales were used that organized symptoms into

domains (Figure 2). These scales can be described as follows: (i) Verbal Descriptive Scale,

which classifies symptoms according to the methods of assessment that include measurements

of mild/moderate/severe symptoms, (ii) Visual Analog Scale, a scale used frequently in the

measurement of DED symptoms (85), is used for describing pain that cannot be characterized

by words with the use of visual images on a scale of one to ten, (iii) Numerical Rating Scale

classifies symptoms based on numerical scales such as 0 to 20 or 0 to 75, and is used as a

mode of assessment of symptoms in dry eye studies, and (iv) Verbal Rating Score, which

describes the occurrences of symptoms as none of the time, some of the time, most of the time,

or all of the time.

The four-domain symptom burden tool that we developed is shown in Figure 3. A visual

rating scale was used for the persistence, activity, and affective interference domains, and a

combination of scales (visual analog and numerical) was used for the intensity domain. The

visual analog scale is commonly used for assessing severity of symptoms (acuteness of pain) in

a variety of settings. After generating the symptom burden tool, a cross-sectional pilot study was

performed where the DED symptom burden tool and the OSDI questionnaire were administered

to 48 patients.

A

B
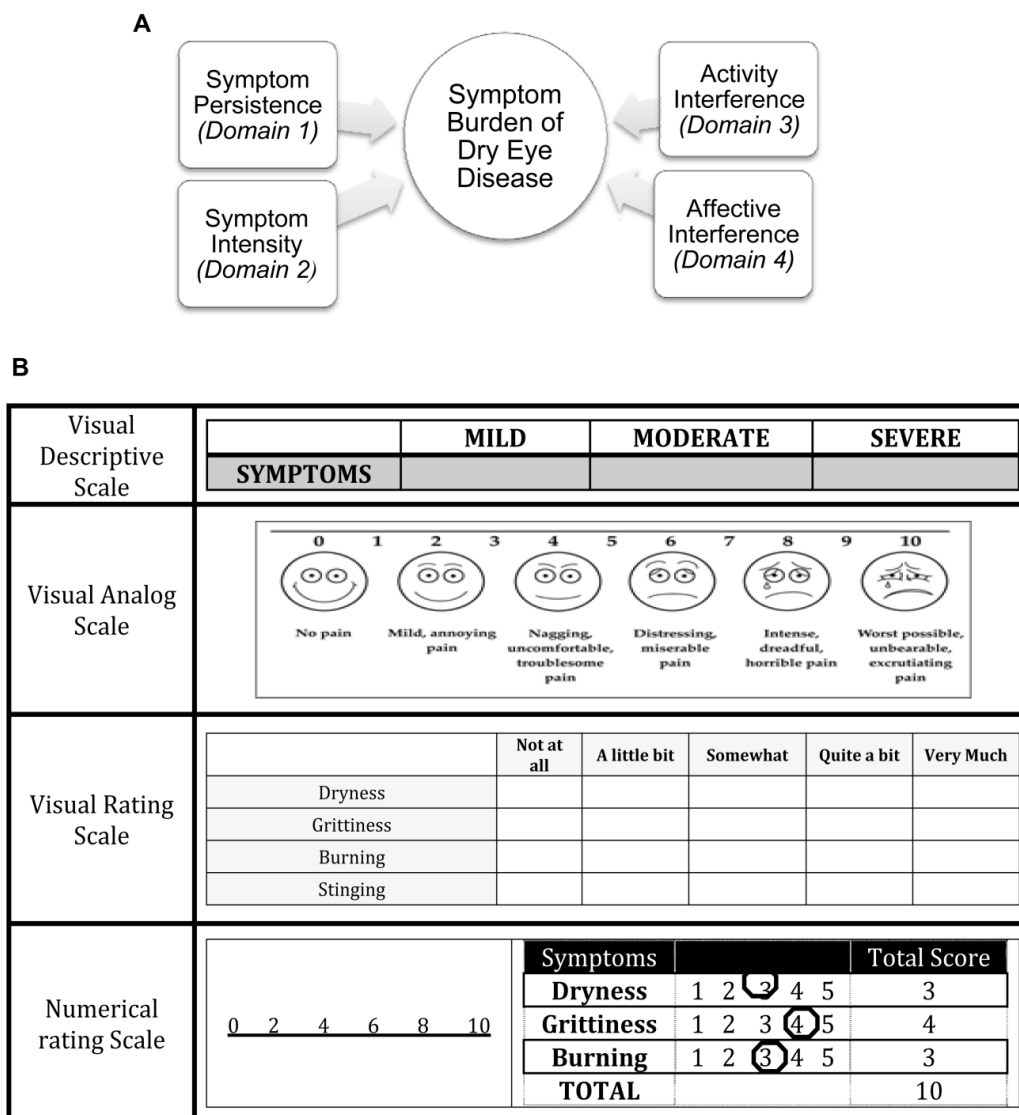
Figure 2. Symptom burden domains and measurement scales. (A). Domains of Symptom Burden of Dry Eye Disease. The symptom burden of dry eye disease is divided into two main dimensions: sensory dimension and reactive dimension. The sensory dimension is divided into two domains, symptom persistency and symptom intensity, while the reactive dimension is divided into activity interference and affective interference [Adapted from reference # 84]. (B). Scales for Measuring Symptoms. The visual analog and numerical scales are used to measure intensity of symptoms, whereas the visual rating scale is used to measure persistence of symptoms.

Domain 1: Symptom Persistence
In the table below, check the persistence of your symptoms, at its worst, over the past week?

	None	Some of the days	Half of the days	Most of the days	All of the days
Dryness					
Irritation / Burning or Stinging / Grittiness or Foreign body sensation					
Light sensitivity / Blurred or Poor Vision					
Pain, Soreness or Discomfort in eye					

Domain 2: Symptom Intensity
On a scale of 0–10 what was the intensity of your symptoms, at its worst, over the past 24 hours?

	Overall	Morning	Afternoon	Evening	Night Do you wake up at night due to symptoms?
Intensity 0-10					YES/ NO Number of times:

0   1   2   3   4   5   6   7   8   9   10

Domain 3: Activity Interference
How much have the symptoms interfered with how you function?

	Not at all	A little bit	Somewhat	Quite a bit	Very Much
Reading					
Watching television or movies					
Using a computer					
Daytime Driving					
Night Driving					
Performing Household Work					
Performing Professional Work					

Domain 4: Affective Interference
How much have the symptoms interfered with how you feel?

	Not at all	A little bit	Somewhat	Quite a bit	Very Much
Mood					
Enjoyment of Life					
Relations with others / socializing					

Figure 3. Dry Eye Symptom Burden Tool consisting of four domains: Symptom persistence, symptom intensity, activity interference, and affective interference.

4.1.2 **Data Collection and Patient Population**

Patients were recruited from the Dry Eye Clinic of the Department of Ophthalmology and Visual Sciences, University of Illinois at Chicago. New and established patients, diagnosed with DED through the assessment of symptoms and clinical signs, were recruited over the course of a six-month period, from October 2012 through March 2013. The symptom burden tool and the OSDI questionnaires were administered to patients in an interview during one visit. Subjects were asked about the persistence of their symptoms over the past week to minimize recall limitation.

Clinical examination included measuring tear production by the Schirmer test (without anesthesia) at five minutes, using Whatman filter strips #41 (Haag-Streit, Essex, UK). Severity of ocular surface disease was assessed using Rose Bengal dye. Saline moistened (1%) Rose Bengal-impregnated strips were used to instill the dye on the inferior palpebral conjunctiva, and scoring of corneal and conjunctival staining was performed by a slit lamp examination after 15 seconds.

The inclusion criteria were patients with Schirmer test results of <10 mm in either eye and Rose Bengal corneal and conjunctival staining of ≥1. Patients who were less than 18 years and women who were pregnant were excluded from the study.

To determine whether intensity of symptoms correlated with treatment decision, out of the 48 subjects included in the study, we randomly selected 9 pairs (18 patients), where each pair had equal symptom persistence scores but varying intensity scores. Prescribed treatments for each patient pair were collected. We scored each treatment option as either 1 point or 2 points as follows: artificial tears (1 point); Restasis (1 point); doxycycline/erythromycin eye ointment (1 point); steroids (2 points); therapeutic contact lens use (2 points); serum/DNase/other (2 points). Total treatment scores were then computed for each patient.

A weighted item response analysis was performed for the symptom burden tool: items from the persistence domain were summed and multiplied with the intensity, and the sum of activity and affective scores was then added to compute a total symptom burden score. Intensity scores were computed by multiplying the overall intensity with the number of times a patient reported waking up at night due to symptoms. The OSDI (index) score was calculated from OSDI item responses following standard procedures (32).

4.1.3 **Statistical Analysis**

Items in each domain were summed to generate domain scores. Domain scores were then standardized by subtracting the mean from each individual score in each domain and dividing by the SD to generate normalized comparative scores. The Q-Q plots and Shapiro Wilk tests were run to determine whether the data were normally distributed. Inter-domain correlations were performed using the nonparametric Spearman test for each of the symptom burden and OSDI questionnaires, to determine whether persistence of symptoms with or without intensity correlated with activity and affective interference. Pearson correlation was not utilized because the data were not normally distributed; however fitted lines with scatter plots are shown for data representation. Cross-domain and subscale correlations were also performed. Subscales A and B in the OSDI were considered to represent persistence of symptoms and activity interference, respectively.

To determine whether intensity of symptoms correlated with treatment decision, the total treatment score assigned to subjects in each pair were compared using a matched paired t-test.

Bland-Altman analyses were performed to determine agreement between normalized symptom burden scores and normalized OSDI scores. A range of agreement was defined as mean ± 2 SD. All analyses were performed using SAS software (SAS Institute Inc., Cary, North Carolina) and STATA (StataCorp LP., College Station, Texas) software. Confidence intervals at the 95% level were computed, and significance was determined if the interval did not include 0.

4.2   **<u>Results</u>**

The patient population consisted of 32 females and 16 males with mean age of 52.8 years. Ten patients were diagnosed with autoimmune DED, 32 with non-autoimmune DED, and 7 with graft-versus-host disease (GVHD)-related DED.

Within the symptom burden tool, higher correlations were observed between persistence of symptoms and affective interference than persistence of symptoms and activity interference (r =0.62; 95% CI [0.39, 0.77] versus r =0.58; 95% CI [0.35, 0.75]) (Table IV). The correlation between the OSDI persistence subscale and affective interference domain in the symptom burden tool was r =0.73; 95% CI [0.56, 0.84] (Table IV). Multiplying the persistence of symptoms with the intensity did not improve the correlation in the symptom burden tool for activity interference (r =0.54) and for affective interference (r =0.56). Correlations between intensity of symptoms alone, and activity and affective interference were low, r =0.37 [95% CI 0.08, 0.60] and r =0.38 [95% CI 0.09, 0.60], respectively, with the symptom burden tool.

TABLE IV

SPEARMAN CORRELATION BETWEEN AND ACROSS DOMAINS IN THE SYMPTOM BURDEN TOOL (SB) AND THE OSDI QUESTIONNAIRE

Domain r [95% CI]	Persistence SB	Intensity SB	Persistence x Intensity (SB)	Activity Interference SB	Affective Interference SB	Persistence OSDI	Activity OSDI
Persistence SB	1.00	0.27 [-0.03,0.53]	0.75 [0.59,0.86]	0.58 [0.35,0.75]	0.62 [0.39,0.77]	0.76 [0.60,0.86]	0.53 [0.29,0.72]
Intensity SB	0.27 [-0.03,0.53]	1.00	0.81 [0.67,0.89]	0.37 [0.08,0.60]	0.38 [0.09,0.60]	0.47 [0.21,0.67]	0.26 [-0.03,0.52]
Persistence x Intensity	0.75 [0.59,0.86]	0.81 [0.67,0.89]	1.00	0.54 [0.3,0.73]	0.56 [0.32,0.74]	0.76 [0.59,0.86]	0.55 [0.30,0.73]
Activity Interference SB	0.58 [0.35,0.75]	0.37 [0.08,0.60]	0.54 [0.3,0.73]	1.00	0.68 [0.48,0.81]	0.60 [0.37,0.76]	0.79 [0.65,0.88]
Affective Interference SB	0.62 [0.39,0.77]	0.38 [0.09,0.60]	0.56 [0.32,0.74]	0.68 [0.48,0.81]	1.00	0.73 [0.56,0.84]	0.64 [0.43,0.79]
Persistence OSDI	0.76 [0.60,0.86]	0.47 [0.21,0.67]	0.76 [0.59,0.86]	0.60 [0.37,0.76]	0.73 [0.56,0.84]	1.00	0.55 [0.30,0.72]
Activity OSDI	0.53 [0.29,0.72]	0.26 [-0.03,0.52]	0.55 [0.30,0.73]	0.79 [0.65,0.88]	0.64 [0.43,0.79]	0.55 [0.30,0.72]	1.00

SB: Symptom Burden; OSDI: Ocular Surface Disease Index
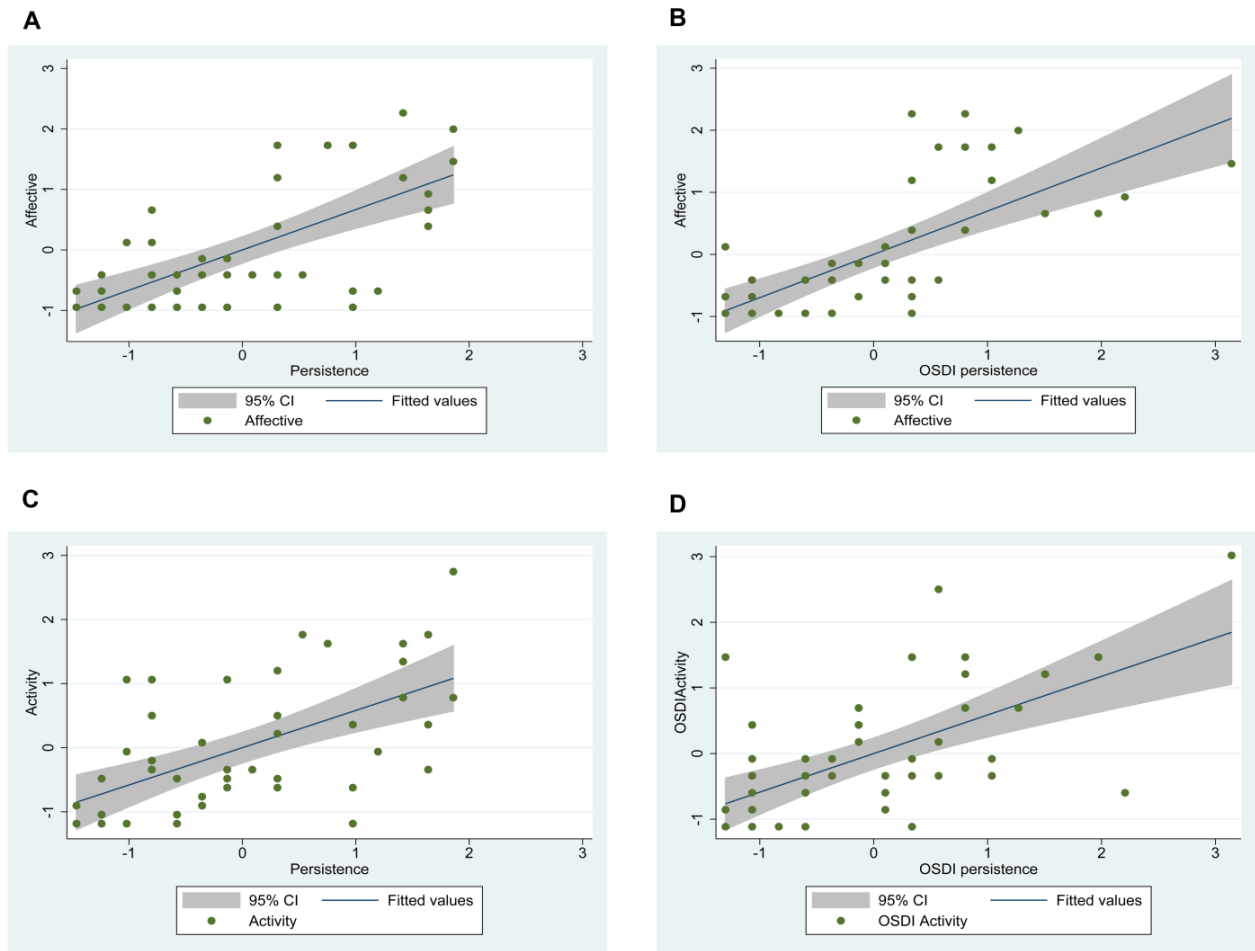
Figure 4. A−D show the scatter plots, with the best fitted lines and the 95% CI between scores of persistence of symptoms and affective interference and persistence of symptoms and activity interference with the symptom burden tool and OSDI questionnaires. The best fitted linear relationship is shown between persistence of symptoms as measured by the OSDI subscale A and affective interference as measured with the symptom burden tool (R^2 =0.49) (Figure 4B).

Bland-Altman analysis showed that most values are between +/- 2 SD of the mean difference between the symptom burden tool scores and OSDI scores. The 95% confidence limits of agreement between the two methods ranged from -1.7 to 1.7, depicting good agreement between OSDI total scores and the symptom burden total scores (Figure 5). With regard to the effect of intensity of symptoms on treatment decision, 6 out of the 9 pairs (66.7%) had patients reporting high-intensity symptom burden 33.3% (3 of 9 pairs) were patients reporting low-intensity symptom burden (A difference between high and low intensity of >4 was used as a cutoff point). The mean difference in symptom burden score between high and low intensity was 10.56 (SD=5.68) (14.89 high versus 4.33 low, p<.0001). The mean treatment score for patients with high-intensity symptoms was 6.33 (SD=1.32) and 3.78 (SD=1.92) for patients with low-intensity symptoms, for a mean difference in treatment score of 2.56 (SD=2.96, p=.03). There were three patients with low intensity who received aggressive treatment, mainly due to either neuropathic pain (one patient with post-LASIK DED reported high intensity), or due to a marginal difference in intensity with equal persistence.
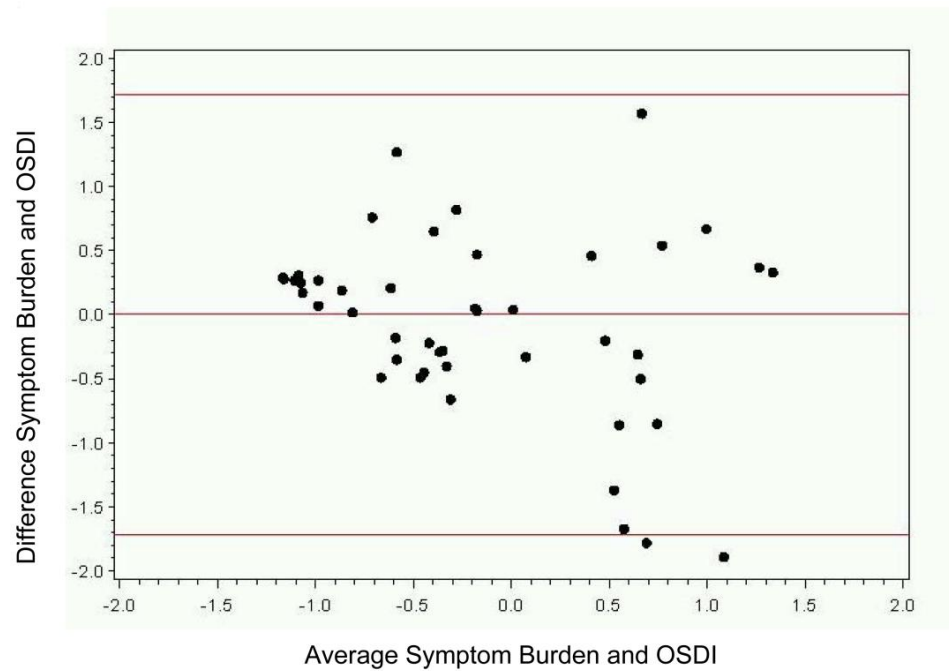
Figure 5. Bland-Altman plot for assessing symptoms of DED with the symptom burden tool and OSDI. The 95% confidence limits of agreement between the two methods ranged from -1.7 to 1.7. As shown, 46 out of 48 scores are within the 95% CI, indicating good agreement between the OSDI and symptom burden scores.

4.3   **Discussion**

This study revealed three main findings: (i) affective interference correlates more strongly with persistence of DED symptoms, (ii) the synergistic effect of intensity of symptoms with persistence of symptoms did not increase the correlation, and (iii) intensity of symptoms may play a role in treatment decisions. The persistence of symptoms in the OSDI did show a moderate correlation with activity interference. However, activity interference alone may not be a good index of the overall suffering of DED patients because it overlooks the emotional and psychological aspects (affective interference). Our results showed that the persistence of symptoms in OSDI actually correlated better with affective interference in the symptom burden tool rather than with activity interference. Therefore, our pilot data make a case for including affective interference in tools that assess DED symptoms. Evaluating the symptom burden of DED in its entirety will allow us to better delineate responses to treatments.

Dry eye disease has been shown to negatively impact the quality of life of patients, including general quality of life and vision-related quality of life (86,87). Furthermore, DED has been shown to be correlated with anxiety and depression (4,5,27). The negative impact on the quality of life is mainly due to the progression of dry eye symptoms, creating a complex situation that interferes with daily activities and the emotional state of DED patients (1). The OSDI activity interference has been utilized by studies to measure the impact of DED on quality of life (33). However, the OSDI does not include an affective component. It is a disease-specific questionnaire that includes three subscales or domains: ocular discomfort (OSDI symptoms, equivalent to persistence); functioning (OSDI function, equivalent to activity interference); and environmental triggers (OSDI triggers) (32,88). In addition to the OSDI, studies have also used more generic instruments such as the National Eye Institute Visual Function Questionnaire (NEI-VFQ) to measure the quality of life of DED patients (33,89). The NEI-VFQ is a 25-item questionnaire with 11 subscales/domains, of which mental functioning is one. Vitale et al. compared the use of the NEI-VFQ and the OSDI to examine the associations between the

quality of life measures and ocular surface measures in patients with Sjögren's syndrome (33). They examined subscale/domain correlations between the two instruments. Associations between OSDI and NEI-VFQ subscales were modest and the report concluded that both instruments were similar in their ability to measure the impact of Sjögren's syndrome-related dry eye on vision-targeted health-related quality of life (33). Li et al. have simultaneously used both the OSDI and NEI-VFQ instruments to measure the quality of life (27), and more recently a new instrument known as the Impact of Dry Eye on Everyday Life (IDEEL) has been developed (40). The IDEEL questionnaire includes 57 items that assess dry eye impact in three modules: symptom-bother, impact on daily life, and dry eye treatment satisfaction. The impact on daily life module included an emotional aspect. While the IDEEL was described as the only comprehensive questionnaire that assesses the entire scope of dry eye on patient outcomes, it is more useful in research settings as its regular clinical utility is limited by the time required to administer the questionnaire. Abetz et al. do mention the reduction of items and the use of specific—but not necessarily all—modules to assess dry eye related quality of life (40).

The concept of symptom assessment of dry eye has been used elsewhere. Schaumberg et al. developed and evaluated a short questionnaire based on a visual analog scale called the "Symptom Assessment iN Dry Eye (SANDE)" to quantify the frequency and severity of DED. While this instrument exhibited good reliability, it did not measure the symptom burden of DED in its entirety (85). In this study, we developed a tool adapting a variety of scales (visual analog scale, visual rating scale, and numerical rating scale) to measure the entire symptom burden of DED in domains used in other chronic studies that were deemed to be necessary components to measure the impact of symptoms on quality of life. We believe that persistence and intensity of DED symptoms affect daily activities and the mood of individuals. However, our results show that intensity of symptoms did not correlate with activity and affective interference, whereas the persistence of symptoms showed much higher correlations, especially with affective interference. The importance of measuring the impact of DED symptoms on affective

interference is consistent with recent studies showing an association between depression, anxiety, and DED (4,5,90).

To further understand the role of intensity of symptoms, we determined whether intensity of symptoms would correlate with physician treatment of choice (aggressive versus nonaggressive). Our results showed that, irrespective of clinical signs, the majority of patients reporting more intense symptoms received aggressive treatments, whereas patients reporting low-symptom intensity received less aggressive treatments. Physicians rely upon symptom analysis to make treatment decisions. The more symptomatic patients are during a clinical visit, the more aggressive treatment they will receive. It becomes fundamentally important to analyze symptoms reliably and in their totality to guide treatment decisions.

The problems in evaluating efficacy of treatment in DED are related to incomplete understanding of symptom burden analysis. Traditional therapies for DED replace or conserve the patient's tears without correcting the underlying disease process. These include tear replacement by topical artificial tears and punctal occlusion to prevent the drainage of natural or artificial tears (91). The development of pharmacological therapies has been limited by our incomplete understanding of the mechanism, pathogenesis, and clinical manifestation of DED. Whether treatment is helpful or not is based on improvements in signs and symptoms. However there is a well-established disconnect between signs and symptoms (86,92,93). The disconnect makes it difficult to determine whether the treatment is efficient. In addition, recent outcome studies and reviews on dry eye therapies have shown that dry eye treatment needs to be tailored to the type and severity of DED (94). This can only be done by effectively developing a multi-symptom patient-reported outcome tool for DED. Dry eye symptoms can persist for years and may worsen over time. Thus, there is a need to collectively assess the symptoms of dry eye and measure its symptom burden.

The symptom burden tool for DED, developed in our study, provides an efficient and easy method to measure the impact of symptom persistence and intensity on activity and affective interference and treatment decisions, respectively. There are several limitations to this study, including the assessment of symptoms at one time point only and the small sample size. This is a pilot study and results cannot be broadly generalized. Studies with a larger sample size in this population, as well as other dry eye population groups, are required to further determine the content, construct, and criterion validity of the symptom burden tool. Specifically, a predictive validity study is required to measure the association between the burden domains with one or two outcome measures over time, such as changes in symptoms over time or the effects of treatment. Additionally, prospective studies where the symptom burden is measured at several time points are needed to measure the reliability of the symptom burden tool. Despite these limitations, we believe that adding an affective component to standardized questionnaires for DED, such as the OSDI, may allow us to determine the effect of persistence of DED symptoms on psychological and social wellbeing. Measuring the intensity of symptoms will allow us to further understand treatment responses and develop treatment decisions.

5. STUDY 2
DEPRESSIVE SYMPTOMS IN DRY EYE DISEASE PATIENTS: A CASE-CONTROL STUDY USING THE BECK DEPRESSION INVENTORY[2]

The aggressiveness of DED treatment is based on the severity of symptoms, which include pain, dryness, grittiness, itchiness, redness, burning or stinging, foreign body sensation, and light sensitivity (1). It is known that DED symptoms do not correlate with clinical signs, where certain clinical tests such as the Schirmer test do not correlate with patient-reported dryness (92). However, the chronic discomfort observed in DED patients may be associated with a decrease with the quality of life correlating it with affective interference (95).

Recent case-control and cross-sectional studies have reported an association between depression and DED, post-traumatic stress disorder and DED, and anxiety and DED (2–5,28,96,97). Examination of the association between depression and DED started when some researchers revealed that depressive mood is one of the underlying causes of subjective dry mouth (29,30). Others suggested that dry eye symptoms and mood status may influence each other (31). The 2007 DEWS report included a discussion on the debilitating symptoms of DED and their result in both psychological and physical effects that impact the quality of life (1). Subsequently, Li et al. assessed vision-related quality of life and psychosocial impacts and found correlations with depression and anxiety (27). Labbe et al., using subjects from the Beijing Eye Study, showed that depression was associated with DED in particular with dry eye symptoms (96).

We hypothesize that the presence of depression in DED may cause patients to perceive symptoms in an anomalous fashion compared to patients without depression. This is similar to the relationship between psychological and psychophysiological characteristics with fibromyalgia (98). This means that if depression was treated independently and its contribution

---

to patient dry eye symptoms removed from the equation, then it may be possible to manage DED with less aggressive treatments (i.e., the frequency of medication intake and type of medication).

In this case-control study, we used the BDI to measure depressive symptoms in DED patients and controls to determine the association between depressive and DED symptoms.

5.1   **Methods**

5.1.1 **Study Overview**

Study approval was obtained from the Institutional Review Board of the University of Illinois at Chicago. Symptomatic patients with DED 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. Eligible patients completed two DED symptom questionnaires and the BDI questionnaire to measure depressive symptoms. The DED symptom questionnaires were completed by the interviewer and the BDI questionnaire was completed by self-administration. Sociodemographic data and psychological and medication history were obtained by chart review. All subjects included in our study were over 18 years of age.

5.1.2 **Study Population**

Fifty-three patients were recruited from our Dry Eye Clinic at the Department of Ophthalmology and Visual Sciences, University of Illinois at Chicago. The DED sample included newly diagnosed and established patients with DED who visited our clinic between November 2012 and June 2014. The diagnostic criteria were: (i) a reporting of symptoms: burning sensation, irritation, grittiness or foreign body sensation, light sensitivity, pain, dryness, soreness, or discomfort in the eye; (ii) a Schirmer value of <10 mm/5 min in either eye using

Whatman filter strips #41 (Haag-Streit, Essex, UK); or (iii) positive corneal staining and/or Rose

Bengal corneal and conjunctival staining of $\geq$1 (1). A saliva sample was collected from all DED

patients attending the clinic between the dates mentioned above. However, given that subjects

were asked whether they would like to participate in this study, the final sample was

characterized as a convenience sample.

Forty-one control patients who visited our general eye clinic with refraction-related

complaints were recruited to the study. The inclusion criteria included no clinical diagnosis of

DED, a Schirmer value of $\geq$10 mm/5 min, and no corneal staining. None of the control subjects

enrolled were using tear supplements. Sampling was similar to cases where an all-sample

technique was intended; however, taking into consideration the willingness to participate

characterized this as more of a convenience sample.

5.1.3 **Assessment of Depressive Symptoms and History of Depression**

Depressive symptoms were measured using the BDI, which is a widely used tool for

measuring the severity of depressive symptoms (50). The tool is a 21-question multiple-choice

self-report inventory that was created by Aaron T. Beck and first published in 1961 (51). The

items for the BDI cover emotional, behavioral, and somatic symptoms. Beck, Steer, and Grabin

concluded that reviews of factors analyses have identified three factors: Negative attitude

toward self, performance impairment, and somatic disturbances (52). The standard cutoff points

for the BDI are 0–9, indicating minimal depression; 10–18, indicating mild depression; 19–29,

indicating moderate depression; 30–63, indicating severe depression. In this study, the BDI

score was divided into $\leq$9 or >9. Depression status was determined as a composite variable

through chart review. The variable was determined as "ever having depression" through medical

and psychological history and/or through any documented history of prescribed psychiatric

medications. This composite variable was determined because for some patients a diagnosis of

clinical depression in their charts was not indicated; however psychiatric medication was listed among their medications. Psychiatric medication was also determined as a separate variable.

5.1.4 **Assessment of Dry Eye Disease Symptoms**

The symptoms of DED were assessed using two tools: the symptom burden tool and the OSDI tool (32). The symptom burden tool assesses four domains: persistence, intensity, activity, and affective interference. The OSDI tool measures persistence, activity interference, and environmental triggers. Scores on the OSDI range from 0 to 100 and from 0 to 520 for the symptom burden tool. The use of the two tools is complementary. The OSDI is the most frequently used survey instrument in DED research for the assessment of ocular surface disease and its severity. It has demonstrated discriminant and concurrent validity and been shown effective for discriminating people with varying levels of DED (32,43). The symptom burden tool is a recent tool that provides a more comprehensive symptom assessment using four domains (persistence, intensity, activity, and affective) adapted from chronic diseases with pain (95).

5.1.5 **Statistical Analysis**

Histograms with the normal curve, Q-Q plots, and the statistical tests for normality were used to determine if the data were normally distributed. Demographic data were summarized as means $\pm$ SD and percent distribution. For data representation and clinical interpretation, OSDI scores, DED symptom burden questionnaire scores, and BDI scores were summarized as mean $\pm$ SD. Regression diagnostics (studentized residuals and leverage) were performed to detect any outliers and unusual influential data. Scatter plots were generated with fitted lines between DED symptoms and depression symptoms. Linear statistical models and polynomial regression were run to determine the type of relationship between depressive symptoms and DED symptoms.

Independent t-test was performed to determine differences of BDI scores between cases and controls. Similarly, independent t-test comparison of DED symptom scores among cases diagnosed with depression and those without depression was also performed. Linear regression was used to estimate the association between DED symptom continuous scores and BDI depression scores. Logistic regression was used for the DED dichotomous outcome and depression status as exposure. Unadjusted and adjusted regression analysis was performed. Chi-square and Fisher's exact tests were used for categorical variables. Medians were also calculated for symptom scoring variables. Statistical significance was set at 0.05. Data were analyzed using STATA/SE v12 software.

5.2   **Results**

5.2.1   **Demographics**

Table V shows the demographic distribution for case and control subjects. Regression diagnostics revealed three major outliers that influenced the data. These three cases were excluded from the analysis, making the total sample equal to 91 (50 cases and 41 controls). Mean age was comparable between the two groups: 52.6 for DED cases and 50.0 for controls (P=.43). In total, there were 32 males (16 DED case and 16 controls) and 62 females (37 DED cases and 25 controls) (P=.39). The distribution for race was different between White and non-White. Among DED cases, 58.0% were White 42.0% were non-White; and among controls, 26.8% were White and 73.2% were non-White (P<.01). Among cases, 38.0% were diagnosed with depression diagnosis and 62.0% were not. Among the controls, 17.1% were diagnosed with depression and 82.9% were not diagnosed with depression (P=.03). Thirty-six percent of cases had depressive symptoms >9 as measured by the BDI and 64.0% has depressive symptoms <9. Among the controls, 17.1% had depressive symptoms >9 and 82.93% had depressive symptoms <9 (P=.04). Seventy-nine percent of cases diagnosed with depression

had documented antidepressant or antianxiety medications in their medical charts, and %21 of

cases did not have documented antidepressant or antianxiety medication.

TABLE V
DEMOGRAPHIC CHARACTERISTICS OF CASES AND CONTROLS
FOR DED AND DEPRESSIVE SYMPTOM ANALYSIS

Variable	DED Cases (n=50) Mean + SD	Controls (n=41) Mean + SD	P-Value
Age	52.6 + 16.23	50.0 + 14.75	0.43
Gender			
Male	16 (32.0%)	16 (39.0%)	0.49
Female	34 (68.0%)	25 (61.0%)	
Race			
White	29 (58.0%)	11 (26.8%)	<.01
Non-White	21 (42.0%)	30 (73.2%)	
Depression			
Yes	19 (38.0%)	7 (17.1%)	0.03
No	31 (62.0%)	34 (82.9%)	
BDI Depressive symptoms			
>9	18 (36.0%)	7 (17.1%)	0.04
<9	32 (64.0%)	34 (82.9%)	

5.2.2 **Dry Eye Disease and Depressive Symptoms Scores**

Figures 6–8 show scatter plots of depression symptoms against OSDI-DED symptoms

for all subjects (Figure 6), for cases and controls (Figure 7), and further by depression status

(Figure 8). Regression models revealed that the association was linear more than quadratic or

cubic. The unadjusted regression coefficient for BDI depressive symptoms was 1.73 (95% CI

1.01–2.45) for all subjects. This means that with every one unit increase in BDI depression

score, we expect a 1.73 unit increase in DED symptoms. After adjusting for age, gender, race,

and psychiatric medication, the regression coefficient was 1.71 (95% CI 1.02, 2.40). Stratified by

DED status, the regression coefficient for depressive symptoms was 1.11 (95% CI 0.17, 2.04)

for cases and 0.50 (95% CI -0.05, 1.06) for controls. The adjusted regression coefficient

between DED symptoms and depressive symptoms among DED cases was 1.22 (95% CI 0.27,

2.18).
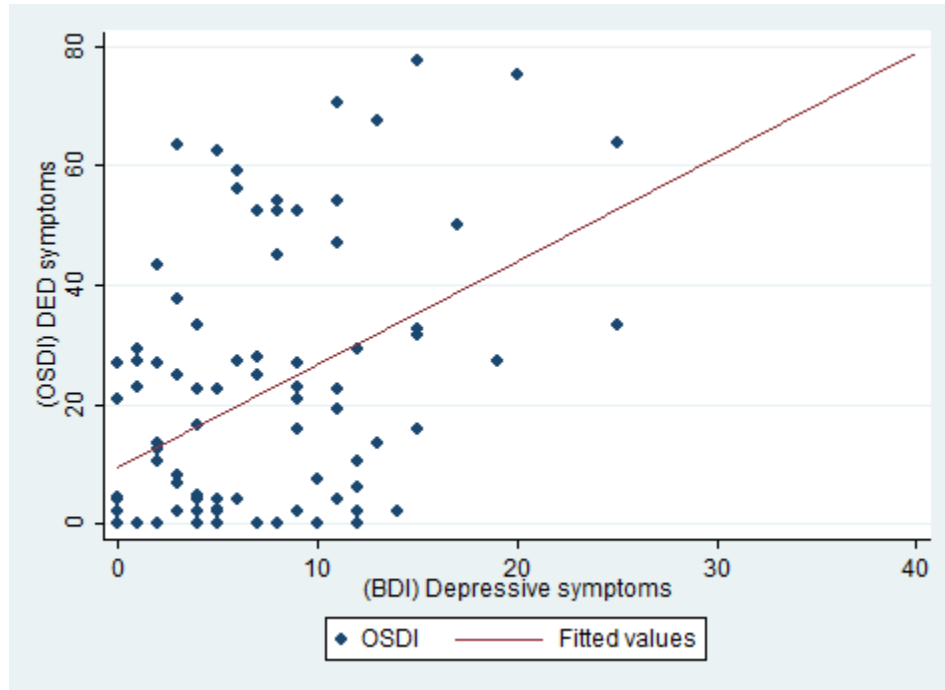
Figure 6. Scatter plots for the relationship between depressive symptoms as measured by the BDI questionnaire and DED symptoms as measured by the OSDI in the entire sample. The unadjusted regression coefficient for BDI depressive symptoms was 1.73 (95% CI 1.01–2.45). After adjusting for age, gender, race, and psychiatric medication, the regression coefficient was 1.71 (95% CI 1.02, 2.40).
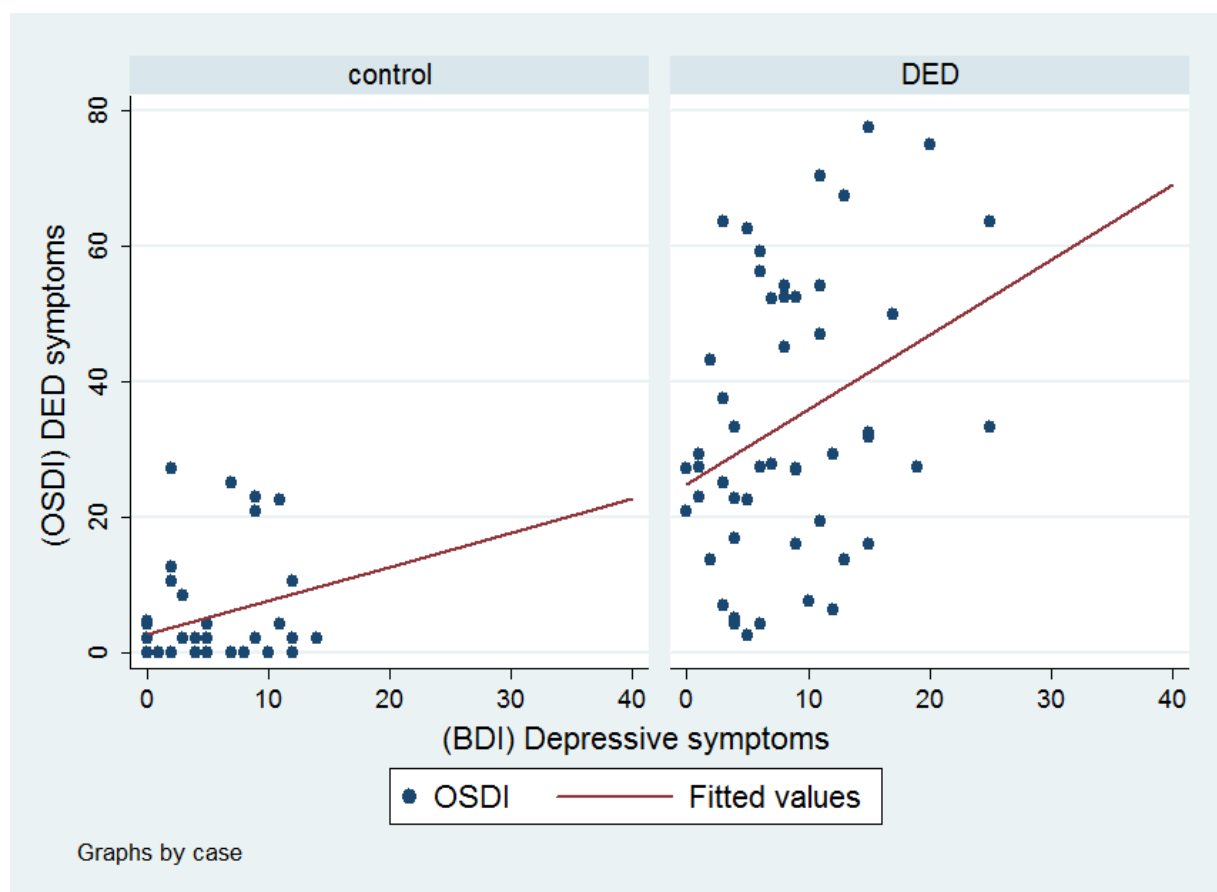
Figure 7. Scatter plots for the relationship between depressive symptoms as measured by the BDI questionnaire and DED symptoms as measured by the OSDI between DED cases and controls. A simple linear regression between DED symptom scores and depressive symptom scores revealed a regression coefficient of 1.11 among DED cases and a regression coefficient of 0.50. The association was significant for DED cases (95% CI 0.17, 2.04) but not significant for controls (95% CI -0.05, 1.06). After controlling for age, gender, race, and psychiatric medication, the regression coefficient between DED symptoms and depressive symptoms among DED cases was 1.22 (95% CI 0.27, 2.18).
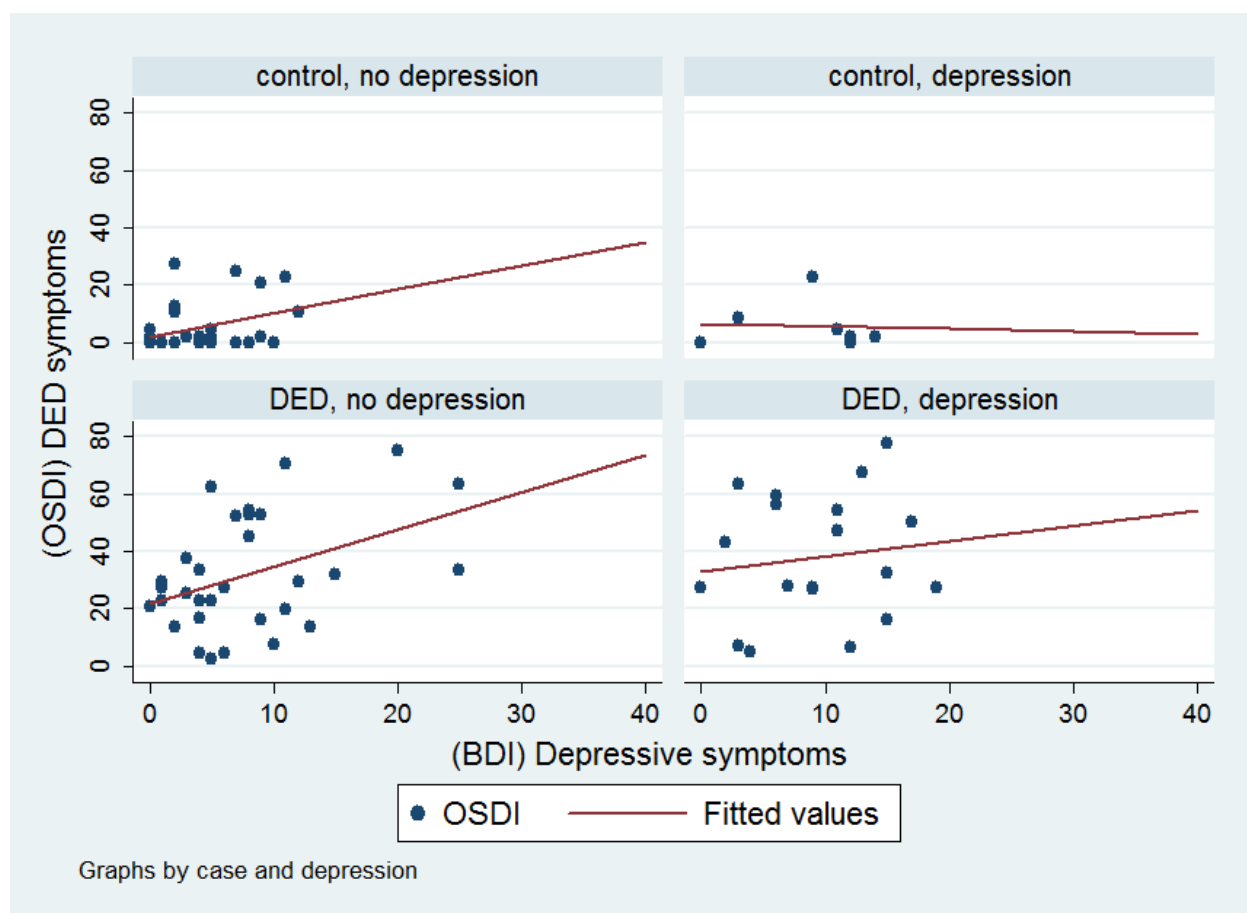
Figure 8. Scatter plots for the relationship between depressive symptoms as measured by the BDI questionnaire and DED symptoms as measured by the OSDI between DED cases and controls and clinical diagnosis of depression.

Table VI lists the means for DED symptom scores and depression scores. The mean total OSDI score among DED cases was 34.18 compared to 4.78 among controls (P<.001) and the mean total symptom burden questionnaire among DED cases was 16.95 versus 1.07 in controls (P<.001) (Table VI). The mean BDI score was 8.44 $\pm$ 6.07 among DED cases and 4.32 $\pm$ 4.38 in controls (P<.001). Among DED cases that have been diagnosed with depression, the mean OSDI scores and symptom burden scores were 37.94 and 19.38 compared to 31.88 and 15.46 among those without depression. Differences, however, were not statistically significant (P=.32 and P=.39). Mean BDI score among DED cases with depression was 9.32 compared to 7.9 among DED cases with no depression diagnosis. This result was also not statistically significant (P=.43). Mean BDI score among controls with depression was 8.71 versus 3.41 among controls without depression (P=.002). Logistic regression revealed an OR of 2.86 (95% CI 1.04, 7.87) for the association between DED and diagnosis of depression after controlling for age, gender, and race. Unadjusted logistic regression between DED status and BDI clinical category of >9 revealed an OR of 2.73, 95% CI (1.0–7.4). Adjusted logistic regression revealed an OR of 2.79, 95% CI (0.96–8.12). This means that cases are 2.79 times more likely to report depressive symptoms of >9 than controls, after adjusting for age, gender, race, and psychiatric medication.

TABLE VI
DED AND DEPRESSIVE MEAN SYMPTOM SCORES

Measure	DED Cases (n=50) Mean $\pm$ SD	Controls (n=41) Mean $\pm$ SD	P-Value
OSDI			
Persistence	6.14 $\pm$ 4.49	1.29 $\pm$ 2.87	<.001
Activity	4.22 $\pm$ 3.35	0.51 $\pm$ 1.19	<.001
Environmental	4.46 $\pm$ 3.36	0.83 $\pm$ 2.12	<.001
Total Score	34.18 $\pm$ 20.71	4.78 $\pm$ 1.22	<.001
Symptom Burden			
Persistence	1.97 $\pm$ 1.10	0.22 $\pm$ 0.40	<.001
Intensity	6.98 $\pm$ 5.47	1.10 $\pm$ 1.91	<.001
Activity	1.45 $\pm$ 1.10	0.15 $\pm$ 0.37	<.001
Affective	1.07 $\pm$ 1.13	0.04 $\pm$ 0.15	<.001
Total Score	16.95 $\pm$ 15.41	1.07 $\pm$ 2.38	<.001
BDI	8.44 $\pm$ 6.07	4.32 $\pm$ 4.38	<.001

5.3   **<u>Discussion</u>**

This case-control study revealed three main findings: (i) a linear association between DED symptoms and depressive symptoms, which is more apparent among DED cases; (ii) DED cases with depression have higher DED symptom scores than DED cases without depression; and (iii) clinical diagnosis of DED is associated with depression status and marginally associated with depressive symptoms, after controlling for age, gender, race, and psychiatric medication. These findings support the hypothesis regarding an association between DED and depressive symptoms. However, the mechanisms that underlie the association between depression, depressive symptoms, DED, and DED symptoms are unclear. We do not know whether DED and its symptoms are causing depression through chronic pain and the negative impact on daily activities, whether depression and its medication is causing DED, or is the relationship due to some other unmeasured factor causing both.

Several population-based studies have reported on the association between DED and depression (3,4,97). The strength of these studies lies in their representative samples and large sample sizes. However, the symptomatic assessment of both DED and depression was lacking. Additionally, these population studies were not hypothesis-based and relied on ICD-9 codes for ascertaining cases. Two population-based retrospective studies in the United States Veterans Affairs population in Miami reported high prevalence of depression in subjects with DED. In the first study, 17% of patients with a diagnosis of depression had DED as opposed to 10% without this diagnosis (3). In the second study, 24% of patients with a diagnosis of depression had DED as opposed to 18% without this diagnosis (4). The reported adjusted ORs for depression in both studies were comparable, 1.91 and 1.92 respectively (3,4).

We reported an adjusted association between clinical diagnosis of DED and depression status of 2.86 in our study. This is comparable to other studies reporting on the association between depression and DED. In a population-based cross-sectional study of 657 Korean elders $\geq$65 years of age randomly selected from an official household registration database in

Yongin, Korea, Kim et al. investigated the association between DED and depression and sought to evaluate the impact of comorbid depression on the agreement of DED signs and symptoms (2). Symptoms of DED were assessed using the 6-item dry eye questionnaire, and depressive symptoms were assessed using the Korean version of the Short Geriatric Depression Scale. Depression was more prevalent in patients with DED (33.3% versus 18.1%). Adjusted analyses revealed depression as an independent risk factor for DED (OR 3.08; 95% CI 1.93–4.93). The authors of this study listed several limitations including the lack of assessment of medication, the cross-sectional nature of the study, and the severity of DED was assessed using the dry eye questionnaire. Subjective symptoms may be better quantified using tools including the visual analog scale and the ocular surface disease index score, especially when using symptom-based diagnostic criteria for DED (2,32,43).

In another recent population-based case-control study in Taiwan, Wang et al. investigated the comorbidities of DED (34). They used a nationwide subset database released by the NHRI in 2006. The program to create the database covered 22 million enrollees, representing more than 98% of the island's population. The NHRI used a systematic, random sampling method to extract 5% of the enrollees (n=1,073,891). The DED cases consisted of 12,007 patients (after excluding patients under 18 years of age) who sought ambulatory care with a principal diagnosis of DED and 36,021 randomly selected controls. The prevalence of psychiatrically diagnosed depression was higher in patients with DED (7.20% versus 3.55%) and the adjusted OR reported was 2.11 (95% CI 1.93–2.31) (34). ICD-9 CM codes were used for the diagnosis of DED and depression but the symptoms were not assessed. Additionally, depression was included among other comorbidities such as heart disease, SLE, asthma, pulmonary circulation disorders, diabetes, liver diseases, and solid tumors and metastasis. A more recent retrospective case-control study performed at the University of North Carolina outpatient clinics, reported an adjusted OR for DED and anxiety of 2.8 and an adjusted OR for

DED and depression of 2.9. Outcome and exposure variables were also assessed using ICD-9 diagnosis codes (97).

In this study, we recruited patients from our DED clinic and assessed the symptoms of both DED and depression using validated questionnaires. Additionally, depression status was determined as a composite variable through chart review as "ever having depression" through medical and psychological history and/or through current prescribed medications or any history of prescribed medication. Seventy-seven percent of cases diagnosed with depression were actively on antidepressant or antipsychotic medications at the time of questionnaire administration, and 23% either had some history of depression or were prescribed antidepressant medication. This allowed us to control for psychiatric medication in our analysis between depressive symptoms and DED symptoms. Due to the possibility of underrecording of medication use in charts, we also analyzed the data according to depression status and the interpretability of our results was the same. We also showed that depressive symptom scores among DED patients do not vary between depressed and not-depressed patients. This means that patients with DED exhibit depressive symptoms regardless of depression status.

There are several limitations to our study. First the sample size is not large to make definite conclusions and our results cannot be generalized to the general population. Additionally, our medication assessment for depression is not complete; while current use was used to determine depression status, we did not determine the frequency of medication, dosage, and the various types of medication. These medications may have different effects on symptoms at the time of questionnaire administration. This may have impacted our results, where patient symptoms may have varied with different medications. Similarly, this applies to our assessment of DED symptoms without assessing aggressiveness of treatment. Additionally, we did not control for other comorbidities that may be related to both DED and depression. This study also lacked information on the socioeconomic and insurance statuses of the patients.

Despite our limitations, this study provides further evidence regarding the association between DED and depression and their symptoms. Prospective studies that account for medication usage, comorbodities, and socioeconomic status are needed to understand the mechanisms underlying the association between symptoms of depression and symptoms of DED.

6. STUDY 3
SINGLE NUCLEOTIDE POLYMORPHISMS IN THE BRAIN DERIVED NEUROTROPHIC FACTOR, VITAMIN-D RECEPTOR, AND DEOXYRIBONUCLEASE 1 GENES IN DRY EYE DISEASE PATIENTS: A CASE-CONTROL STUDY[3]

Dry Eye Disease is a complex multifactorial common phenotype resulting from interactions of genetic and nongenetic factors, with prevalence in adult populations ranging from 5% to more than 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 (2–5,96,97) post-traumatic stress disorder and dry eye (4), and anxiety and dry eye (5, 97). 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. have demonstrated in a cohort of British middle-aged and elderly female twins that there is a heritability of 40% for a DED diagnosis, and a varying heritability of 25% to 80% for DED (6). The DED gene studies have included some small candidate gene studies and some genome-wide association studies on Sjögren's syndrome (99,100). The candidate gene studies have identified polymorphisms in interleukin proinflammatory cytokine genes (101), and killer cell immunoglobulin-like receptor and human leukocyte antigen-C (102). However, results have not been replicated. In a review, Burbelo et al. summarized the genetic findings from genome-wide association studies associated with Sjögren's and described the disease relevance of newly

---

[3] Preliminary data for this study were accepted as an abstract and presented as a poster at the 2014 Association for Research in Vision and Ophthalmology meeting

identified genes and their corresponding pathways (99). More than 15 robust susceptibility loci have now been associated with Sjögren's. Many of these risk genes play important roles in immune activity and many are often shared with SLE (99). 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 other 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 BDNF, VDR, and DNASE 1 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 (103). Serum levels of BDNF have been shown to be higher in patients with primary Sjögren's syndrome as compared to controls (104). Additionally, findings from studies support a complex and functional role of BDNF in depression and antidepressant action (105,106). The DNASE1 gene and the VDR gene 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 (79,107). We have shown that eDNA production and clearance mechanisms are dysregulated in DED (8). 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 SNPs in the DNASE1 gene that may be involved in the pathogenesis of DED and depression. Also, VDR gene SNPs have been investigated in the risk of SLE (80), and as with similar autoimmune diseases, SLE has been associated with both depression and DED (81,108).

The purpose of this case-control study was to identify SNPs in the BDNF, VDR, and DNASE 1 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.

6.1   **Methods**

6.1.1   **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 >18 years of age.

6.1.2   **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 DED sample included established patients of DED who visited our clinic between November 2012 and June 2014. The diagnostic criteria were: (i) a reporting of symptoms: burning sensation, irritation, grittiness or foreign body sensation, light sensitivity, pain, dryness, soreness, or discomfort in the eye; (ii) a Schirmer value of <10 mm/5 min in either eye using Whatman filter strips #41 (Haag-Streit, Essex, UK) ; or (iii) 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.

6.1.3 **Selection of Polymorphisms**

The SNPs were selected from the BDNF, VDR, and DNASE 1 genes. The BDNF gene is located on human chromosome 11 (11p13) and contains 11 exons. This gene may play a role in stress response and mood disorders (109). 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.

The VDR is located on human chromosome 12 (12q13.11) and contains 11 exons. 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 (110). These same four SNPs were included in our study.

The DNASE 1 gene 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. Mutations in this gene have been associated with SLE, an autoimmune disease (111). The following seven SNPs from the DNASE 1 gene were included for analysis: rs8176927, rs34923865, rs8176919, rs1799891, rs1053874, rs8176920, and rs34186031.

6.1.4 **Saliva and Genotyping**

A saliva sample of 2 ml was collected, following a routine eye exam, in a saliva collection kit (Oragene Sample DNA collection kit) 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 Oragene-Purifier and centrifuged to precipitate and pellet various impurities from the sample. The aqueous phase was then transferred to fresh tubes. The DNA present in the aqueous phase was precipitated using 95%–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 NanoDrop.

Following DNA extraction, the samples were sent to the Duke Molecular Physiology Institute. They were diluted to 1ng/ul using UltraPure™ DNase/RNase-Free Distilled Water (Life Technologies, Grand Island, New York), and then 3 ng of each sample were transferred to a 384 well plate using an epMotion 5075 TMX automated pipetting system (Eppendorf North America, Hauppauge, NY).

Genotyping was performed using both custom and predesigned TaqMan® SNP Genotyping Assays (Life Technologies, Grand Island, New York) for the 12 SNPs. Table III lists the SNPs and their primers. The PCR was performed according to manufacturer protocols on 3 ng of genomic DNA in 5 µl reaction volumes, using the GeneAmp® 9700 Dual 384-Well PCR system (Life Technologies, Grand Island, NY) and subsequently scanned on a ViiA™ 7 Real-Time PCR System (Life Technologies, Grand Island, NY). Data were assessed on, and exported from, ViiA 7 RUO Software v1.2.1. The CEPH samples (NIGMS Repository, Coriell Institute for Medical Research, Camden, NJ), study sample replicates, and NTCs were used for QC. The QC replicates were required to match 100%, and NTCs were required to have no amplification.

6.1.5 **Sociodemographic Variables and Assessment of Depression**

Demographic data (date of birth, gender, 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.

6.1.6 **Statistical Analysis**

The SNPs were tested for HWE using χ2-test. Any SNPs that deviated from HWE (P<.05) were excluded from further analysis. Genotype and allele frequencies of cases and controls were evaluated. The trend P-value for additive effects was determined for genotypes, and the allele frequencies were evaluated using chi-square. Odds ratios and 95% CIs were also calculated for allele frequencies. Logistic regression was also performed to determine the association between SNPs and DED. Stratified analysis was performed to determine if the association between SNPs and DED varied by depression status. All analyses were performed using PLINK 1.07 (http://pngu.mgh.harvard.edu/~purcell/plink/index.shtml), and STATA SE 12.0 (StataCorp LP., College Station, Texas) software.

6.2 **Results**

6.2.1 **Demographics**

Table VII shows the demographic distribution for patients and control subjects. Mean age was 54.6 in cases compared to 46.8 in controls and was statistically significantly different using independent t-test (P=.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% versus 45.1% among controls, P=.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<.001).

TABLE VII

DEMOGRAPHIC CHARACTERISTICS OF CASES AND CONTROLS
FOR SNPS IN DED

Variables	Dry Eye cases N=64 n(%)	Controls N=51 n(%)	P-value*
Mean Age in years	54.56 ± 15.48	46.75 ± 15.48	P=.01
Gender			P=.01
Male	14 (21.9%)	23 (45.1%)	
Female	50 (78.1%)	28 (54.9%)	
Race			P=.11
White	28 (43.8%)	15 (29.4%)	
Non-White	36 (56.3%)	36 (70.6%)	
Depression			P<.001
Yes	27 (42.2%)	6　(11.8%)	
No	37 (57.8%)	45 (88.2%)	

*Chi-square test for categorical variables, Fisher's exact test p-value when cell size <50, and student's t-test for continuous variables

6.2.2 <u>**Association of Polymorphisms with Dry Eye Disease**</u>

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 6 SNPs were analyzed. Allelic frequencies were calculated and their genotypic distributions tested for HWE. No significant deviations were detected at 0.05. Table VIII shows the allelic and genotypic distributions between cases and controls. The Rs6265 in the BDNF gene was the most significant, where the number of the minor allele A was higher in cases compared to controls (22 versus 9). Cases were 2.22 times more likely to have the minor allele A in SNP rs6265 as compared to the controls (P=.05; 95% CI 0.97–5.08) (Table VIII). Genotypes GA and AA were higher in cases as compared to controls, 29.5% and 3.3% among the cases, and 18.0% and 0.0% 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. Cases were 1.72 times more likely to have the minor allele A for rs2228570 compared to controls (P=.06; 95% CI 0.98–3.01), and cases were 1.66 times more likely to have the minor allele C for rs7975232 compared to controls (P=.06; 95% CI 0.97–2.84) (Table VIII). Genotype AA for rs2228570 was higher in cases as compared to controls, 24.6% among cases and 12.0% among controls. The trend P-value was 0.08. Genotype CC for rs7975232 was higher in cases as compared to controls, 28.3% among cases and 14.0% among controls. The trend P-value of 0.064. After adjusting for age, gender, race, and depression status, cases were 1.62 times more likely to have the GA or AA genotype compared to controls. This OR, however, was not significant (95% CI 0.59–4.45).

Table VIII
GENOTYPE AND ALLELE DISTRIBUTION BETWEEN CASES AND CONTROLS

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

*P-value: Trend; **P-value: Allelic distribution; OR and 95% CI is for the allelic distribution

6.2.3 **Association of Polymorphisms with Depression and Stratified Analysis**

The distribution of SNPs with depression is shown in Table IX. 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=.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 to 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 non-depressed group (Tables X and XI).

TABLE IX
DISTRIBUTION OF SNPS WITH DEPRESSION

SNPs	Depression	No Depression	*P**
BDNF rs6265			
AA	0 (0.0%)	2 (2.5%)	0.07
GA	12 (38.7%)	15 (18.8%)	
GG	19 (61.3%)	63 (78.8%)	
VDR rs2228570			
AA	7 (23.3%)	13 (16.9%)	0.55
GA	13 (43.3%)	30 (39.0%)	
GG	10 (33.3%)	34 (44.2%)	
VDR rs7975232			
CC	8 (27.6%)	16 (19.8%)	0.65
AC	14 (48.3%)	41 (50.6%)	
AA	7 (24.1%)	24 (29.6%)	

*P-value: chi-square

TABLE X
DED AND RS6265 AMONG PATIENTS WITH DEPRESSION

Depression Yes	DED cases	Controls	Total
rs6265 A	11 (44.0%)	1 (16.7%)	12 (38.7%)
rs6265 G	14 (56.0%)	5 (83.3%)	19 (61.3%)
Total	25 (100.0%)	6 (100.0%)	31 (100.0%)

OR=3.93 [95% CI: 0.35–202.4]

TABLE XI
DED AND RS6265 AMONG PATIENTS WITHOUT DEPRESSION

Depression No	DED cases	controls	Total
rs6265 A	9 (25.0%)	8 (18.2%)	17 (100.0%)
rs6265 G	27 (75.0%)	36 (81.2%)	63 (100.0%)
Total	36 (100.0%)	44 (100.0%)	80 (100.0%)

OR=1.5 [95% CI: 0.45–5.1]

6.3   **Discussion**

This study revealed two main findings. Val66Met (rs6265) in the BDNF gene and two SNPs, FokI (rs2228570) and ApaI (rs7975232), in the VDR gene may potentially be associated with DED. Additionally, the association between DED and rs6265 (Val66Met) varies by depression status. Together DED and depression are characterized as environmental and psychosomatic stress-related diseases. This study cannot make definite conclusions given its limited sample size; however, these results shed light on a possible common biological link that may explain the association between DED and depression. Further, it is important to further understand this association along the duration and prognosis of DED.

There are three main types of DED that patients present with: (i) tear-deficient DED stemming from Sjögren's, autoimmune diseases, and keratoconjunctivitis sicca; (ii) evaporative DED, which mainly stems from meibomian gland dysfunction as can be seen in patients with rosacea, and (iii) mixed DED as seen in GVHD patients. 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 that presents as ocular rosacea and evaporative DED (112). The identification of the cationic antimicrobial peptide cathelicidin as a vitamin D target gene (113) 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 meibomian gland dysfunction, a deficiency in androgens results in loss of the lipid layer, specifically triglycerides, cholesterol, and monounsaturated essential fatty acids exacerbating DED. Vitamin D receptor polymorphisms BsmI, ApaI and TaqI, wild variants of the VDR gene, were associated with lower vitamin D levels (114), which in turn affect levels of androgen. In addition, there is evidence that VDR is also expressed in the brains of several species during development (115). We have found that VDR FokI and ApaI SNPs are more

common among patients with DED. Larger prospective studies are needed to look at VDR gene variants to further discover their potential operative mechanisms.

In addition to the VDR SNPs, we found that Val66Met, an 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 that 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 is the "injury switch" that leads to corneal allodynia and hyperalgesia.

Additionally, Val66Met is a BDNF prodomain SNP resulting in a valine-to-methionine substitution that has been shown to be associated with depressive disorder and depression-related phenotypes (66–69). In this study, we have found that patients with depression are more likely to have DED; additionally patients with Val66Met and depression are more likely to have DED than patients with Val66Met alone. Temporality between DED and depression has not been established. We are not sure whether DED causes depression or whether depression causes DED. Does SNP Val66Met predispose individuals to DED which may lead to depression? Or Does SNP Val66Met predispose individuals to depression which may lead to DED?

This study has several limitations. The first is the low 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, supported by the budget. 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 were characterized as having the most difficult and complicated prognosis. This limited 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 cohort studies studying the biological link between DED and depression. These studies will help identify temporality and the interplay of all factors involved in the pathogenesis of DED, ultimately improving treatment decisions.

7. STUDY 4
FOLLOW-UP OF SYMPTOMS IN DRY EYE DISEASE PATIENTS AND THE POTENTIAL ROLE OF VAL66MET SNP

The diagnosis, prognosis, and treatment of DED are heavily based on symptoms and the patient perception of those symptoms. Treatment efficacy varies widely among DED patients. One potential explanation is that DED patients may be genetically predisposed to varying treatment responses and to different pain perceptions.

Single nucleotide polymorphisms have been used as markers for treatment response in chronic disease such as cancer and response to radiotherapy (116), antidepressant treatment, antihypertensive treatment (117), and chronic HCV-infection (118). Studies have also looked at the association between polymorphisms and the biological response to drugs for autoimmune diseases (119–121). Marquez et al. studied whether rs6822844 G/T polymorphism at the IL2–IL21 region contributes to the observed variation in response to rituximab in patients with SLE (119). They showed that rs6822844 seems to play a role in response to treatment in SLE patients. Given that BDNF is associated with depressive mood disorders (122) and that depression has been shown to be associated with DED (96,97), we hypothesize that polymorphisms in the BDNF may predispose individuals to increased sensitivity to pain and DED symptoms.

Findings from studies support a complex and functional role of BDNF in depression and antidepressant action. Val66Met (rs6265) is an SNP in the BDNF gene. Not only has it been shown to be associated with depression, it has also been studied in treatment response and treatment resistance in antidepressant and antipsychotic treatment (123,124). Yu et al. discovered that desipramine but not fluoxetine has antidepressant effects on BDNF (+/Met) mice, suggesting that specific classes of antidepressant may be a more effective treatment

77

option for depressive symptoms in humans with Val66Met (123). More recently, a study by El-Hage et al. highlighted a significant association between Val66Met and the treatment response in severely depressed patients (125). We have found that Val66Met is associated with DED and with depression (chapter 6).

The purpose of this study was to investigate the symptoms of DED over time following treatment at our clinic and examine whether symptoms varied by Val66Met genotype status. This study will allow us to identify individuals who may not benefit from standard DED treatment due to the presence of SNPs. This may bring us a step closer to establishing targeted personalized treatments to susceptible individuals.

7.1 **Methods**

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 at the baseline visit and DED symptom questionnaires were administered at subsequent visits. All subjects included in our study were >18 years of age.

7.1.1 **Selection of Study Population**

A sample from a larger set of 64 DED patients was selected. These patients were mainly established patients who either have been attending the clinic prior to initiation of the study, have been diagnosed with DED in a previous clinic during the study and again in our clinic, or have been suffering from DED symptoms and using over-the-counter drugs. The intent was to select 50% of patients included in this larger DED study looking at SNPs. Taking into consideration that some of these patients may be lost to follow-up, a simple random sample of

thirty-six patients was performed. These patients were recruited from our Dry Eye Clinic at the Department of Ophthalmology and Visual Sciences, University of Illinois at Chicago. They visited our clinic between November 2012 and December 2014. The diagnostic criteria used for DED based on the criteria developed in DEWS included: (i) a reporting of symptoms: burning sensation, irritation, grittiness or foreign body sensation, light sensitivity, pain, dryness, soreness, or discomfort in the eye; (ii) a Schirmer value of <10 mm/5 min in either eye using Whatman filter strips #41 (Haag-Streit, Essex, UK); or (iii) positive corneal staining and/or Rose Bengal corneal and conjunctival staining of $\geq$1.

First administration of DED symptom questions was considered the baseline time point. Patients were then followed up over time between November 2012 and June 2014 for clinical and symptom assessment. Patients were asked to rate their symptoms on a scale of 0 to 4 related to: dryness, irritation, light sensitivity, and pain at baseline and at their follow-up. Additionally, during each follow-up, patients were asked about their symptom experience as follows: (i) no change; (ii) 25% better; (iii) 50% better; (iv) 75% better; or (v) worse. Prescribed DED treatments for each patient were also collected. We scored each treatment option as either 1 point or 2 points as follows: artificial tears (1 point); Restasis (1 point); doxycycline/erythromycin eye ointment (1 point); steroids (2 points); therapeutic contact lens use (2 points). Points were summed, ranging from 0 to 7 and were compared with genotype status. Patients were followed up for a minimum of 6 months as at requires a minimum of 6 months for treatment efficacy and patient response. For this analysis, the last follow-up time for each patient was selected for analysis.

Sociodemographic data and depression status were determined from the medical charts. Depression status was determined as a composite variable. The variable was determined as "ever having depression" through medical and psychological history and/or through any history of prescribed medications specific to depression.

7.1.2 **Saliva and Genotyping**

As mentioned in the larger case-control study (the previous chapter), 2 ml of saliva were collected in a saliva collection kit (Oragene Sample DNA collection kit) and transported to the laboratory for extraction. Samples were then sent to the Duke Molecular Physiology Institute for genotyping. The following Primer was used for genotyping for rs6265 (Primer ACTCTGGAGAGCGTGAATGG).

7.1.3 **Statistical Analysis**

Histograms with the normal curve, Q-Q plots, and the statistical tests for normality were used to determine if the data were normally distributed. Demographic data were summarized as means $\pm$ SD and percent distribution. Mean and median baseline scores and follow-up scores were generated. Baseline symptom scores were calculated and compared with scores from the last follow-up time point for each patient using a paired t-test. To test our hypothesis, we stratified change in symptom score by phenotype. Given that data from some of our patients were incomplete and that some were lost to follow-up, we compared baseline characteristics of those who were included in the follow-up analysis and those who were not (missing).

7.2 **Results**

Table XII shows the demographics for the 36 DED patients. Mean age was 53 years $\pm$ 15.7. Twenty-five percent were males and 75% were females. Forty-seven percent were Whites and 53% were non-Whites. Forty-two percent were clinically diagnosed with depression. The genotype distribution for Val66Met was 0.1% for AA, 30.6% for GA, and 63.9% for GG.

TABLE XII
DEMOGRAPHIC CHARACTERISTICS

Variable	DED Patients (n=36)
Age	53.1 $\pm$ 15.7
Gender	
Male	9 (25.0%)
Female	27 (75.0%)
Race	
White	17 (47.2%)
Non-White	19 (52.8%)
Depression	
Yes	15 (41.7%)
No	21 (58.3%)
Val66Met BDNF	
A/A	2 (0.1%)
G/A	11 (30.6%)
G/G	23 (63.9%)

7.2.1   **Dry Eye Disease Baseline and Follow-Up Symptom Scores**

For this pilot study we enrolled the maximum feasible number of patients supported

under our budget. Despite the recruitment of 36 patients, complete follow-up data after initial

assessment were only available for 21 patients. The scores for these patients were not normally

distributed. While medians were generated and compared, we calculated and compared means

for clinical relevance and interpretability. The average mean follow-up of those patients was

15.52 $\pm$ 8.4 months and the median follow-up was 20.3 months. Table XIII shows the average

symptom scores of dryness, irritation, light sensitivity, and pain at baseline and average of last

follow-up time point for all 36 patients. Baseline DED symptom scores and genotype distribution

between patients with and without complete data were comparable and not statistically

significantly different (Table XIV).

TABLE XIII
DED BASELINE AND AVERAGE LAST FOLLOW-UP SYMPTOM SCORES (N=36)

Variable	Average Baseline	Average Last Follow	P-Value
Dryness	2.8 $\pm$ 1.3	1.8 $\pm$ 1.3	0.01
Irritation	2.1 $\pm$ 1.4	1.0 $\pm$ 0.9	<.01
Light Sensitivity	1.9 $\pm$ 1.6	2.2 $\pm$ 1.5	0.6
Pain	1.8 $\pm$ 1.6	0.4 $\pm$ 0.6	<.01

TABLE XIV
MEAN BASELINE DED SYMPTOM SCORES FOR PATIENTS WITH COMPLETE DATA AND
THOSE WITH INCOMPLETE DATA OR THOSE WHO WERE LOST-TO-FOLLOW-UP

Variable	Complete n=21	Incomplete n=15	P-Value
Dryness	2.8 $\pm$ 1.4	2.7 $\pm$ 1.3	0.9*
Irritation	2.0 $\pm$ 1.3	2.1 $\pm$ 1.4	0.4
Light Sensitivity	2.2 $\pm$ 1.5	1.4 $\pm$ 1.7	0.2
Pain	1.9 $\pm$ 1.6	1.7 $\pm$ 1.6	0.8
Val66Met BDNF			
A/A	0 (0.0%)	2 (5.6%)	0.33
G/A	8 (38.1%)	11 (30.6%)	
G/G	13 (61.9%)	23 (63.9%)	

Paired t-tests were performed for patients with complete follow-up data on all symptoms stratified by genotype status (Tables XV and XVI). Patients with GG genotype showed significant decreases in dryness and pain symptoms between baseline and the last follow-up time point (dryness: 3.0 to 1.7, P=.005; pain: 2.2 to 0.5, P=.002), whereas patients with the GA genotype did not exhibit significant decreases in the dryness and pain symptoms (2.5 till 2.0 P=.4 for dryness; 1.5 to 0.4 P=.1 for pain). Thirty-one percent of patients with the GG genotype said that their symptoms improved by 25%, 46.2% reported no change in symptoms, and 23.1% reported that their symptoms became worse. As for patients with the GA genotype, 25.0% said that their symptoms improved by 25%, 62.5% reported no change in symptoms, and 12.5% reported that their symptoms became worse (P=.90). One patient who had the GG genotype

said that they are doing "ok." They were classified as doing 25% better given that they were not

doing "ok" at baseline. Additionally, 62.5% of DED patients with the GA genotype were also

clinically diagnosed with depression, whereas 30.8% of DED patients with the GG genotype

were clinically diagnosed with depression. As for prescribed treatments, the mean level of

aggressiveness between GG and GA genotype did not differ clinically nor statistically (2.7 and

2.8, respectively, P=.8). This means that prescribed treatment did not vary with genotype status.

TABLE XV
DED BASELINE AND LAST FOLLOW-UP SYMPTOM SCORES PAIRED T-TEST GG
GENOTYPE (N=13)

Variable	Baseline	Last Follow	P-Value
Dryness	**3.0 $\pm$ 1.3**	**1.7 $\pm$ 1.3**	**0.005**
Irritation	1.6 $\pm$ 1.2	1.1 $\pm$ 1.1	0.25
Light Sensitivity	1.9 $\pm$ 1.4	1.6 $\pm$ 1.8	0.4
Pain	**2.2 $\pm$ 1.5**	**0.5 $\pm$ 0.5**	**0.002**

TABLE XVI
DED BASELINE AND LAST FOLLOW-UP SYMPTOM SCORES PAIRED T-TEST GA
GENOTYPE (N=8)

Variable	Baseline	Last Follow	P-Value
Dryness	**2.5 $\pm$ 1.6**	**2.0 $\pm$ 1.3**	**0.4**
Irritation	2.6 $\pm$ 1.4	0.9 $\pm$ 0.4	0.004
Light Sensitivity	2.6 $\pm$ 1.7	1.5 $\pm$ 1.1	0.1
Pain	**1.5 $\pm$ 1.7**	**0.4 $\pm$ 0.7**	**0.1**

Tables XVII and XVIII show the median scores between genotypes. Results were similar

to means, however the magnitudes of change were greater.

TABLE XVII
DED BASELINE AND LAST FOLLOW-UP SYMPTOM SCORES WILCOXON RANK SUM
GG GENOTYPE (N=13)

Variable	Baseline	Last Follow	P-Value
Dryness	**3.0**	**1.0**	**0.02**
Irritation	1.0	1.0	0.5
Light Sensitivity	1.0	0.0	0.3
Pain	**3.0**	**0.0**	**0.02**

TABLE XVIII
DED BASELINE AND LAST FOLLOW-UP SYMPTOM SCORES WILCOXON RANK SUM
GA GENOTYPE (N=8)

Variable	Baseline	Last Follow	P-Value
Dryness	**3.0**	**1.5**	**1.0**
Irritation	3.0	**1.0**	0.03
Light Sensitivity	1.0	1.0	0.3
Pain	**1.0**	**0.0**	**0.3**

7.3   **Discussion**

Genes are involved in the complex biological processes of drug response, such as absorption, distribution, and target interaction. Their efficacy largely depends on interactions with environmental and psychological variables. However, there is also evidence that key mutations, located in specific genes, may play a big role in the proportion of drug response (126). In this pilot study, we investigated the role of SNP Val66Met in the BDNF gene on treatment response for patients with DED and revealed that symptom reports may vary by genotype status.

The selection of Val66Met in the BDNF gene is largely because of the association between DED and depression. We have reported that 42.4% of our patients have been clinically diagnosed with depression (chapter 6). We have also revealed an OR of 3.46 for the association between DED and depression. Therefore it seemed plausible to investigate common polymorphisms between depression and DED and to look at the effect of treatment by

examining the DED symptom change over time. One of the most studied genetic variations within the BDNF gene is Val66Met (rs6265), which results in a Val66-to-Met (Val66Met) change in the 50-pro region of the protein. It has been reported that this variation is associated depression (127) and anxiety (128). Additionally, Met (minor allele a) carriers exhibit a higher risk of suicide attempts (129) and higher vulnerability to stressful life events than Val individuals (130,131). Even though Met carriers exhibit worse disease symptoms, studies have shown that this polymorphism interacts with antidepressant efficacy where Met carriers appear to exhibit a better response to classical antidepressants (132,133). Niitsu et al. in a comprehensive meta-analysis of published candidate gene studies focusing on antidepressant efficacy in major depression, revealed that BDNF Val66Met heterozygous genotype was associated with better Selective serotonin reuptake inhibitors response compared to the homozygous genotypes (133). Nevertheless, a better response to treatment in Met carriers may be highly dependent on the type of antidepressant, gender, ethnicity, and the presence of other polymorphisms (134).

Looking at the role of Val66Met in DED symptoms over time, we found that some symptoms like dryness and pain do not decrease significantly in patients with the minor allele a (Met carriers) of Val66Met despite treatment, whereas these symptoms get significantly reduced in patients who do not have the minor allele a. As such, there are some sensations that respond differentially to the presence of this SNP. The relationship of BDNF with the different sensation in DED is not known. This biological mechanism could be similar to the one of BDNF and antidepressant activity. Thus, the modification of DED treatment response by genotype is biologically plausible, and potential mechanisms should be investigated, building on these known mechanisms.

There are several limitations to our study. First, this is a pilot study and power would be improved with a larger sample size. Due to the small sample size, our data were not normally distributed. With distributions that are non-normal medians are presented. We did compare median scores between genotypes and the interpretation of the data was similar to means but

the magnitude was different. This may largely be due to precision issues. Second, our assessment of self-reported symptom change was weighted toward improvement. Similar to asking the extent of symptom improvement, we should ask the extent of how much worse the symptoms became. Additionally, we did not have a clinical measure over time, such as Schirmer and Rose Bengal staining, which would have provided objective measure. However, there is a disconnect between clinical signs and symptoms in DED, and physicians heavily rely on reported symptoms for treatment. Moreover, our analysis included only one follow-up time point given the allotted study time frame. The natural progression of DED is best described as a "waxing and waning" course; unlike diabetes, DED does not progress linearly. Patients with DED may show improvement at one follow-up time point and may not at another time point, despite treatment. Therefore, patients with a 10-year diagnosis of DED may show a similar pattern to a newly diagnosed patient. In order to describe the trajectory of DED treatment response and the role of Val66Met, a study with patients enrolled early after initial diagnosis who are followed up at multiple time points is necessary. Despite these limitations, this initial study may shed light for considering the role of Val66Met in the BDNF gene in regulating the efficacy of DED treatment.

8. CONCLUSIONS

8.1 <u>Summary of Findings and Public Health Significance</u>

This thesis on the epidemiological and biological links between DED and depression revealed the following main findings: (i) the persistence of DED symptoms correlates the most with affective interference of mood and social life, as shown through a new tool to measure the entire symptom burden of DED (Study 1: chapter 4); (ii) clinical diagnosis of DED is associated with depression as assessed through clinical diagnosis and medication (study 2: chapter 5); (iii) DED symptoms are correlated with depressive symptoms, where patients with DED symptoms exhibit more depressive symptoms than patients without DED (Study 2: chapter 5); (iv) Val66Met (rs6265), a SNP in the BDNF gene and two SNPs FokI (rs2228570) and ApaI (rs7975232) in the VDR gene were found to be potentially associated with DED, and that the association between Val66Met and DED may vary by depression status (Study 3: chapter 6); and (v) DED symptom follow-up for at least six months revealed that DED symptoms like dryness and pain persist in patients with the GA genotype (Met carries) of the Val66Met SNP whereas GG genotype patients showed improved DED symptoms (Study 4: chapter 7).

The burden of DED on patients and clinics is high; it is one of the leading causes of patient visits to ophthalmologists and optometrists in the United States. It is prevalent in more than 50% of patients of the American population aged 50 years or older (15). Symptoms of DED have consistently affected the quality of life of patients. The efficacy of DED treatment varies between patients. The reasons for this variation remain unknown. Like DED, depression is a chronic disease that waxes and wanes, affecting more than 120 million people worldwide (135). According to the US Centers for Disease Control in 2009–2012, 7.6% of Americans aged 12 and over had depression (136). The World Health Organization also reports on the rate of depression and its increasing burden (137). Specifically, in the United States, major depression accounts

for: 3.7% of all disability-adjusted life years; and 8.3% of all US years lived with disability (137).

Additionally, "absenteeism" and "presenteeism" due to depression have been estimated to result

in a loss of $36.6 billion per year in the United States (138). Causes of depression have yet to

be pinned down precisely. Studies have shown that depression is a disease with a biological

basis along with psychological and social implications. Similarly, patients with DED suffer from

inexplicable pain and patients with both depression and DED exhibit a significant reduction in

their quality of life. The work in this thesis focused on these two debilitating chronic diseases to

lay the foundation for studies that may help clinicians more effectively treat patients and reduce

their suffering.

There are several limitations of the studies in this thesis, which are highlighted below.

Our findings need to be confirmed by additional observational studies (case-control with larger

sample sizes and cohort studies); the specific biological interactions need to be delineated, and

several additional factors need to be considered.

8.2. **Limitations**

8.2.1 **Sample Size and Convenience Sampling**

Each of the studies in this thesis has a lower sample size than desired. An initial sample

size calculation was performed and determined for the entire thesis, especially for Specific Aims

B and C. This analysis took into consideration that we needed three types of cases (DED alone,

DED and depression, and depression alone) along with the controls. However, the calculation

revealed a large number beyond our able target. As such, we enrolled the maximum number of

patients that was feasible and budget-supported. The ideal goal was to have 250 cases and 250

controls using the following criteria: 1:1 ratio of cases to controls, α error of 0.05 (two-sided) and

β error of 0.2 (1–0.2=80% power), expected effect size (1.5), frequency of risk allele (SNP) in

sample population. For example, the minor allele frequency for Val66Met as taken from The

National Center for Biotechnology Information is 0.228. Using the CaTS Power Calculator

software developed at the Center of Statistical Genetics at the University of Michigan (http://www.sph.umich.edu/csg/abecasis/CaTS/index.html), the parameters above revealed a sample size of 250 for cases and 250 for controls. A low sample size affected our results with regard to precision and detection of modest associations.

In addition to low sample size, our method of sampling is characterized as a convenience sample, where we enrolled subjects attending one clinic. The advantages of convenience sampling are related to time and cost. The relative cost and time required to have a convenience sample are small compared to other sampling techniques. Given our budget and the relatively novel hypotheses, we were able to collect data on a sample of patients than if we were to use other systematic sampling. However, convenience sampling may suffer from selection bias leading to overrepresentation or underrepresentation of DED patients in the study sample. In our case, we recruited subjects from a dry eye clinic that treats the most advanced and complicated cases. Our patients may overrepresent the severity of DED in the population. This limits our ability to generalize our findings to the other populations who may be suffering from the milder form of DED.

8.2.2 **Additional Confounding and Effect Modification**

As mentioned in the background of this thesis, the consistent risk factors for DED include: female gender, older age, contact lens use, postmenopausal estrogen therapy, a diet that is low in omega 3 essential fatty acids or has a high ratio of omega 6 to omega 3 fatty acids, refractive surgery, vitamin A deficiency, radiation therapy, bone marrow transplantation, hepatitis C, and certain classes of systemic and ocular medications, including antihistamines. Throughout our analyses, we controlled for sociodemographic variables: age, gender, and race. Additionally, we determined depression diagnosis from medical charts by past history and medication use. Medication use for depressive symptoms was also determined as a separate variable and used as a control for the association between DED symptoms and depressive

symptoms. Given that sociodemographic data and depression status were determined from medical charts, the chances of information bias may have been higher than a study where these factors were more actively collected and if patients were referred from psychiatric clinics. Additionally, our studies do not control for additional risk factors, such as comorbidity, hormonal imbalance and therapy, and systemic medications.

Dry eye disease is not a single disease but is related to a heterogeneous group of conditions that present with common ocular findings. Amongst these conditions, some may also have common biological underpinnings with depression. For example, SLE, GVHD, and Sjögren's are all associated with DED and depression. Conditions like GVHD may cause more depression independent of how severe the DED is, and simply because overall these patients are more ill. Controlling for those comorbidities is important to determine whether the association between DED and depression is distorted by their presence. Additionally, stratifying by these comorbidities may allow us to identify whether the relationship between DED and depression differentially varies and if the estimates are different between those with and those without comorbidities. This will allow us to identify patients who are most likely to have depression with DED or not, ultimately allowing us to treat them differently.

Another important consideration is that of the role of hormones with DED and hormones with depression. Studies have shown that hormonal imbalance is associated with evaporative DED and meibomian gland dysfunction. Women are 1.5 to 2 times more likely than men to have DED (16,139,140). This occurs mostly after menopause. Additionally, hormone replacement therapy in postmenopausal women, especially estrogen alone, has been shown to be associated with DED (23). Controlling for hormonal imbalance in the association between DED and depression is needed because it may affect both diseases differently.

8.3   **Future Directions**

The results in this thesis may very well serve as preliminary data for larger multicenter case-control and cohort studies that further dissect the relationship between DED and depression and confirm the findings. We were limited in interpretation with regard to temporality. It is still unknown whether DED causes depression or depression causes DED, or whether their onset is simultaneous. To answer this question and address the primary limitations mentioned above, multicenter case-control studies followed by a longitudinal cohort study are needed. These multicenter studies would include DED clinics and psychiatric clinics collaborating on recruitment of patients, data collection, design, and analysis. For example the multicenter case-control study would consist of four groups: (i) patients with DED and no depression; (ii) patients with depression and no DED; (iii) patients with DED and depression; and (iv) healthy controls, with a minimum sample size of 250 in each group.

Additionally, a cohort study would specifically entail collecting baseline and genetic data and following up subjects for a minimum of five years. To minimize selection and sampling bias, systematic sampling techniques will be employed instead of convenience sampling to ensure that the cohort population is as representative as possible with regard to DED severity. Patients will be recruited from clinics that specialize in DED and that include a broad range of severity of DED patients. Patients will be followed up for a minimum of five years to determine whether DED patients develop depression, for example, compared to healthy controls. Study visits will occur every six months and detailed clinical testing will be performed. The inclusion criteria would primarily be broad to include a large sample of patients. To address recruitment challenges, incentives will be given to subjects. Examples of incentives include: free eye exams, reimbursement for clinic visits, and free health education.

The following data will be collected at baseline: sociodemographic data (age, gender, race, occupation, education, income); detailed medical history and comorbidities; detailed medication intake (current and past); saliva samples for genetic analysis clinical parameters for

DED (Schirmer, Rose Bengal staining); depression status and history; DED and depression symptom questionnaires; and dietary habits (e.g., foods rich in omega 3 have been shown to alleviate DED). At each visit, patients will undergo routine clinical eye exams for DED and depression and will complete the symptom questionnaires. Patients will also be asked about changes in their diet and medication.

Incidence rates and relative risks will be calculated and results summarized. Statistical modeling would entail controlling for all confounding variables mentioned above and stratifying by variables that are hypothesized to differentially affect the association between DED and depression.

Based on our preliminary data, we hypothesize that patients with DED will more likely develop depression than will healthy controls. Since we are interested in knowing if DED leads to depression or depression leads to DED through other mechanisms, we will observe whether patients with depression and no DED will be more likely to develop DED compared to healthy controls.

8.4   **<u>Conclusions</u>**

Together the studies in this thesis have uncovered that there may be a biological and epidemiological interrelationship between DED and depression. It is known that DED and depression occur independently, but these preliminary studies suggest that together they may have a bigger impact on clinical outcomes and treatment decisions. This field of research is relatively innovative and has been initiated as a result of public health concerns related to patient suffering of significant symptoms and a diminished quality of life stemming from both diseases.

CITED LITERATURE

1.   [No authors listed]. "The Epidemiology of Dry Eye Disease: Report of the Epidemiology Subcommittee of the International Dry Eye WorkShop (2007)." *Ocul Surf* 5, no. 2 (2007): 93–107.

2.   Kim, K. W., S. B. Han, E. R. Han, S. J. Woo, J. J. Lee, J. C. Yoon, and J. Y. Hyon. "Association between Depression and Dry Eye Disease in an Elderly Population." *Invest Ophthalmol Vis Sci* 10; 52, no. 11 (2011): 7954–7958.

3.   Galor, A., W. Feuer, D. J. Lee, H. Florez, D. Carter, B. Pouyeh, W. J. Prunty, and V. L. Perez. "Prevalence and Risk Factors of Dry Eye Syndrome in a United States Veterans Affairs Population." *Am J Ophthalmol* 152, no. 3 (2011): 377–384.

4.   Galor, A., W. Feuer, D. J. Lee, H. Florez, A. L. Faler, K. L. Zann, and V. L. Perez. "Depression, Post-Traumatic Stress Disorder, and Dry Eye Syndrome: A Study Utilizing the National United States Veterans Affairs Administrative Database." *Am J Ophthalmol* 154, no. 2 (2012): 340–346.

5.   Li, M., L. Gong, X. Sun, and W. J. Chapin. "Anxiety and Depression in Patients with Dry Eye Syndrome." *Curr Eye Res* 36, no. 1 (2011): 1–7.

6.   Vehof, J., B. Wang, D. Kozareva, P. G. Hysi, H. Snieder, and C. J. Hammond. "The Heritability of Dry Eye Disease in a Female Twin Cohort." *Invest Ophthalmol Vis Sci* 23; 55, no. 11 (2014): 7278–83.

7.   Chaudhary, S., A. Namavari, L. Yco, J. H. Chang, S. Sonawane, V. Khanolkar, J. Sarkar, and S. Jain. "Neurotrophins and Nerve Regeneration-Associated Genes Are Expressed in the Cornea after Lamellar Flap Surgery." *Cornea* 12 (2012): 1460–1467

8.   Sonawane, S., V. Khanolkar, A. Namavari, S. Chaudhary, S. Gandhi, S. Tibrewal, S. H. Jassim, B. Shaheen, J. Hallak, J. H. Horner, M. Newcomb, J. Sarkar, and S. Jain. "Ocular Surface Extracellular DNA and Nuclease Activity Imbalance: A New Paradigm for Inflammation in Dry Eye Disease." *Invest Ophthalmol Vis Sci* 53, no. 13 (2012): 8253–63.

9.   Stoll, T., Y. Kauer, S. Büchi, R. Klaghofer, T. Sensky, and P. M. Villiger. "Prediction of Depression in Systemic Lupus Erythematosus Patients Using SF-36 Mental Health Scores." *Rheumatology (Oxford)* 40, no. 6 (2001): 695–698.

10. Su, D., and P. D. Drummond. "Blushing Propensity and Psychological Distress in People with Rosacea." *Clin Psychol Psychother* 19, no. 6 (2012): 488–95.

11. Wlodarczyk, J., and C. Fairchild. "United States Cost-Effectiveness Study of Two Dry Eye Ophthalmic Lubricants." *Ophthalmic Epidemiol* 16, no. 1 (2009): 22–30.

12. Gayton, J. L. "Etiology, Prevalence, and Treatment of Dry Eye Disease." *Clin Ophthalmol* 3 (2009): 405–12.

13. Lemp, M. A. "Report of the National Eye Institute/Industry Workshop on Clinical Trials in Dry Eyes." *CLAO J* 21, no. 4 (1995): 221–32.

14. Williamson, J. F., K. Huynh, M. A. Weaver, and R. M. Davis. "Perceptions of Dry Eye Disease Management in Current Clinical Practice." *Eye Contact Lens* 40, no. 2 (2014): 111–115.

15. Lemp, M. A. "Dry Eye (Keratoconjunctivitis Sicca), Rheumatoid Arthritis, and Sjögren's Syndrome." *Am J Ophthalmol* 140, no. 5 (2005): 898–909.

16. Schaumberg, D. A., D. A. Sullivan, J. E. Buring, and M. R. Dana. "Prevalence of Dry Eye Syndrome among US Women." *Am J Ophthalmol* 136, no. 2 (2003): 318–26.

17. Schaumberg, D. A., R. Dana, J. E. Buring, and D. A. Sullivan. "Prevalence of Dry Eye Disease among US Men: Estimates from the Physicians' Health Studies. *Arch Ophthalmol* 127, no. 6 (2009): 763–768.

18. McCarty, C. A., A. K. Bansal, P. M. Livingston, Y. L. Stanislavsky, and H. R. Taylor. "The Epidemiology of Dry Eye in Melbourne, Australia." *Ophthalmology* 105, no. 6 (1998): 1114–1119.

19. Lin, P. Y., S. Y. Tsai, C. Y. Cheng, J. H. Liu, P. Chou, and W. M. Hsu. "Prevalence of Dry Eye among an Elderly Chinese Population in Taiwan: The Shihpai Eye Study." *Ophthalmology* 110, no. 6 (2003): 1096–101.

20. Reddy, P., O. Grad, and K. Rajagopalan. "The Economic Burden of Dry Eye: A Conceptual Framework and Preliminary Assessment." *Cornea* 23, no. 8 (2004): 751–61.

21. Yu, J., C. V. Asche, and C. J. Fairchild. "The Economic Burden of Dry Eye Disease in the United States: A Decision Tree Analysis." *Cornea* 30, no. 4 (2011): 379–87.

22. Yamada, M., Y. Mizuno, and C. Shigeyasu. "Impact of Dry Eye on Work Productivity." *Clinicoecon Outcomes Res* no. 4 (2012): 307–12.

23. Schaumberg, D. A., J. E. Buring, D. A. Sullivan, and M. R. Dana. "Hormone Replacement Therapy and Dry Eye Syndrome." *JAMA* 7; 286, no. 17 (2001): 2114–2119.

24. Miljanović, B., K. A. Trivedi, M. R. Dana, J. P. Gilbard, J. E. Buring, and D. A. Schaumberg. "Relation between Dietary n-3 and n-6 Fatty Acids and Clinically Diagnosed Dry Eye Syndrome in Women." *Am J Clin Nutr* 82, no. 4 (2005): 887–93.

25. Hovanesian, J. A., S. S. Shah, and R. K. Maloney. "Symptoms of Dry Eye and Recurrent Erosion Syndrome after Refractive Surgery." *J Cataract Refract Surg* 27, no. 4 (2001): 577–84.

26. Sommer, A. "Vitamin A Deficiency and the Global Response." *Forum Nutr* 56 (2003): 33–35.

27. Li, M., L. Gong, W. J. Chapin, and M. Zhu. "Assessment of Vision-Related Quality of Life in Dry Eye Patients." *Invest Ophthalmol Vis Sci* 53, no. 9 (2012): 5722–5727.

28. Wen, W., Y. Wu, Y. Chen, L. Gong, M. Li, X. Chen, M. Yan, Z. Xiao, and X. Sun. "Dry Eye Disease in Patients with Depressive and Anxiety Disorders in Shanghai." *Cornea* 31, no. 6 (2012): 686–92.

29. Anttila, S. S., M. L. Knuuttila, and T. K. Sakki. "Depressive Symptoms as an Underlying Factor of the Sensation of Dry Mouth." *Psychosom Med* 60, no. 2 (1998): 215–218.

30. Bergdahl, M., J. Bergdahl, and I. Johansson. "Depressive Symptoms in Individuals with Idiopathic Subjective Dry Mouth." *J Oral Pathol Med* 26, no. 10 (1997): 448–50.

31. Vriezekolk, J. E., R. Geenen, A. Hartkamp, G. L. Godaert, H. Bootsma, A. A. Kruize, J. W. Bijlsma, and R. H. Derksen. "Psychological and Somatic Predictors of Perceived and Measured Ocular Dryness of Patients with Primary Sjögren's Syndrome." *J Rheumatol* 32, no. 12 (2005): 2351–2355.

32. Schiffman, R. M., M. D. Christianson, G. Jacobsen, J. D. Hirsch, and B. L. Reis. "Reliability and Validity of the Ocular Surface Disease Index." *Arch Ophthalmol* 118, no. 5 (2000): 615–21.

33. Vitale, S., L. A. Goodman, G. F. Reed, and J. A. Smith. "Comparison of the NEI-VFQ and OSDI Questionnaires in Patients with Sjögren's Syndrome-Related Dry Eye." *Health Qual Life Outcomes* 1, no. 2 (2004): 44.

34. Wang, T. J., I. J. Wang, C. C. Hu, and H. C. Lin. "Comorbidities of Dry Eye Disease: A Nationwide Population-Based Study. *Acta Ophthalmol* 90, no. 7 (2012): 663–668.

35. Goren, M. B., and S. B. Goren. "Diagnostic Tests in Patients with Symptoms of Keratoconjunctivitis Sicca." *Am J Ophthalmol* 106, no. 5 (1998): 570–574.

36. van Bijesterveld, O. P. "Diagnostic Tests in Sicca Syndrome." *Arch Ophthalmol* 82 (1969): 10–14.

37. Farris, R. L. "Tear Osmolarity—A New Gold Standard?" *Adv Exp Med Biol* 350 (1994): 495–503.

38. Rajagopalan, K., L. Abetz, P. Mertzanis, D. Espindle, C. Begley, R. Chalmers, B. Caffery, C. Snyder, J. D. Nelson, T. Simpson, and T. Edrington. "Comparing the Discriminative Validity of Two Generic and One Disease-Specific Health-Related Quality of Life Measures in a Sample of Patients with Dry Eye." *Value Health* 8, no. 2 (2005): 168–74.

39. Gothwal, V. K., K. Pesudovs, T. A. Wright, and C. W. McMonnies. "McMonnies Questionnaire: Enhancing Screening for Dry Eye Syndromes with Rasch Analysis." *Invest Ophthalmol Vis Sci* 51, no. 3 (2010): 1401–1407.

40. Abetz, L., K. Rajagopalan, P. Mertzanis, C. Begley, R. Barnes, and R. Chalmers. "Impact of Dry Eye on Everyday Life (IDEEL) Study Group. Development And Validation of the Impact of Dry Eye on Everyday Life (IDEEL) Questionnaire, A Patient-Reported Outcomes (PRO) Measure for the Assessment of the Burden of Dry Eye on Patients." *Health Qual Life Outcomes* 9 (2011): 111.

41. Gulati, A., R. Sullivan, J. E. Buring, D. A. Sullivan, R. Dana, and D. A. Schaumberg. "Validation and Repeatability of a Short Questionnaire for Dry Eye Syndrome." *Am J Ophthalmol* 142, no. 1 (2006): 125–131.

42. Hunter, M. S. "The Women's Health Questionnaire (WHQ): Frequently Asked Questions (FAQ)." *Health Qual Life Outcomes* 10, no. 1 (2003): 41.

43. Dougherty, B. E., J. J. Nichols, and K. K. Nichols. "Rasch Analysis of the Ocular Surface Disease Index (OSDI)." *Invest Ophthalmol Vis Sci* 52, no. 12 (2011): 8630–8635.

44. Gürdal, C., O. Saraç, I. Genç, H. Kırımlıoğlu, T. Takmaz, and I. Can. "Ocular Surface and Dry Eye in Graves' Disease." *Curr Eye Res* 36, no. 1 (2011): 8–13.

45. Rossi, G. C., C. Tinelli, G. M. Pasinetti, G. Milano, and P. E. Bianchi. "Dry Eye Syndrome-Related Quality of Life in Glaucoma Patients." *Eur J Ophthalmol* 19, no. 4 (2009): 572–579.

46. Adatia, F. A., A. Michaeli-Cohen, J. Naor, B. Caffery, A. Bookman, and A. Slomovic. "Correlation between Corneal Sensitivity, Subjective Dry Eye Symptoms and Corneal Staining in Sjögren's Syndrome." *Can J Ophthalmol* 39, no. 7 (2004): 767–71.

47. Luchs, J. I., D. S. Nelinson, J. I. Macy, and LAC-07-01 Study Group. "Efficacy of Hydroxypropyl Cellulose Ophthalmic Inserts (LACRISERT) in Subsets of Patients with Dry Eye Syndrome: Findings from a Patient Registry." *Cornea* 29, no. 12 (2010): 1417–27.

48. Kim, T. H., J. W. Kang, K. H. Kim, K. W. Kang, M. S. Shin, S. Y. Jung, A. R. Kim, H. J. Jung, S. D. Lee, J. B. Choi, and S. M. Choi. "Acupuncture for Dry Eye: A Multicentre Randomised Controlled Trial with Active Comparison Intervention (Artificial Tear Drop) Using a Mixed Method Approach Protocol." *Trials* 11 (2010): 107.

49. Monaco, G., V. Cacioppo, D. Consonni, and P. Troiano. "Effects of Osmoprotection on Symptoms, Ocular Surface Damage, and Tear Film Modifications Caused by Glaucoma Therapy." *Eur J Ophthalmol* 21, no. 3 (2011): 243–50.

50. Shafer, A. B. "Meta-Analysis of the Factor Structures of Four Depression Questionnaires: Beck, CES-D, Hamilton, and Zung." *J Clin Psychol* 62, no. 1 (2006): 123–46.

51. Beck, A. T., C. H. Ward, M. Mendelson, J. Mock, and J. Erbaugh. "An Inventory for Measuring Depression." *Arch Gen Psychiatry* 4 (1961): 561–71.

52. Beck, A. T., N. Epstein, G. Brown, and R. A. Steer. "An Inventory for Measuring Clinical Anxiety: Psychometric Properties." *J Consult Clin Psychol* 56, no. 6 (1988): 893–897.

53. Yeung, A., S. Howarth, R. Chan, S. Sonawalla, A. A. Nierenberg, and M. Fava. "Use of the Chinese Version of the Beck Depression Inventory for Screening Depression in Primary Care." *J Nerv Ment Dis* 190, no. 2 (2002): 94–99.

54. Dutton, G. R., K. B. Grothe, G. N. Jones, D. Whitehead, K. Kendra, and P. J. Brantley. "Use of the Beck Depression Inventory-II with African American Primary Care Patients." *Gen Hosp Psychiatry* 26, no. 6 (2004): 437–42.

55. Cohen, S. D., L. Norris, K. Acquaviva, R. A. Peterson, and P. L. Kimmel. "Screening, Diagnosis, and Treatment of Depression in Patients with End-Stage Renal Disease." *Clin J Am Soc Nephrol* 2, no. 6 (2007): 1332–42.

56. Finkelstein, F. O., and S. H. Finkelstein. "Depression in Chronic Dialysis Patients: Assessment and Treatment." *Nephrol Dial Transplant* 15, no. 12 (2000): 1911–1913.

57. Geisser, M. E., R. S. Roth, and M. E. Robinson. "Assessing Depression among Persons with Chronic Pain Using the Center for Epidemiological Studies-Depression Scale and the Beck Depression Inventory: A Comparative Analysis." *Clin J Pain* 13, no. 2 (1997): 163–70.

58. Lustman, P. J., R. E. Clouse, L. S. Griffith, R. M. Carney, and K. E. Freedland. "Screening for Depression in Diabetes Using the Beck Depression Inventory." *Psychosom Med* 59, no. 1 (1997): 24–31.

59. Watnick, S., P. L. Wang, T. Demadura, and L. Ganzini. "Validation of 2 Depression Screening Tools in Dialysis Patients." *Am J Kidney Dis* 46, no. 5 (2005): 919–24.

60. Poole, H., S. White, C. Blake, P. Murphy, and R. Bramwell. "Depression in Chronic Pain Patients: Prevalence and Measurement." *Pain Pract* 9, no. 3 (2009): 173–80.

61. Segal, D. L., F. L. Coolidge, B. S. Cahill, and A. A. O'Riley. "Psychometric Properties of the Beck Depression Inventory II (BDI-II) among Community-Dwelling Older Adults." *Behav Modif* 32, no. 1 (2008): 3–20.

62. Suija, K., U. Rajala, J. Jokelainen, T. Liukkonen, P. Härkönen, S. Keinänen-Kiukaanniemi, and M. Timonen. "Validation of the Whooley Questions and the Beck Depression Inventory in Older Adults." *Scand J Prim Health Care* 30, no. 4 (2012): 259–64.

63. Anderson, J. E., E. E. Michalak, and R. W. Lam. "MD Depression in Primary Care: Tools for Screening, Diagnosis, and Measuring Response to Treatment Issue." *BCMJ* 44, no. 8 (2002): 415–419.

64. Castrén, E., and T. Rantamäki. "The Role of BDNF and Its Receptors in Depression and Antidepressant Drug Action: Reactivation of Developmental Plasticity." *Dev Neurobiol* 70, no. 5 (2010): 289–97.

65. Groves, J. O. "Is It Time to Reassess the BDNF Hypothesis of Depression?" *Mol Psychiatry* 12, no. 12 (2007): 1079–88.

66. Cardoner, N., V. Soria, M. Gratacòs, R. Hernández-Ribas, J. Pujol, M. López-Solà, J. Deus, M. Urretavizcaya, X. Estivill, J. M. Menchón, and C. Soriano-Mas. "Val66met BDNF Genotypes in Melancholic Depression: Effects on Brain Structure and Treatment Outcome." *Depress Anxiety* 30, no. 3 (2013): 225–33.

67. Kanellopoulos, D., F. M. Gunning, S. S. Morimoto, M. J. Hoptman, C. F. Murphy, R. E. Kelly, C. Glatt, K. O. Lim, and G. S. Alexopoulos. "Hippocampal Volumes and the Brain-Derived Neurotrophic Factor Val66met Polymorphism in Geriatric Major Depression." *Am J Geriatr Psychiatry* 19, no. 1 (2011): 13–22.

68. Frodl, T., C. Schüle, G. Schmitt, C. Born, T. Baghai, P. Zill, R. Bottlender, R. Rupprecht, B. Bondy, M. Reiser, H. J. Möller, and E. M. Meisenzahl. "Association of the Brain-Derived Neurotrophic Factor Val66Met Polymorphism with Reduced Hippocampal Volumes in Major Depression." *Arch Gen Psychiatry* 64, no. 4 (2007): 410–416.

69. Montag, C., B. Weber, K. Fliessbach, C. Elger, and M. Reuter. "The BDNF Val66Met Polymorphism Impacts Parahippocampal and Amygdala Volume in Healthy Humans: Incremental Support for a Genetic Risk Factor for Depression." *Psychol Med* 39, no. 11 (2009): 1831–1839.

70. Musil, R., P. Zill, F. Seemüller, B. Bondy, M. Obermeier, I. Spellmann, W. Bender, M. Adli, I. Heuser, J. Zeiler, W. Gaebel, W. Maier, M. Rietschel, D. Rujescu, R. Schennach, H. J. Möller, and M. Riedel. "No Influence of Brain-Derived Neurotrophic Factor (BDNF) Polymorphisms on Treatment Response in a Naturalistic Sample of Patients with Major Depression." *Eur Arch Psychiatry Clin Neurosci* 263, no. 5 (2013): 405–12.

71. Domschke, K., B. Lawford, G. Laje, K. Berger, R. Young, P. Morris, J. Deckert, V. Arolt, F. J. McMahon, and B. T. Baune. "Brain-Derived Neurotrophic Factor (BDNF) Gene: No Major Impact on Antidepressant Treatment Response." *Int J Neuropsychopharmacol* no. 1 (2010): 93–101.

72. Pei, Y., A. K. Smith, Y. Wang, Y. Pan, J. Yang, Q. Chen, W. Pan, F. Bao, L. Zhao, C. Tie, Y. Wang, J. Wang, W. Zhen, J. Zhou, and X. Ma. "The Brain-Derived Neurotrophic-Factor (BDNF) Val66met Polymorphism Is Associated with Geriatric Depression: A Meta-Analysis. *Am J Med Genet B Neuropsychiatr Genet*, 159B, no. 5 (2012): 560–566.

73. Zou, Y. F., Y. Wang, P. Liu, X. L. Feng, B. Y. Wang, T. H. Zang, X. Yu, J. Wei, Z. C. Liu, Y. Liu, M. Tao, H. C. Li, K. Q. Li, J. Hu, M. Li, K. R. Zhang, D. Q. Ye, and X. P. Xu. "Association of Brain-Derived Neurotrophic Factor Genetic Val66Met Polymorphism with Severity of Depression, Efficacy of Fluoxetine and Its Side Effects in Chinese Major Depressive Patients." *Neuropsychobiology* 61, no. 2 (2010): 71–78.

74. Chen, L., D. A. Lawlor, S. J. Lewis, W. Yuan, M. R. Abdollahi, N. J. Timpson I. N. Day, S. Ebrahim, G. D. Smith, and Y. Y. Shugart. "Genetic Association Study of BDNF in Depression: Finding from Two Cohort Studies and a Meta-Analysis." *Am J Med Genet B Neuropsychiatr Genet* 147B, no. 6 (2008): 814–21.

75. Gyekis, J. P., W. Yu, S. Dong, H. Wang, J. Qian, P. Kota, and J. Yang. "No Association of Genetic Variants in BDNF with Major Depression: A Meta- and Gene-Based Analysis." *Am J Med Genet B Neuropsychiatr Genet* 162, no. 1 (2013): 61–70.

76. Verhagen, M., A. van der Meij, P. A. van Deurzen, J. G. Janzing, A. Arias-Vásquez, J. K. Buitelaar, and B. Franke. "Meta-Analysis of the BDNF Val66Met Polymorphism in Major Depressive Disorder: Effects of Gender and Ethnicity." *Mol Psychiatry* 15, no. 3 (2010): 260–71.

77. Jiang, R., B. H. Brummett, M. A. Babyak, I. C. Siegler, and R. B. Williams. "Brain-Derived Neurotrophic Factor (BDNF) Val66Met and Adulthood Chronic Stress Interact to Affect Depressive Symptoms." *J Psychiatr Res* 47, no. 2 (2013): 233–239.

78. Chen, J., X. Li, and M. McGue. "Interacting Effect of BDNF Val66Met Polymorphism and Stressful Life Events on Adolescent Depression." *Genes Brain Behav* [Epub ahead of print], (2012).

79. Skiljevic, D., I. Jeremic, M. Nikolic, S. Andrejevic, M. Sefik-Bukilica, B. Stojimirovic, and B. Bonaci-Nikolic. "Serum Dnase I Activity in Systemic Lupus Erythematosus: Correlation with Immunoserological Markers, the Disease Activity and Organ Involvement." *Clin Chem Lab Med* 51, no. 5 (2013): 1083–91.

80. Mostowska, A., M. Lianeri, M. Wudarski, M. Olesińska, and P. P. Jagodziński. "Vitamin D Receptor Gene Bsmi, Foki, Apai and Taqi Polymorphisms and the Risk of Systemic Lupus Erythematosus." *Mol Biol Rep* 40, no. 2 (2013): 803–10.

81. Mak, A., C. S. Tang, and R. C. Ho. "Serum Tumour Necrosis Factor-Alpha Is Associated with Poor Health-Related Quality of Life and Depressive Symptoms in Patients with Systemic Lupus Erythematosus. *Lupus*, 22, no. 3 (2013): 254–61.

82. Mata, J., R. J. Thompson, and I. H. Gotlib. "BDNF Genotype Moderates the Relation between Physical Activity and Depressive Symptoms." *Health Psychol* 29, no. 2 (2010): 130–133.

83. Cleeland, C. S. "Symptom Burden: Multiple Symptoms and Their Impact as Patient-Reported Outcomes." *J Natl Cancer Inst Monogr* no. 37 (2007): 16–21.

84. mdanderson website Cleeland CS (The MD Anderson Symptom Inventory User Guide. Accessed February 26, 2015. Available: http://www.mdanderson.org/education-and-research/departments-programs-and-labs/departments-and-divisions/symptom-research/symptom-assessment-tools/MDASI_userguide.pdf, 2010.

85. Schaumberg, D. A., A. Gulati, W. D. Mathers, T. Clinch, M. A. Lemp, J. D. Nelson, G. N. Foulks, and R. Dana. "Development and Validation of a Short Global Dry Eye Symptom Index." *Ocul Surf* 5, no. 1 (2007): 50–57.

86. Mertzanis, P., L. Abetz, K. Rajagopalan, D. Espindle, R. Chalmers, C. Snyder, B. Caffery, T. Edrington, T. Simpson, J. D. Nelson, C. Begley. "The Relative Burden of Dry Eye in Patients' Lives: Comparisons to a U.S. Normative Sample." *Invest Ophthalmol Vis Sci* no. 46 (2005): 46–50.

87. Miljanovic, B., R. Dana, D. A. Sullivan, and D. A. Schaumberg. "Impact of Dry Eye Syndrome on Vision-Related Quality of Life." *Am J Ophthalmol* no. 143 (2007): 409–415.

88. Walt, J. G., M. M. Rowe, and K. L. Stern. "Evaluating the Functional Impact of Dry Eye: The Ocular Surface Disease Index [abstract]." *Drug Inf J* 311436, (1997).

89. Mizuno, Y., M. Yamada, Y. Miyake, and Dry Eye Survey Group of the National Hospital Organization of Japan. "Association between Clinical Diagnostic Tests and Health-Related Quality of Life Surveys in Patients with Dry Eye Syndrome." *Jpn J Ophthalmol* 54, no. 4 (2010): 259–65.

90. Fernandez, C. A., A. Galor, K. L. Arheart, D. L. Musselman, and V. D. Venincasa. "Dry Eye Syndrome, Posttraumatic Stress Disorder, and Depression in an Older Male Veteran Population." *Invest Ophthalmol Vis Sci* 54, no. 5 (2013): 3666–3672.

91. Calonge, M. "The Treatment of Dry Eye." *Surv Ophthalmol.* 45, Suppl 2 (2001): S227–39.

92. Nichols, K. K., J. J. Nichols, and G. L. Mitchell. "The Lack of Association between Signs and Symptoms in Patients with Dry Eye Disease." *Cornea* 23, no. 8 (2004): 762–70.

93. Johnson, M. E. "The Association between Symptoms of Discomfort and Signs in Dry Eye." *Ocul Surf* 7, no. 4 (2009): 199–211.

94. Dogru, M., and K. Tsubota . "Pharmacotherapy of Dry Eye." *Expert Opin Pharmacother* 12, no. 3 (2011): 325–34.

95. Hallak, J. A., S. Jassim, V. Khanolkar, and S. Jain. "Symptom Burden of Patients with Dry Eye Disease: A Four-Domain Analysis." *PLoS One* 8, no. 12 (2013): e82805.

96. Labbé, A., Y. X. Wang, Y. Jie, C. Baudouin, J. B. Jonas, and L. Xu. "Dry Eye Disease, Dry Eye Symptoms and Depression: The Beijing Eye Study." *Br J Ophthalmol* 97, no. 11 (2013): 1399–403.

97. van der Vaart, R., M. A. Weaver, C. Lefebvre, and R. M. Davis. "The Association between Dry Eye Disease and Depression and Anxiety in a Large Population-Based Study." *Am J Ophthalmol* 159, no.3 (2014): 470–474.

98. Thieme, K., D. C. Turk, R. H. Gracely, W. Maixner, and H. Flor. "The Relationship among Psychological and Psychophysiological Characteristics Of Fibromyalgia Patients." *J Pain* [Epub ahead of print], (2014).

99. Burbelo, P. D., K. Ambatipudi, I. Alevizos. "Genome-Wide Association Studies in Sjögren's Syndrome: What Do the Genes Tell Us about Disease Pathogenesis?" *Autoimmun Rev* 13, no. 7 (2014): 756–61.

100. Ice, J. A., H. Li, I. Adrianto, P. C. Lin, J. A. Kelly, C. G. Montgomery, C. J. Lessard, and K. L. Moser. "Genetics of Sjögren's Syndrome in the Genome-Wide Association Era." *J Autoimmun* 39, nos. 1–2 (2012): 57–63.

101. Na, K. S., J. W. Mok, J. Y. Kim, and C. K. Joo. "Proinflammatory Gene Polymorphisms Are Potentially Associated with Korean Non-Sjögren Dry Eye Patients." *Mol Vis* no. 17 (2011): 2818–23.

102. Lowe, D.P., M. A. Cook, S. J. Bowman, D. C. Briggs, and UK Sjögren's Interest Group. "Association of Killer Cell Immunoglobulin-Like Receptors with Primary Sjögren's Syndrome." *Rheumatology (Oxford)* 48, no. 4 (2009): 359–62.

103. Kimpton, J. "The Brain Derived Neurotrophic Factor and Influences of Stress in Depression." *Psychiatr Danub* 24, Suppl 1(2012): S169–71.

104. Fauchais, A.L., A. Boumediene, F. Lalloue, G. Gondran, V. Loustaud-Ratti, E. Vidal, and M. O. Jauberteau. "Brain-Derived Neurotrophic Factor and Nerve Growth Factor Correlate with T-Cell Activation in Primary Sjögren's Syndrome." *Scand J Rheumatol* 38, no. 1 (2009): 50–57.

105. Sairanen, M., G. Lucas, P. Ernfors, M. Castrén, and E. Castrén. "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*. 2; 25, no. 5 (2005): 1089–94.

106. Polyakova, M., K. Stuke, K. Schuemberg, K. Mueller, P. Schoenknecht, and M. L. Schroeter. "BDNF as a Biomarker For Successful Treatment Of Mood Disorders: A Systematic & Quantitative Meta-Analysis." *J Affect Disord* [Epub ahead of print], (2014).

107. Tinazzi, E., A. Puccetti, R. Gerli, A. Rigo, P. Migliorini, S. Simeoni, R. Beri, M. Dolcino, N. Martinelli, R. Corrocher, and C. Lunardi. "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* no. 3 (2009): 237–43.

108. Palejwala, N. V., H. S. Walia, and S. Yeh. "Ocular Manifestations of Systemic Lupus Erythematosus: A Review of the Literature." *Autoimmune Dis* [Epub ahead of print], (2012).

109. NCBI Gene Summary. "BDNF Brain-Derived Neurotrophic Factor [Homo Sapiens (Human)] Gene ID: 627, updated on 1-Jan-2015." Accessed Jan 4, 2014 http://www.ncbi.nlm.nih.gov/gene/627.

110. NCBI Gene Summary. "VDR Vitamin D (1,25- dihydroxyvitamin D3) Receptor [Homo sapiens (Human)] Gene ID:7241, updated on 1-Jan-2015." Accessed Jan 4, 2014 http://www.ncbi.nlm.nih.gov/gene/7421.

111. NCBI Gene Summary. "DNASE1 Deoxyribonuclease I [Homo sapiens (Human)] Gene ID:1773, updated on 1-Jan-2015." Accessed Jan 4, 2014 http://www.ncbi.nlm.nih.gov/gene/1773.

112. Yamasaki, K., and R. L. Gallo. "Rosacea As a Disease of Cathelicidins and Skin Innate Immunity. *J Investig Dermatol Symp Proc* no. 1 (2011): 12–15.

113. Gombart, A. F., N. Borregaard, and H. P. Koeffler. "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* no. 9 (2005): 1067–77.

114. Santos, B. R., L. P. Mascarenhas, F. Satler, M. C. Boguszewski, and P. M. Spritzer. "Vitamin D Deficiency in Girls from South Brazil: A Cross-Sectional Study on Prevalence and Association with Vitamin D Receptor Gene Variants." *BMC Pediatr* 12 (2012): 62.

115. Harms, L. R., T. H. Burne, D. W. Eyles, J. J. McGrath. "Vitamin D and the Brain. *Best Pract Res Clin Endocrinol Metab* no. 4 (2011): 657–69.

116. Carles, J., M. Monzo, M. Amat, S. Jansa, R. Artells, A. Navarro, P. Foro, F. Alameda, A. Gayete, B. Gel, M. Miguel, J. Albanell, and X. Fabregat. "Single-Nucleotide Polymorphisms in Base Excision Repair, Nucleotide Excision Repair, and Double Strand Break Genes as Markers for Response to Radiotherapy in Patients with Stage I to II Head-and-Neck Cancer." *Int J Radiat Oncol Biol Phys* 66, no. 4 (2006):1022–30.

117. Liljedahl, U., L. Lind, L. Kurland, L. Berglund, T. Kahan, and A. C. Syvänen. "Single Nucleotide Polymorphisms in the Apolipoprotein B and Low Density Lipoprotein Receptor Genes Affect Response to Antihypertensive Treatment." *BMC Cardiovasc Disord* 4, no. 1 (2004): 16.

118. Schott, E., H. Witt, K. Neumann, A. Bergk, J. Halangk, V. Weich, T. Müller, G. Puhl, B. Wiedenmann, and T. Berg. "Association of TLR7 Single Nucleotide Polymorphisms with Chronic HCV-Infection and Response to Interferon-A-Based Therapy." *J Viral Hepat* 15, no. 1 (2008): 71–78.

119. Márquez, A., C. L. Dávila-Fajardo, G. Robledo, J. L. Rubio, E. de Ramón Garrido, F. J. García-Hernández, R. González-León, R. Ríos-Fernández, J. C. Barrera, M. F. González-Escribano, M. T. García, M. J. Palma, M. del Mar Ayala, N. Ortego-Centeno, and J. Martín. "IL2/IL21 Region Polymorphism Influences Response yo Rituximab in Systemic Lupus Erythematosus Patients." *Mol Biol Rep* 40, no. 8 (2013): 4851–4856.

120. Robledo, G., C. L. Dávila-Fajardo, A. Márquez, N. Ortego-Centeno, J. L. Callejas Rubio, E. de Ramón Garrido, J. Sánchez-Román, F. J. García-Hernández , R. Ríos-Fernández, M. F. González-Escribano, M. T. Camps García, M.J. Castillo Palma, M. Ayala Mdel, and J. Martín. "Association between -174 Interleukin-6 Gene Polymorphism And Biological Response To Rituximab In Several Systemic Autoimmune Diseases. *DNA Cell Biol* 31, no. 9 (2012): 1486–91.

121. Fabris, M., L. Quartuccio, S. Lombardi, M. Saracco, F. Atzeni, A. Carletto, M. Cimmino, C. Fabro, E. Pontarini, R. Pellerito, L. M. Bambara, P. Sarzi-Puttini, M. Cutolo, M. Manfredi, M. Benucci, P. Morassi, F. Fischetti, M. Padovan, M. Govoni, F. Curcio, E. Tonutti, and S. De Vita. "The CC Homozygosis of the -174G>C IL-6 Polymorphism Predicts a Lower Efficacy of Rituximab Therapy in Rheumatoid Arthritis." *Autoimmun Rev* 11, no. 5 (2012): 315–20.

122. Choi, S., K. M. Han, E. Won, B.J. Yoon, M.S. Lee, and B. J. Ham. "Association of Brain-Derived Neurotrophic Factor DNA Methylation and Reduced White Matter Integrity in the Anterior Corona Radiata in Major Depression." *J Affect Disord* 172C (2014): 74–80.

123. Yu, H., D. D. Wang, Y. Wang, T. Liu, F. S. Lee, and Z. Y. Chen. "Variant Brain-Derived Neurotrophic Factor Val66Met Polymorphism Alters Vulnerability to Stress and Response to Antidepressants." *J Neurosci* 32, no. 12 (2012): 4092–101.

124. Zhang, J. P., T. Lencz, S. Geisler, P. Derosse, E. J. Bromet, and A. K. Malhotra. "Genetic Variation In BDNF Is Associated with Antipsychotic Treatment Resistance in Patients with Schizophrenia." *Schizophr Res* 146, nos. 1–3 (2013): 285–288.

125. El-Hage, W., P. Vourc'h, P. Gaillard, J. Léger, C. Belzung, Y. Ibarguen-Vargas, C. R. Andres, and V. Camus. "The BDNF Val(66)Met Polymorphism Is Associated with Escitalopram Response in Depressed Patients." *Psychopharmacology (Berl)* 232, no. 3 (2015): 575–81.

126. Drago, A., D. De Ronchi, A. Serretti. "Pharmacogenetics of Antidepressant Response: An Update." *Hum Genomics* 3, no. 3 (2009): 257–74.

127. Gratacòs, M., V. Soria, M. Urretavizcaya, J. R. González, J. M. Crespo, M. Bayés, R. de Cid, J. M. Menchón, J. Vallejo, and X. Estivill. "A Brain-Derived Neurotrophic Factor (Bdnf) Haplotype Is Associated with Antidepressant Treatment Outcome in Mood Disorders." *Pharmacogenomics J*, 8, no. 2 (2008): 101–12.

128. Hünnerkopf, R., A. Strobel, L. Gutknecht, B. Brocke, K. P. Lesch. "Interaction between BDNF Val66Met and Dopamine Transporter Gene Variation Influences Anxiety-Related Traits." *Neuropsychopharmacology* 32, no. 12 (2007): 2552–60.

129. Sarchiapone, M., V. Carli, A. Roy, L. Iacoviello, C. Cuomo, M. C. Latella, M. di Giannantonio, L. Janiri, M. de Gaetano, and M. N. Janal. "Association of Polymorphism (Val66Met) of Brain-Derived Neurotrophic Factor with Suicide Attempts in Depressed Patients. *Neuropsychobiology* 57, no. 3 (2008): 139–45.

130. Brown, G. W., T. K. Craig, T. O. Harris, J. Herbert, K. Hodgson, K. E. Tansey, and R. Uher. "Functional Polymorphism in the Brain-Derived Neurotrophic Factor Gene Interacts with Stressful Life Events but Not Childhood Maltreatment in the Etiology of Depression." *Depress Anxiety* 31, no. 4 (2014): 326–34.

131. Hosang, G. M., C. Shiles, K. E. Tansey, P. McGuffin, R. Uher. "Interaction between Stress and the BDNF Val66Met Polymorphism in Depression: A Systematic Review and Meta-Analysis." *BMC Med* 12 (2014): 7.

132. Choi, M. J., R. H. Kang, S. W. Lim, K. S. Oh, and M. S. Lee. "Brain-Derived Neurotrophic Factor Gene Polymorphism (Val66Met) and Citalopram Response in Major Depressive Disorder." *Brain Res* 1118, no. 1 (2006): 176–82.

133. Niitsu, T., C. Fabbri, F. Bentini, and A. Serretti. "Pharmacogenetics in Major Depression: A Comprehensive Meta-Analysis." *Prog Neuropsychopharmacol Biol Psychiatry* 1; 45 (2013): 183–94.

134. Duclot, F., and M. Kabbaj. "Epigenetic Mechanisms Underlying the Role of Brain-Derived Neurotrophic Factor in Depression and Response to Antidepressants." *J Exp Biol* 218 (Pt 1) (2015): 21–31.

135. Lépine, J. P., and M. Briley. "The Increasing Burden of Depression." *Neuropsychiatr Dis Treat* 7(Suppl 1) (2011): 3–7.

136. Pratt, L. A., and D. J. Brody. "Depression in the US Household Population, 2009–2012." *NCHS Data Brief* 2014, 172 (2014): 1–8.

137. World Health Organization. Major Depression among Adults. Accessed on February 26, 2015 http://www.nimh.nih.gov/health/statistics/prevalence/major-depression-among-adults.shtml.

138. Kessler, R. C., H. S. Akiskal, M. Ames, H. Birnbaum, P. Greenberg, R. M. Hirschfeld, R. Jin, K. R. Merikangas, G. E. Simon, and P. S. Wang. "Prevalence and Effects of Mood Disorders on Work Performance in a Nationally Representative Sample Of U.S. Workers." *Am J Psychiatry* 163, no. 9 (2006): 1561–1568.

139. Chia, E. M., P. Mitchell, E. Rochtchina, A. J. Lee, R. Maroun, and J. J. Wang. "Prevalence and Associations of Dry Eye Syndrome in an Older Population: The Blue Mountains EyeStudy. *Clin Experiment Ophthalmol* 31, no. 3 (2003): 229–32.

140. Nichols, J. J., C. Ziegler, G. L. Mitchell, K. K. Nichols. "Self-Reported Dry Eye Disease across Refractive Modalities." *Invest Ophth almol Vis Sci* 46, no. 6 (2005): 1911–1914.

Ocular Surface Disease Index© (OSDI©)[2]

Ask your patients the following 12 questions, and circle the number in the box that best represents each answer. Then, fill in boxes A, B, C, D, and E according to the instructions beside each.

Have you experienced any of the following *during the last week*?	All of the time	Most of the time	Half of the time	Some of the time	None of the time
1. Eyes that are sensitive to light? . .	4	3	2	1	0
2. Eyes that feel gritty?	4	3	2	1	0
3. Painful or sore eyes?	4	3	2	1	0
4. Blurred vision?	4	3	2	1	0
5. Poor vision?	4	3	2	1	0

Subtotal score for answers 1 to 5   **(A)**

Have problems with your eyes limited you in performing any of the following *during the last week*?	All of the time	Most of the time	Half of the time	Some of the time	None of the time	N/A
6. Reading?	4	3	2	1	0	N/A
7. Driving at night?	4	3	2	1	0	N/A
8. Working with a computer or bank machine (ATM)?	4	3	2	1	0	N/A
9. Watching TV?	4	3	2	1	0	N/A

Subtotal score for answers 6 to 9   **(B)**

Have your eyes felt uncomfortable in any of the following situations *during the last week*?	All of the time	Most of the time	Half of the time	Some of the time	None of the time	N/A
10. Windy conditions?	4	3	2	1	0	N/A
11. Places or areas with low humidity (very dry)?	4	3	2	1	0	N/A
12. Areas that are air conditioned? . . .	4	3	2	1	0	N/A

Subtotal score for answers 10 to 12   **(C)**

Add subtotals A, B, and C to obtain D
(D = sum of scores for all questions answered)   **(D)**

Total number of questions answered
(do not include questions answered N/A)   **(E)**

106

APPENDIX B

Copyright Rules for Chapter 4 and Author Contributions

Chapter 4 in this thesis has been published in PLOS One as the following citation:

Hallak JA, Jassim S, Khanolkar V, Jain S. Symptom burden of patients with dry eye disease: a four domain analysis. PLoS One. 2013 Dec 13;8(12):e82805. doi: 10.1371/journal.pone.0082805.

Per PLOS one Content License guidelines: "No permission is required from the authors or the publishers to reuse or repurpose PLOS content provided the original article is cited. In most cases, appropriate attribution can be provided by simply citing the original article". The article has been originally cited throughout this thesis.

Author contributions are as follows:

"Conceived and designed the experiments: JAH S. Jassim VK S. Jain. Performed the experiments: JAH S. Jassim VK S. Jain. Analyzed the data: JAH S. Jassim. Contributed reagents/materials/analysis tools: JAH S. Jassim. Wrote the paper: JAH S. Jain".

<div align="center">**VITA**</div>

NAME   Joelle A. Hallak

EDUCATION

- 2011–present   Doctor of Philosophy Student (PhD Candidate since September 2012) in Epidemiology, substantive areas: genetics and ophthalmology. School of Public Health, University of Illinois at Chicago, Chicago, IL

- 2010   Master of Science in Epidemiology: School of Public Health, University of Illinois at Chicago, Chicago, IL
  Thesis title: Risks for Age-related Cataracts: Varying Associations Revealed by Half-Sigma Cut-off Bracketing

- 2005   BSc. Health Sciences, with distinction: University of Balamand (UOB), Beirut, Lebanon

- 2002   Lebanese Baccalaureate, High School Program, Life Sciences focus: Beirut Evangelical School, Beirut, Lebanon

LANGUAGES AND COMPUTER SKILLS

Languages: English (fluent), Arabic (fluent), French (elementary proficiency)
Statistical Software: SAS, STATA, SPSS
Other Software: Adobe Photoshop, Dreamweaver

HONORS AND AWARDS

- 2004, 2005   Dean's Honor List, Faculty of Health Sciences, UOB
- Fall 2004, Fall 2005 Undergraduate Merit Scholarship, Faculty of Health Sciences,
- 2012   Illinois Society to Prevent Blindness Research Grant Award

ACADEMIC EXPERIENCE

- 2011–Current   Research, Resource and Policy Analyst College of Medicine, University of Illinois at Chicago

- Fall 2013   Teaching Assistant for Epi 403-Introduction to Epidemiology: Principles and Methods. Instructor: Dr. Supriya Mehta. School of Public Health, University of Illinois at Chicago.

- 2006–2011   Research Specialist/Project Coordinator, Department of Ophthalmology and Visual Sciences, College of Medicine, University of Illinois at Chicago

- 2005–2006   Research Assistant, Massachusetts Eye and Ear Infirmary, Harvard Medical School

- 2004–2005   Research Intern and Research Assistant, St. Jude Children's Cancer Center of Lebanon, American University of Beirut

- 2003–2005   Research/Student Assistant, Faculty of Health Sciences, University of Balamand

PUBLICATIONS

Peer-Reviewed Publications

1. Tobaigy, F. M., R. C. Ghanem, R. R. Sayegh, **J. A. Hallak**, and D. T. Azar. "A Control-Matched Comparison of Laser Epithelial Keratomileusis and Laser In Situ Keratomileusis for Low to Moderate Myopia." *Am J Ophthalmol* 142, no. 6 (2006): 901–908.

2. Kojima, T., **J. A. Hallak**, and D. T. Azar. "Control-Matched Analyses of LASIK Outcomes in High Myopia." *J Cataract Refract Surg* 34, no. 4 (2008): 544–50.

3. Azar, D. T., R. C. Ghanem, J. de la Cruz, **J. A. Hallak**, T. Kojima, F. M. Al-Tobaigy, and S. Jain. "Thin-Flap (Sub-Bowman Keratomileusis) Versus Thick-Flap Laser In Situ Keratomileusis for Moderate to High Myopia: Case-Control Analysis." *J Cataract Refract Surg* 34, no. 12 (2008): 2073–2078.

4. Lee, J., J. Winokur, **J. Hallak**, and D. T. Azar. "Femtosecond Dovetail Penetrating Keratoplasty: Surgical Technique and Case Report." *Br J Ophthalmol* 93, no. 7 (2009): 861–863.

5. **Hallak, J. A.**, S. Taneri, A. F. Alharbi, and D. T. Azar. "Management of Astigmatism Post Penetrating Keratoplasty." *Saudi Journal of Ophthalmology* 23, no. 1 (2009): 17–24.

6. Ellenberg, D., D. T. Azar, **J. A. Hallak**, F. Tobaigy, K. Y. Han, S. Jain, and J. H. Chang. "Novel Aspects of Corneal Angiogenic and Lymphangiogenic Privilege." *Prog Retin Eye Res* 29, no. 3 (2010): 208–48.

7. Chang, J., E. Koo, E. Agron, **J. Hallak**, T. Clemons, D. Azar, R. D. Sperduto, F. L. Ferris III, and E. Y. Chew. "Risk Factors Associated with Incident Cataracts and Cataract Surgery in the Age-Related Eye Disease Study: AREDS Report #33. *Ophthalmology* 118, no. 11 (2011): 2113–2119.

8. Sarkar, J., S. Chaudhary, A. Namavari, O. Ozturk, J. H. Chang, L. Yco, S. Shankar, V. Khanolkar, **J. Hallak**, and S. Jain. "Corneal Neurotoxicity Due to Topical Benzalkonium Chloride." *Invest Ophthalmol Vis Sci* 53, no. 4 (2012): 1792–802.

9. Namavari, A., S. Chaudhary, O. Ozturk, J. H. Chang, L. Yco, S. Sonawane, N. Katam, V. Khanolkar, **J. Hallak**, J. Sarkar, and S. Jain. "Semaphorin 7a Links Nerve Regeneration and Inflammation in the Cornea." *Invest Ophthalmol Vis Sci* 53, no. 8 (2012): 4575–85.

10. Sonawane, S., V. Khanolkar, A. Namavari, S. Chaudhary, S. Gandhi, S. Tibrewal, S. H. Jassim, B. Shaheen, **J. Hallak**, J. H. Horner, M. Newcomb, J. Sarkar, and S. Jain. "Ocular Surface Extracellular DNA and Nuclease Activity Imbalance: A New Paradigm for Inflammation in Dry Eye Disease." *Invest Ophthalmol Vis Sci* 53, no. 13 (2012): 8253–63.

11. Chow, C. C., R. J. Shah, J. I. Lim, F. Y. Chau, **J. A. Hallak**, and T. S. Vajaranant. "Peripapillary Retinal Nerve Fiber Layer Thickness in Sickle-Cell Hemoglobinopathies Using Spectral-Domain Optical Coherence Tomography." *Am J Ophthalmol* 155, no. 3 (2013): 456–464.

12. Sarkar, J., S. Chaudhary, S. H. Jassim, O. Ozturk, W. Chamon, B. Ganesh, S. Tibrewal, S. Gandhi, Y. S. Byun, **J. Hallak**, D. L. Mahmud, N. Mahmud, D. Rondelli, and S. Jain. "CD11b+GR1+ Myeloid Cells Secrete NGF and Promote Trigeminal Ganglion Neurite Growth: Implications for Corneal Nerve Regeneration." *Invest Ophthalmol Vis Sci* 54, no. 9 (2013): 5920–36.

13. Kapamajian, M. A., J. de la Cruz, **J. A. Hallak**, and T. S. Vajaranant. "Correlation Between Corneal and Scleral Pneumatonometry: An Alternative Method for Intraocular Pressure Measurement." *Am J Ophthalmol* 156, no. 5 (2013): 902–906.

14. Tibrewal, S,, J. Sarkar, S. H. Jassim, S. Gandhi, S. Sonawane, S. Chaudhary, Y. S. Byun, Y. Ivanir, **J. Hallak**, J. H. Horner, M. Newcomb, and S. Jain. "Tear Fluid Extracellular DNA: Diagnostic and Therapeutic Implications in Dry Eye Disease." *Invest Ophthalmol Vis Sci* 54, no. 13 (2013): 8051–61.

15. **Hallak, J. A.**, S. Jassim, V. Khanolkar, and S. Jain. "Symptom Burden of Patients with Dry Eye Disease: A Four-Domain Analysis." *PLoS One* 8, no. 12 (2013): e82805.

16. Prickett, A. L., K. Bui, **J. Hallak**, P. Bakhtiyari, J. de la Cruz, D. T. Azar, and W. Chamon. "Cyclotorsional and Non-Cyclotorsional Components of Eye Rotation Observed from Sitting to Supine Position. *Br J Ophthalmol* 99, no. 1 (2015): 49–53.

17. Cortina, M. S., and **J. A. Hallak**. "Vision-Related Quality-of-Life Assessment Using NEI VFQ-25 in Patients after Boston Keratoprosthesis Implantation." *Cornea* 34, no. 2 (2015): 160–164.

Book Chapters

1. Kojima, T., T. Ongucci, **J. A. Hallak**, and D. T. Azar. "A Ten-Year Classified Review of the Peer Review Literature on Excimer Laser Refractive Complications." In *Management of Complications in Refractive Surgery*, edited by J. L. Alio, and D. T. Azar, 329–337. Berlin Heidelberg: Springer-Verlag, 2008.

2. De la Cruz, José, **J. A. Hallak**, D. T. Azar, and S. Jain. "Infections after Refractive Surgery." In *Management of Complications in Refractive Surgery*, edited by J. L. Alio, and D. T. Azar, 179–185. Berlin Heidelberg: Springer-Verlag, 2008.

3. Assil, K. K., **J. A. Hallak**, and D. T. Azar. "Radial and Astigmatic Keratotomy." In *Ophthalmology*, 3rd ed., edited by M. Yanoff, and J. Duker, 122–130. London: Mosby, 2009.

4. Assil, K. K., **J. A. Hallak**, and D. T. Azar. "Radial and Astigmatic Keratotomy." In *Ophthalmology*, 4th ed., edited by M. Yanoff, and J. Duker, 141–146. London: Mosby, 2014.

5. Barnes, S. D., **J. Hallak**, D. Pavan-Langston, and D. T. Azar. "Microbial Keratitis." In *Principles and Practices of Infectious Diseases*, 8th ed., edited by G. L. Mandell, J. E. Bennett, and R. Dolin, 1402–1414. New York: Elsevier Science, 2015.

6. Fahd, D., **J. Hallak**, and D. T. Azar. "Late-Onset Unilateral Irregular Mixed Astigmatism Following Thick-Flap LASIK." *In Difficult and Complicated Cases in Refractive Surgery*, edited by J. Alio, D.T. Azar, A. Abbouda, and A.E. Aswad. Berlin Heidelberg: Springer-Verlag, 2015. (In Press).

7. **Hallak, J. A.**, and D. T. Azar. "Combined Post-Keratoplasty LASIK/AK to Treat High Astigmatism." *In Difficult and Complicated Cases in Refractive Surgery*, edited by J. Alio, D.T. Azar, A. Abbouda, and A.E. Aswad. Berlin Heidelberg: Springer-Verlag, 2015. (In Press).

Abstracts and Papers Presented at Academic Conferences

1. Abboud, Miguel MD, **J. A. Hallak**, Samar Muwakkit MD, Pierre Zalloua PhD, Adlette Inati MD, Ayman Tawil MD, and Ibrahim Dabbous MD, Beirut, Lebanon and Tripoli, Lebanon. "Increased Rates of Blood Transfusion Among Lebanese Patients with Sickle Cell Disease and Splenomegaly." Paper presented at the 29th Annual Meeting of the National Sickle Cell Disease Program: Clinical Care, Research, and Education, Thriving in the City of the Blues, Memphis, TN, April 2006.

2. Kulchaiyawat, V, V. K. Aakalu, K. Sajja, S. Gupta, **J. Hallak**, and P. A. Setabutr. "Clinicopathological Correlation Between Müller's Muscle-Conjunctival Resection and Corneal Staining Pattern." ARVO Abstract. No. 231 (2010).

3. Vasaiwala, R., J. de la Cruz, **J. Hallak**, and D. Azar. "Evaluation of Direct and Indirect Techniques of LASIK Flap Thickness Measurement Using IntraLase Femtosecond Laser." ARVO Abstract. No. 323 (2010).

4. Nayeb-Hashemi, N., I. W. Porter, **J. Hallak**, R. Vasaiwala, and D. T. Azar. "Delayed Efficacy and Predictability Outcome Measures in Epi-LASIK and LASIK: A Cox Proportional Hazard Model for the Assessment of Related Risk Factors." ARVO Abstract. No. 423 (2010).

5. Shah, R., C. C. Chow, **J. A. Hallak**, J. I. Lim, and T. Vajaranant. "Retinal Nerve Fiber Layer (RNFL) Thickness Analysis in Sickle Cell Disease: Implications for Glaucoma Evaluation." ARVO Abstract. No. 248 (2011).

6. Ostheimer, T., C. C. Chow, R. Shah, **J. A. Hallak**, F. Y. Chau, J. I. Lim, and T. S. Vajaranant. "Rate of Retinal Nerve Fiber Layer Thinning by Heidelberg SD-OCT in Sickle Cell Disease: Implications for Glaucoma Evaluation." ARVO Abstract. (2012).

7. Chow, C. C., R. J. Shah, J. I. Lim, F. Y. Chau, **J. A. Hallak**, and T. S. Vajaranant. "Effect of Macular Thinning, Vessel Tortuosity, and Vessel Diameter in Peripapillary RNFL Thickness by Spectral Domain OCT in Sickle Cell Disease: Implications for Glaucoma Evaluation." ARVO Abstract. (2012).

8. Sarkar, J., S. V. Chaudhary, A. Namavari, O. Ozturk, L. Yco, S. Sonawane, V. Khanolkar, P. Batta, **J. Hallak**, and S. Jain. "Topical Benzalkonium Chloride Treatment Causes Corneal Neurotoxicity." ARVO Abstract. (2012).

9. Namavari, A., J. Sarkar, S. V. Chaudhary, O. Ozturk, L. Yco, S. Sonawane, V. Khanolkar, N. Katam, **J. Hallak**, and S. Jain. "Corneal Reinnervation Following Surgical Transection." ARVO Abstract. (2012).

10. Joslin, C. E., **J. A. Hallak**, and T. S. Vajaranant. "Five- and Ten-Year Glaucoma Incidence in the Age-Related Eye Disease Study (AREDS)." ARVO Abstract. (2012).

11. Prickett, A. L., W. Chamon, K. M. Bui , **J. Hallak**, P. Bakhtiari, and D. T. Azar."A Novel Approach to Determine Theoretical Head Tilt Effect on Ocular Cyclotorsion Measurements during Laser Refractive Surgery." ARVO Abstract. (2012).

12. Vajaranant, T. S., **J. A. Hallak**, and C. E. Joslin. "Intraocular Pressure and Ocular Perfusion Pressure among 10-Year Incident Glaucoma Cases in the Age-Related Eye Disease Study (AREDS)." ARVO Abstract. (2012).

13. **Hallak, J.**, S. Jassim, and S. Jain. "Symptom Burden of Dry Eye Disease: A Four-Domain Analysis." ARVO Abstract. (2013).

14. **Hallak, J. A.**, X. Gao, and S. Jain. "The Brain-Derived Neurotrophic Factor Rs6265 (Val66Met) Polymorphism and Dry Eye Disease: Potential Association with Stress-Related Disorders (Depression/Anxiety)." ARVO Abstract. (2014).

15. **Hallak, J. A.**, S. Tibrewal, and S. Jain. "Depressive Symptoms in Dry Eye Disease Patients: A Case-Control Study using the Beck Depression Inventory." [Accepted ARVO Abstract]. (2015).