TFOS DEWS III: Digest

FIONA STAPLETON, PABLO ARGÜESO, PENNY ASBELL, DIMITRI AZAR, CHARLES BOSWORTH, WEI CHEN, JOSEPH B. CIOLINO, JENNIFER P. CRAIG, JUANA GALLAR, ANAT GALOR, JOSÉ A.P. GOMES, ISABELLE JALBERT, YING JIE, LYNDON JONES, KENJI KONOMI, YANG LIU, JESUS MERAYO-LLOVES, FABIOLA R. OLIVEIRA, VICTOR L. PEREZ, EDUARDO M. ROCHA, BENJAMIN D. SULLIVAN, DAVID A. SULLIVAN, JELLE VEHOF, SUSAN VITALE, MARK WILLCOX, JAMES S. WOLFFSOHN, AND MURAT DOGRU

This digest summarizes the interdisciplinary research in dry eye disease (DED) published since the 2017 TFOS DEWS II reports. It comprises 7 topics including Sex, Gender, and Hormones; Epidemiology; Pathophysiology; Tear Film; Pain and Sensation; Iatrogenic Dry Eye; and Clinical Trial Design and explores how each of these inform diagnostic methodology, disease subtype, and management of DED.

Sex- and gender-related differences significantly influence the ocular surface due to hormones, sex chromosomes, sex-specific autosomal factors, epigenetics, care-seeking behaviors, and service use. Epidemiologic data reveal that DED prevalence varies by age and sex, influenced by diagnostic criteria and the multifactorial nature of the disease. New risk factors for DED include environmental, iatrogenicity, systemic diseases, and lifestyle domains.

Pathophysiological distinctions between aqueous deficient and more evaporative forms of DED have been clarified, with the latter most commonly characterized by a muted inflammatory response at the ocular surface, meibomian gland dysfunction, and conceivably phenotypic changes in corneal epithelial cells. There is an expanding role for metabolic, hormonal, physical, neural and cellular stresses, including hyperosmolarity, mitochondrial stress, and neurogenic inflammation.

Advancements in tear film research recommend new approaches to understanding DED pathogenesis and identifying biomarkers, such as microRNAs. Ocular pain perception is linked to structural integrity of corneal nerves, functional capacities of neurons, and activity of the central and peripheral nervous systems. Iatrogenic DED can result from medications, contact lenses, and surgical procedures. Clinical trials now emphasize aligning de-

AJO.com Supplemental Material available at AJO.com.
Accepted for publication May 23, 2025.

From the School of Optometry and Vision Science (F.S., P.As., I.J., M.W.), UNSW Sydney, New South Wales, Australia; Department of Ophthalmology (P.Ar.), Tufts Medical Center, Tufts University School of Medicine, Boston, Massachusetts, USA; Biomedical Engineering (P.As.), University of Memphis, Memphis, USA; Chicago College of Medicine (D.A.), University of Illinois, Chicago, IL, USA; Azura Ophthalmics (C.B.), Tel Aviv, Israel; Eye Hospital of Wenzhou Medical University (W.C.), National Eye Clinic Research Center, People's Republic of China; Cornea Service (J.B.C.), Massachusetts Eye and Ear, Department of Ophthalmology, Harvard Medical School, Boston, Massachusetts, USA; Department of Ophthalmology (J.P.C.), Aotearoa, New Zealand National Eye Centre, The University of Auckland, Auckland, New Zealand; Ocular Neurobiology Group (J.G.), Instituto de Neurociencias, Universidad Miguel Hernández-Consejo Superior de Investigaciones Científicas, San Juan de Alicante, Spain; Surgical Services (A.G.), Miami Veterans Affairs Hospital, Bascom Palmer Eye Institute, University of Miami, Miami, Florida, USA; Department of Ophthalmology and Visual Sciences (J.A.P.G.), Paulista School of Medicine / Federal University of São Paulo, São Paulo, Brazil; Beijing Institute of Ophthalmology (Y.J.), Beijing Tongren Eye Center, Beijing Tongren Hospital, Capital Medical University, Beijing, China; Beijing Ophthalmology & Visual Sciences Key Laboratory (Y.J.), Beijing, China; Centre for Ocular Research & Education (CORE) (L.J.), School of Optometry and Vision Science, University of Waterloo, Waterloo, Ontario, Canada; Clinical and Translational Research Center (K.K.), Keio University Hospital, Tokyo, Japan; Department of Ophthalmology (Y.L.), Zhongnan Hospital of Wuhan University, Wuhan, China; Instituto Universitario Fernandez-Vega (J.M.-L.), Universidad de Oviedo, Principality of Asturias, Spain; Department of Clinical Medicine (F.R.O.), Division of Rheumatology and Clinical Immunology, Ribeirao Preto Medical School, University of Sao Paulo, Ribeirao Preto, Sao Paulo, Brazil; Department of Ophthalmology (V.A.P.Q.), University of Miami Miller School of Medicine, Miami, Florida, USA; Department of Ophthalmology (E.M.R.), Otorhinolaryngology and Head & Neck Surgery, Ribeirao Preto Medical School, University of Sao Paulo, Ribeirao Preto, Sao Paulo, Brazil; Lμbris BioPharma (B.D.S.), LLC, Naples, Florida, USA; Bausch & Lomb (B.D.S.), Bridgewater, New Jersey, USA; Tear Film and Ocular Surface Society (D.A.S.), Boston, Massachusetts, USA; Departments of Ophthalmology and Epidemiology (J.V.), University of Groningen, University Medical Center Groningen, Groningen, the Netherlands; Department of Ophthalmology (J.V.), Vestfold Hospital Trust, Tønsberg, Norway; Division of Epidemiology and Clinical Applications (S.V.), National Eye Institute, National Institutes of Health, Bethesda, Maryland, USA; College of Health & Life Sciences (J.W.), School of Optometry, Aston University, Birmingham, United Kingdom; Dept of Ophthalmology (M.D.), Ichikawa General Hospital, Tokyo Dental College, Ichikawa, Chiba, Japan

Abbreviations: ADDE, Aqueous-deficient dry eye; BALB/c, BALB/C wildtype (mice); CI, confidence interval; CXL, Corneal cross-linking; DED, Dry eye disease; DEQ-5, 5-Item Dry Eye Questionnaire; DEWS, Dry Eye Workshops; DNA, Deoxyribonucleic acid; EDE, Evaporative dry eye; FDA, Food and drug administration; (f)MRI, (Functional) magnetic resonance imaging; GVHD, Graft-versus-host disease; HLA, Human leukocyte antigen; HPMC, Hydroxypropyl methylcellulose; ICAM-1, Intercellular Adhesion Molecule 1; IL, Interleukin; IGF, Insulin-like growth factor; IVCM, *In vivo* confocal microscopy; LASIK, Laser-assisted *in situ* keratomileusis; LIPCOF, Lid-parallel conjunctival folds; LLT, Lipid layer thickness; MGD, Meibomian gland dysfunction; MGYLS, Meibomian glands yielding liquid secretion; MMP, Matrix metalloproteinase; MUC4, Mucin 4; NGF, Nerve growth factor; NIBUT, Non-invasive tear film breakup time; NF-kB, Nuclear factor kappa-light-chain-enhancer of activated B cells; NRS, Numerical rating scale; OCT, Optical coherence tomography; OR, Odds ratio; OSDI, Ocular Surface Disease Index; PRK, Photorefractive keratectomy; QoL, Quality of Life; SANDE, Symptom Assessment iN Dry Eye questionnaire; SPEED, Standard Patient Evaluation of Eye Dryness questionnaire; TBUT, Tear film breakup time; TED, Thyroid eye disease; TFOS, Tear Film & Ocular Surface Society; TRPM8, Transient Receptor Potential cation channel subfamily M member 8; VAS, Visual analogue scale.

Inquiries to Fiona Stapleton, University of New South Wales Sydney, Sydney, New South Wales, Australia; e-mail: f.stapleton@unsw.edu.au

0002-9394/$36.00
https://doi.org/10.1016/j.ajo.2025.05.040

sign and end points with DED subtypes and therapeutic mechanisms, with new therapeutics and trial designs under consideration. (Am J Ophthalmol 2025;279: 451–553. © 2025 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/))

1. INTRODUCTION

This review updates the evidence for interdisciplinary aspects of dry eye disease (DED). It considered novel, human and animal evidence-based research published between 2017 and 2024. The goal of this report was to identify key research published since the 2017 TFOS DEWS II Workshop reports to underpin the evidence described in the TFOS DEWS III Diagnostic Methodology[1] and Management and Therapy[2] reports. The topics include sex, gender, and hormones; epidemiology including prevalence and risk factors; novel concepts and findings associated with pathophysiology; relevant changes to the tear film and interactions; the mechanisms related to ocular pain and sensation; iatrogenic causes of DED and their unique management; and a synthesis of clinical trial designs to inform exploration of new treatment modalities.

2. SEX, GENDER, AND HORMONES

2.1. INTRODUCTION: The TFOS DEWS II report on Sex, Gender, and Hormones[3] addressed many sex- and gender-related differences that significantly influence the ocular surface in health and DED. Many of these differences appeared to be due to the effects of hormones, sex chromosomes, sex-specific autosomal factors, epigenetics, care-seeking behaviors, and service use.[3] The purpose of this section is to highlight some of the relevant research since the publication of that report. The focus is primarily on studies published after July 1, 2017.

Additional studies related to sexual health and DED may be found in the recent detailed review titled "TFOS Lifestyle: Impact of Lifestyle Challenges on the Ocular Surface."[4]

2.2. SEX-RELATED DIFFERENCES IN THE OCULAR SURFACE AND ADNEXA: Significant sex-related differences in the lacrimal gland, meibomian gland, cornea and eyelid, reported since publication of the TFOS DEWS II report on Sex, Gender, and Hormones,[3] are briefly highlighted below. These differences may contribute to the increased prevalence of DED in females.

2.2.1. Lacrimal gland

Significant, sex-related differences exist in the gene expression, morphology, and pathophysiology of the lacrimal gland. The sex-associated differences in lacrimal gland gene expression may be very important in promoting lymphocyte accumulation in this tissue and contributing to the onset, progression, and/or severity of the inflammatory disease process in Sjögren's disease.[5] This condition is a multisystem autoimmune disease affecting the exocrine glands including the salivary and lacrimal glands that occurs primarily in women, and is associated with aqueous-deficient DED.[6] Murine models of autoimmune lacrimal gland disease, such as MRL/MpJ-Tnfrsf6^{lpr} and non-obese diabetic/LtJ mice, have provided critical insights into sex-related immune differences. The extent of lacrimal gland inflammation in MRL/MpJ-Tnfrsf6^{lpr} mice is, as in humans, far greater in females as compared with males, whereas the magnitude of lacrimal gland inflammation is far worse in nonobese diabetic/LtJ males.[7] Results showed that sex significantly influences the expression of thousands of genes and that the immune nature of the glandular response is very dependent on the Sjögren's disease model. Lacrimal tissues of female, as compared with male, MRL/MpJ-Tnfrsf6^{lpr} mice featured a significant increase in the expression of genes related to inflammatory responses, antigen processing, and chemokine pathways. In contrast, it was the lacrimal glands of nonobese diabetic/LtJ males, and not females, that presented with a significantly greater expression of immune-related genes. These data suggest that factors in the lacrimal gland microenvironment may be critically important in mediating these sex-associated immune effects and in promoting lacrimal gland inflammation.[5]

Analyses of single-cell transcriptomes from lacrimal glands of MRL/MpJ-Tnfrsf6^{lpr}, nonobese diabetic/LtJ and wild-type (BALB/c) mice have also defined the location of multiple cell type–specific messenger RNA (mRNA) markers and proteins, the latter of which may be secreted into the tear film in a sex-specific manner.[8]

As concerns morphology and pathophysiology, significant, sex-linked differences occur during aging in the magnitude of acinar atrophy, periacinar fibrosis, periductal fibrosis, ductal dilation, ductal proliferation, fatty infiltration, and lymphocyte infiltration in human lacrimal glands.[9] Female tissues had a higher frequency of all observed degenerative changes, except for ductal dilation, which was significantly more prevalent in male glands.[9] The authors concluded that female lacrimal glands are more susceptible to degeneration, conceivably because of hormonal influences, estrogen withdrawal, or genetic susceptibility, and that this susceptibility may play a significant role in the higher prevalence of DED in older women.[9]

2.2.2. Meibomian gland

Significant sex-related differences exist in meibomian gland gene expression, but the nature of these differences may be primarily species-dependent.[10] Analysis of the 500 most

highly expressed genes from human and BALB/c mouse meibomian glands demonstrated that only 24.4% were the same. Further, analysis of 100 genes with the greatest sex-associated differences in the human and mouse meibomian glands showed that none were the same, indicating that mice are not optimal models for understanding sex-associated differences in gene expression of the human meibomian glands.[10]

The prevalence of meibomian gland dysfunction (MGD) may vary by sex but results are inconsistent. Population- and hospital-based studies report that MGD may occur more frequently in males, or in females, or in neither sex. A recent prospective cross-sectional study indicated that older males had more severe eyelid margin abnormities and decreased gland number, height, and area compared with females.[11] Some studies suggest that the influence of sex on MGD may depend on the type (eg, obstructive vs hypersecretory forms,[12] the patient's age,[13] and/or the individual's medical condition[14]). The inconsistency in the sex-related prevalence of MGD is notable, given that DED is more common in females[15-24] and that MGD is a major cause of DED.[6,15,25,26] The prevalence of any MGD and clinically significant MGD are separately reported in a meta-analysis by age and sex (see Section 3.3, Figure 3, F and G). In brief, any MGD including asymptomatic gland changes is more prevalent in men than in women in older age groups but sex differences in clinically significant MGD are equivocal. There remains a need to explore the impact of more detailed diagnostic criteria to understand sex-related effects on MGD.

2.2.3. Cornea

Significant, sex-related differences exist in corneal thickness, sensitivity, reepithelization and DED-induced damage. As has been found previously, males have greater corneal epithelial thickness in all but the peripheral nasal zone[27] and females have higher corneal sensitivity,[28] and corneal nerve regeneration,[29] and slower corneal epithelial wound healing.[30] In addition, aqueous-deficient DED elicits more ocular pain, anxiety, and severe corneal damage in female mice.[31]

2.2.4. Eyelid blinking

A sexual dimorphism also has been identified in eyelid blinking. DED appears to increase sex-related differences in blinking, including heightened exaggeration of excitability in males and enhanced modifiability of the female trigeminal complex. This latter modifiability is proposed to explain the female predominance in the development of focal dystonia and benign essential blepharospasm.[32]

2.3. SEX-RELATED DIFFERENCES AND IMMUNITY: As stated in the TFOS Sex, Gender, and Hormones report,[3] sex-related differences are well known to occur in both innate and adaptive immunity and lead to differences in the severity and frequency of infections (male > female) and the risk of developing autoimmune diseases (female > male). These sex-based variations appear to be due to several factors, including sex steroid hormones, genetics, the microbiome, and nonbiological factors.

Since that report was published, almost 6000 articles have been cited in PubMed addressing the phrase "sex differences and immune." These have continued to show that sex as a biological factor significantly influences the distribution of lymphocyte subsets, quality of T cell responses, development of regulatory T cells, formation of the germinal centers, and the epigenetic accessibility of B cell loci.[33,34] Sex also affects transcriptional differences that are often highly immune cell–specific.[35] For example, more than 50 monocyte transcripts linked to the interferon pathway, inflammatory cytokines, and chemokines display sex-associated expression that are prominent in some female subjects.[35] In addition, changes in sex steroid hormone concentrations over the human lifespan contribute to sex-related differences in immune profiles and disease susceptibility patterns.[36]

There are also significant sex-related differences in regulatory processes between transcription factors and their target genes in multiple tissues.[37] Different transcription factors may regulate genes in males and females, irrespective of whether those target genes are differentially expressed. This sex-associated pattern of gene regulation may help to explain why males and females do not often manifest disease in the same way, or respond in the same way to treatment.[37]

All of these sex-related differences in immunity should have relevance to the ocular surface and adnexa in health and disease.

2.4. SEX AND GENDER DIFFERENCES IN PAIN ASSESSMENT: The intersection of sex and pain responses has been widely studied at clinical, psychological, and social levels. However, pain research often conflates sex and gender, and little is known about how gender identity diversity, such as gender-affirming medical procedures (hormonal or surgical therapies), environmental exposures, and minority status, impact pain.[38]

Despite chronic pain and DED being more common in women, most pain mechanism studies are based on male rodents.[39] With the inclusion of sex as a biological variable in preclinical research, studies on the influence of sex on pain and analgesia have increased. Female sex and older age are still the main factors associated with chronic pain. Experimental pain response differences suggest a biological mechanism rather than sociocultural gender-related issues.[40]

Studies have investigated whether pain sensitivity influences DED symptoms differently between sexes, accounting for ocular parameters. In a cross-sectional study of a young and healthy cohort (194 women and 93 men), intersex differences in ocular surface and pain sensitivity were linked to higher DED symptoms in women.[41] This finding helps explain the disparity between DED symptom intensity and signs previously described in females.

2.5. HORMONAL REGULATION OF THE OCULAR SURFACE AND ADNEXA:

2.5.1. Androgens

As detailed in the TFOS DEWS II report,[3] androgens are extremely important in the regulation of the ocular surface and adnexa and appear to mediate many of the sex-related differences in these tissues. Androgen deficiency, in turn, is associated with, and a risk factor for, both aqueous-deficient and evaporative DED.

A 1-month administration of the antiandrogen finasteride led to the development of rat lacrimal gland inflammation and aqueous tear deficiency.[42] Conversely, testosterone treatment of female MRL/MpJ-Tnfrsf6^{lpr} mice led to a striking downregulation of the lacrimal gland expression of more than 60 immune-associated biological process ontologies (≥20 genes/ontology), including those related to immune system processes, lymphocyte activation, cytokine production, and inflammatory response.[43] The nature of this androgen effect was dependent on murine strain, and the data indicate a major role for the lacrimal gland microenvironment in mediating androgen effects on immune gene expression.[43]

In human meibomian gland epithelial cells *in vitro*, dihydrotestosterone administration suppressed proinflammatory gene expression,[44] a hormone action that may contribute to the typical absence of inflammation within the human glands.[6,25] Dihydrotestosterone also inhibited the hyperosmolar-induced expression of tumor necrosis factor alpha (TNF-α), interleukin (IL)-8, and IL-6 mRNAs in human corneal epithelial cells[45] but had no influence on proinflammatory gene expression in unchallenged human corneal epithelial cells.[44] In contrast, dihydrotestosterone significantly increased 33 gene ontologies linked to the immune system in human conjunctival epithelial cells.[44]

Antiandrogen therapy for the treatment of prostate cancer and benign prostate hyperplasia led to a significant increase in the signs and symptoms of MGD and DED.[46,47] Transdermal androgen therapy applied to the lower abdomen, in turn, alleviated DED signs and symptoms in androgen-deficient individuals,[48] as well as DED signs in rabbits with combined androgen deficiency and MGD.[49]

Androgenetic alopecia was associated with decreased tear film breakup times, increased meiboscores, ocular surface symptoms, and MGD.[50] However, this form of hair loss is not necessarily linked to androgen excess but is associated with an increased risk of polycystic ovary syndrome.[51]

2.5.2. Estrogens

It has recently been reported that estrogen receptor-1 deficiency in mice induces inflammation and lipid deposition in the meibomian gland and lacrimal gland.[52] Estrogen receptor 1 loss, however, does not block estrogen receptor activity but results in an abnormal endocrine environment (e.g., increased luteinizing hormone, estradiol, testosterone, and progesterone levels) and may lead to insulin resistance.[53-55]

Ovariectomy of monkeys[56] and rats[57] engenders the signs of DED and these can be reversed with estrogen administration.[57] Other studies show that ovariectomy leads to rat anxiety and depressive-like behavior,[58] conditions that promote DED[4] and that estrogen treatment may amplify rat ocular hyperalgesia.[59] Some of these disparate findings regarding the role of estrogens might be explained by differences in experimental design, hormone dosage, or animal model.[60] However, complete estrogen absence does not cause lacrimal gland inflammation, gross alterations in meibomian gland histology, or aqueous-deficient DED in mice, and does not play a major role in the sex-related differences of the mouse meibomian gland.[60,61]

However, is it possible that this animal research does not reflect the situation in humans? This question is prompted by the results from aromatase inhibitor studies, which report an increased prevalence of DED.[62-65] Aromatase inhibitors block the synthesis of estrogens, induce estrogen deficiency, and are used as therapy in women with hormone receptor–positive breast cancer post initial chemotherapy.[66] However, these studies had no control groups. Treatment with aromatase inhibitors is known to promote anxiety (>112 PubMed articles), depression (>243 PubMed articles), and sleep disturbance (>25 PubMed articles),[67,68] all of which are associated with DED.[4] It may be that these mental health factors and sleep disorders contribute to, and possibly account for, these DED effects seen in those treated with aromatase inhibitors.

Investigators have also suggested that the increased estrogen levels following *in vitro* fertilization are responsible for the *in vitro* fertilization–associated DED symptoms[69] and/or signs.[69,70] However, there were no controls with these studies, and the effects may also have been linked to the known *in vitro* fertilization–induced anxiety, depression, and sleep disturbance.[71]

Clinicians continue to test whether topical estradiol might serve as a DED treatment. One of the most recent clinical trials showed no significant differences in effects between any of the estrogen dosages and the placebo.[72]

Lastly, estradiol may inhibit the conjunctival goblet cell response *in vitro* to an inflammatory stimulus.[73] This effect may be dose-dependent, given that the estrogen concentration used (0.1 μM) was considerably higher than the physiological range. Very high doses of estrogen often suppress, whereas physiological doses often enhance, immune responses.[74]

2.5.3. Progestins

Progesterone appears to suppress ocular pain and discomfort, which are common features of DED. Within 30 minutes after application to the rat forehead, 1% progesterone gel (ie, 10 mg/mL) produced corneal antinociception.[75] Forehead application of the same dose, twice per day, for 10 weeks led to a significant decrease in the frequency and severity of ocular symptoms in ocular graft-vs-host patients.[76] Researchers speculated that this hormone effect

may be mediated by the V1 branch of the trigeminal nerve that innervates the forehead skin, and that progesterone modifies the signal relay in the rostral and caudal trigeminal nucleus to dampen nociception.[76] However, given that the applied progesterone concentration in these studies was so much higher than that typically found in blood (eg, ng/mL levels), it might be possible that hormone action also involved other receptors. Progestins are known to bind glucocorticoid, androgen, and mineralocorticoid receptors, as well membrane receptors, oxytocin receptors, and γ-aminobutyric acid ($GABA_A$).[77,78]

2.5.4. Sex steroids in the tear film

Investigators continue to try to measure 17β-estradiol, progesterone, and testosterone in the human tear film.[79-81] However, as explained previously,[82] such measurements are of questionable, or no, relevance, and do not necessarily reflect the concentration of sex steroids or their metabolites in any ocular tissue. The processes by which sex steroids are synthesized and metabolized in humans are addressed in detail in the TFOS DEWS II Sex, Gender, and Hormones report.[3]

2.6. INSULIN-LIKE GROWTH FACTOR AND INSULIN: Insulinlike growth factor (IGF)-1 levels in tears may be an indicator of ocular surface health. Higher IGF-1 levels in tears were found in young adults compared with older adults, correlating positively with tear film breakup time (TBUT) and Schirmer test results.[83] In experimental studies, IGF-binding protein-3 plays a role in delivering IGF to target cells and works independently of IGF. IGF-binding protein-3 levels decrease in response to hyperosmolarity, a marker of DED, potentially causing epithelial damage in DED.[84]

Insulin insufficiency, as found in diabetes mellitus, has adverse effects on the ocular surface. A study in South Africa found significantly worse tear film parameters and a higher frequency of DED in children with diabetes mellitus compared with healthy controls.[85] An investigation in Turkey found that obese children had lower tear meniscus parameters and worse TBUT and Schirmer test scores than healthy children, and these results correlated with insulin resistance.[86] A study in India showed worse ocular surface parameters in those with diabetes mellitus compared with healthy controls.[87]

Additional research has linked the DED in diabetes mellitus to insulin impairment. For example, elevated serum opioid growth factor levels in diabetes are associated with DED and corneal damage, which can be mitigated by controlling glucose levels with insulin or opioid receptor antagonists.[88] Topical insulin therapy, in turn, has shown promise for treating the signs and symptoms of DED.[2,89-91]

For insulin topical therapy to be widely adopted for DED, questions about the optimal concentration, vehicle, and suitable DED subgroups need to be addressed.[92] Novel formulations using nanotechnology to improve insulin permeability and exposure to the ocular surface have shown promise in experimental models.[93,94]

2.7. THYROID HORMONE REGULATION OF THE OCULAR SURFACE AND ADNEXA: Thyroid eye disease (TED) or thyroid autoimmune orbitopathy, includes a spectrum of conditions like Hashimoto thyroiditis, Graves disease, and Schmidt syndrome.[95]

The association between TED and DED is debated. A study in the United States found no link between TED and DED.[96] However, studies in India, Saudi Arabia, Spain, Taiwan, and Russia found TED to be a risk factor for DED.[97-100] The variable clinical manifestations of TED (Figure 1) and different definitions of DED likely contribute to these discrepancies.

The mechanisms that cause DED in TED are broad and include anatomical changes in the orbit leading to proptosis and excessive corneal exposure, as well as inflammation of the ocular adnexa, including the main lacrimal gland and the eyelids.[101] Animal models deprived of thyroid hormone have shown clinical responses consistent with DED. As an example, rats made hypothyroid with methimazole developed higher tear film osmolarity and hypoesthesis.[102]

In TED, changes in the lacrimal gland have been observed via magnetic resonance imaging, with smaller lacrimal glands correlating with more severe clinical signs and higher inflammatory indices.[103] A study on the overlap of Hashimoto thyroiditis and Sjögren's disease found higher expression of 4 genes involved in both diseases, which may have diagnostic value.[104] These observations highlight the role of anatomical changes, genetic factors, and molecular inflammation mediators in linking TED, thyroid hormone dysfunction, and DED.

Recent studies have confirmed higher Ocular Surface Disease Index (OSDI) scores, lower tear TBUT, and more MGD in TED patients, compared with healthy controls. These symptoms and signs were correlated with worse proptosis, higher Clinical Activity Scores, incomplete blinking, as well as corneal damage.[100,105-108] Changes were also observed in euthyroid or inactive TED, indicating mixed inflammatory and anatomical factors.[109,110] Superior limbic keratoconjunctivitis was observed more frequently in TED patients (31%) and was associated with worse ocular surface conditions, younger age, and smoking.[111] Worse Schirmer test results, TBUT, and goblet cell density were found in children with Hashimoto thyroiditis compared with healthy children, even without symptoms.[112] In a study of 38 individuals with moderate to severe TED in Iran, more than 70% had DED.[113]

Noninvasive imaging techniques have recently improved the diagnosis and monitoring of TED. Magnetic resonance imaging and computed tomography, commonly used to assess orbital parameters in TED, are now used to study lacrimal gland volumetry and activity.[114,115] Evaluation of the lids and meibomian glands has shown an asso-

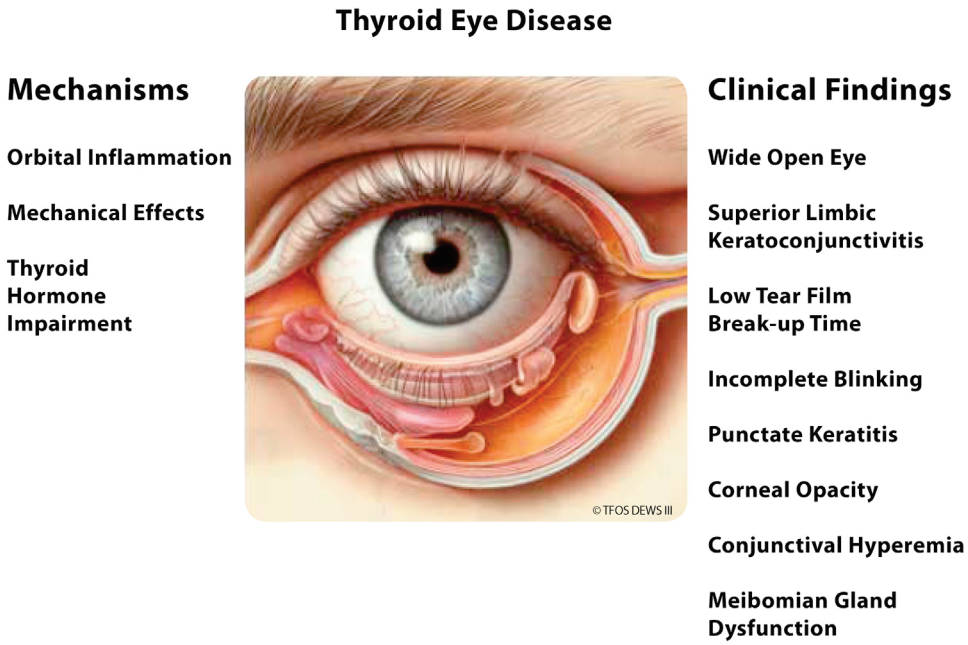

FIGURE 1. Thyroid eye disease.

ciation between MGD scores and clinical activity scores in TED.[116]

2.8. GENDER AND DED: As reported in the Sex, Gender, and Hormones report of TFOS DEWS II,[3] "gender" refers to an individual's self-representation as a man or woman, and how social institutions respond to that person based on the person's gender presentation.[3] Gender is not the same as "sex." Sex distinguishes males and females based on their biological characteristics, whereas gender reflects socially constructed characteristics such as behaviors related to being a woman, feminine, or being a man, masculine.[3] Both sex and gender affect health and disease, and gender also affects people's access to and interactions with the health care system.[3] Many health disparities are associated with gender, and both gender and biological sex influence DED risk and presentation, care-seeking behaviors, and service use.

Transgender individuals experience difficulties accessing appropriate health care.[117] A case series reported ocular findings in a transgender and gender-diverse population receiving gender-affirming hormone therapy. The treatments involved daily doses of estrogen and spironolactone or testosterone to 10 male-to-female and 7 female-to-male individuals, respectively. The major findings included intracranial hypertension in female-to-male and chorioretinal diseases in male-to-female. Regarding the ocular surface, 1 female-to-male patient developed dendritic keratitis, 2 male-to-female patients had prior DED, and 2 others were diagnosed with *de novo* DED due to low tear film TBUT and punctate keratitis.[118]

Providing appropriate health care for transgender and gender-diverse individuals is challenging because of a lack of specialized knowledge and social and cultural barriers. Clinical research and education for health care professionals are essential to address these challenges.[119-121] The interactions of sex hormones with ocular tissues and their documented effects on systemic health indicate that the impact of gender-affirming hormone therapy on vision and ocular health needs further investigation (Table 1). This includes exploring potential associations with DED and other ocular surface diseases and developing specific preventive and therapeutic strategies, such as perceived in geriatrics and gastroenterology conditions.[122,123]

2.9. FUTURE DIRECTIONS: There have been significant research advances linking sex, hormones, and gender to DED. Aging, cancer, and hormone therapy increasingly broaden the interdisciplinarity in this field over time. Despite the significant impact of gender-affirming hormone therapy on the entire endocrine system and its effects on physical and mental health, there is limited information on its impact on ocular health. Variations in age, health profile, gender-affirming hormone therapy compliance, and barriers to accessing regular health care limit the documentation of side effects. Clinicians and future research should consider these variations, as recommended in a recent systematic review on the medical aspects of the transgender and gender-diverse population.[123]

3. EPIDEMIOLOGY

3.1. SCOPE OF THE UPDATE: This epidemiology update aimed to assess and summarize knowledge on the prevalence and incidence of DED from well-designed population stud-

TABLE 1. Systemic Effects of Gender-Affirming Hormone Therapy With Potential Impact on Ocular Surface Health and DED

Reference	Gender-Affirming Hormone Therapy Type	Systemic Manifestation	Relationship With DED and Ocular Surface Disease
Betsi et al, 2024[124]	Puberty suppression with GnRH	Bone mass retardation, mood fluctuation	Anxiety and depression
Heng et al, 2024[125]	Testosterone	Breast atrophy and reduction of breast epithelia	Potential risk of other exocrine glands atrophy
Hashemi et al, 2024[126]	Testosterone	Higher risk for metabolic syndrome	Potential impact of metabolic syndrome on DED
de Silva et al, 2024[127]	Estrogen	Venous thromboembolism	Ischemic damage to ocular and adnexal tissues
Nieves-Rios et al, 2023[118]	Estrogen and spironolactone for MTF or testosterone for FTM	—	Corneal epitheliopathy attributed to HSK in FTM and DED and Punctate keratitis in MTF
Tienforti et al, 2024[128]	Hormonal	Sex hormone imbalance, menopause, andropause	Sexual hormone imbalance and DED

DED = dry eye disease, FTM = female-to-male transgender, GnRH = gonadotrophin release hormone, HSK = herpes simplex keratitis, MTF = male-to-female transgender.

ies and to perform a meta-analyses of existing study data to determine prevalence of DED using different diagnostic approaches, stratified by age and sex.

3.2. OPERATIONAL DEFINITIONS: As per the TFOS DEWS II report,[15] the subcommittee examined data from a large range of cohort studies and considered different methods of disease ascertainment and definition, including studies involving the type, frequency, and severity of symptoms, patient self-report of a diagnosis of DED by an eyecare practitioner, and studies that involved a clinical examination.

An updated search of published peer-reviewed literature was conducted using PubMed (https://pubmed.ncbi.nlm.nih.gov/), which includes MEDLINE for articles that reported the prevalence or incidence of DED. The following terms (dry eye syndrome, OR dry eye disease, OR meibomian gland dysfunction, OR keratoconjunctivitis sicca, OR blepharitis) AND (prevalence, OR epidemiology, OR incidence) were used to identify potential additional articles. Human studies published since the date applied in the previous TFOS DEWS II update (September 18, 2015, onward) were considered for inclusion. For the meta-analyses, human studies captured in the TFOS DEWS II meta-analysis report and those published subsequently were included.

Eligible studies included those reporting prevalence of either or both dry eye symptoms and signs. Observational studies (cross-sectional or cohort) were included if they were population-based and presented the study outcome as DED vs non-DED. Studies were excluded if no variance in the measure of prevalence was available in the manuscript, if it was not possible to calculate it from the data presented, if the sampling criteria were not explicitly stated or if no denominator was reported. Detailed exclusion criteria are described in Figure 2. Article author and date, setting (region; population or hospital), numbers and characteristics of participants within each study group (age, sex, ethnicity), prevalence, and incidence data were extracted from each article. When required, data were extracted from manuscript figures using open-source software (available at https://plotdigitizer.com/app).[129] Where multiple studies were published from the same data set, those with minimal overlap, distinctly different diagnostic criteria, and age/sex disaggregation were preferentially chosen.

A meta-analysis was conducted to determine the prevalence of DED for different diagnostic criteria stratified by age and sex. Using the search strategy described above, population-based prevalence studies published since 1980 (per TFOS DEWS II 2015 search) were included. Prevalence data were extracted firstly by age group as 6-9 years, 10-19 years, 20-29 years, 30-39 years, 40-49 years, 50-59 years, 60-69 years, 70-79 years, and ≥80 years and secondly by sex. For studies that had age categories that overlapped with the decade-based categorizations (eg, 35-44, 45-54, and 55-64), the weighted averages of data from each contributing interval were computed for each decade. CIs for the measures of prevalence were computed by using standard methods for computing the SE of a proportion or, if prevalence was zero, by computing the Poisson 95% CI, dividing it by 2 to provide an estimate of the SE as above. Studies were combined where the diagnostic criteria were broadly similar and in line with the 2017 approach and included a new category for studies consistent with the diagnostic methods described in the TFOS DEWS II Diagnostic Methodology report[130] as follows:

1. Women's Health Study criteria

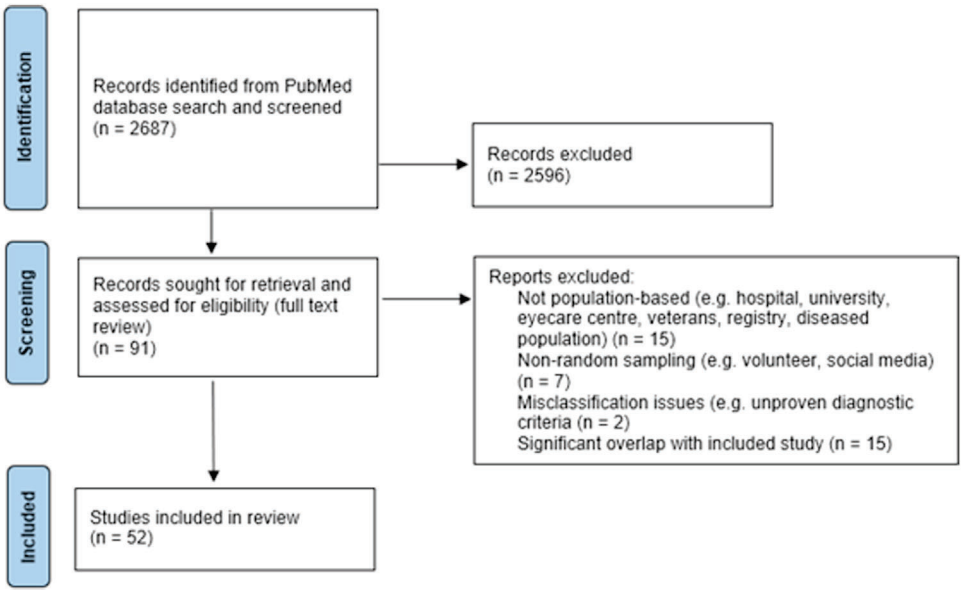

FIGURE 2. PRISMA flowchart of the literature search outcome for prevalence studies published between September 18, 2015, and June 29, 2024.

2. Symptomatic DED (OSDI ≥ 13 or significant ocular or visual symptoms, where signs are not reported)
3. Insurance claims data supporting diagnosis or treatment of DED
4. Symptoms and signs (eg, fluorescein staining, TBUT, and Schirmer score)
5. Diagnostic criteria broadly aligned with those described in the TFOS DEWS II Diagnostic Methodology Report[130]
6. Clinical diagnosis or prior diagnosis of DED
7. Any MGD reported and separately, clinically significant (grade 2 and higher, as reported) MGD

A random effects model was used to combine prevalence data. To compute the SE, the formula $SE = \sqrt{p \times q/n}$, where p was the proportion with DED was used. For studies where prevalence was 0, the exact Poisson confidence limits for the proportion computed and the width of that interval divided by 3.92 to approximate the SE.

For descriptive analyses, box plots were produced using SAS, version 9.5 (SAS Institute). For most diagnostic categories, the number of studies available for each age category was small. When there was only a single study in the category, the prevalence is reported as originally reported by the study. If there were 2 or more studies in the category, a pooled rate was computed using a suite of SAS macros models developed by Weir and Senn.[1006] These macros include summary statistics for the DerSimonian and Laird model[1007]. The Weir and Senn macro forest was used to produce forest plots of the results.

The flowchart describes the studies identified since 2015 and reasons for exclusion (Figure 2). A total of 2687 articles were identified through PubMed search (updated 29 June 2024). The extracted data summary is available here (Supplementary Table S1). For the overall prevalence estimation and meta-analysis, the final data set combined both 2015 and 2024 results. Subsequently, meta-analyses were conducted by age and sex by decade where data were available.

Estimates of the prevalence of DED from 76 large international cohort studies (24 and 52 from the 2015 and 2024 data sets, respectively) are summarized in Figure 2 and later. Supplementary Table S1 describes the characteristics of the additional included studies from the 2024 data set.

Table 2 summarizes the overall prevalence range by diagnostic criteria and Figure 3 disaggregates the prevalence by age and sex where there were sufficient data and includes data from both the 2015 and 2024 data sets.

3.3. PREVALENCE OF DED

3.3.1. Prevalence of DED based on the Women's Health Study criteria

Figure 3, A, represents prevalence by age and sex based on report of severe symptoms and/or a diagnosis of DED by a practitioner (n=12).[131-142] The prevalence ranges from 2.7% in those aged 20-29 years to 30.1% in women older than 80 years. The rate increases by age particularly after the age of 40 years in both sexes, and women have a higher rate of DED above the age of 50 years, with the sex difference in prevalence becoming more marked with age. One study has shown high prevalence rates in those 10-19 years old without sex-related differences. (See Supplementary Table S1 for individual study data.)

TABLE 2. Prevalence Range for DED for Each of the Diagnostic Criteria

Diagnostic criteria	Prevalence range (%)[a]
Women's Health Study criteria	2.7 - 30.1
Symptomatic DED	7.3 - 31.6
Claims data	2.8 - 8.5
Symptoms and signs of DED	4.7 - 62.9
TFOS DEWS II criteria	5.4 - 44.2
Clinical or prior diagnosis of DED	1.0 - 15.3
Any MGD	0.0 - 66.3
Clinically significant MGD	1.8 - 23.3

[a]Noting the lower end of the range relates to the rates in children. See Figure 3 below for prevalence disaggregated by age.

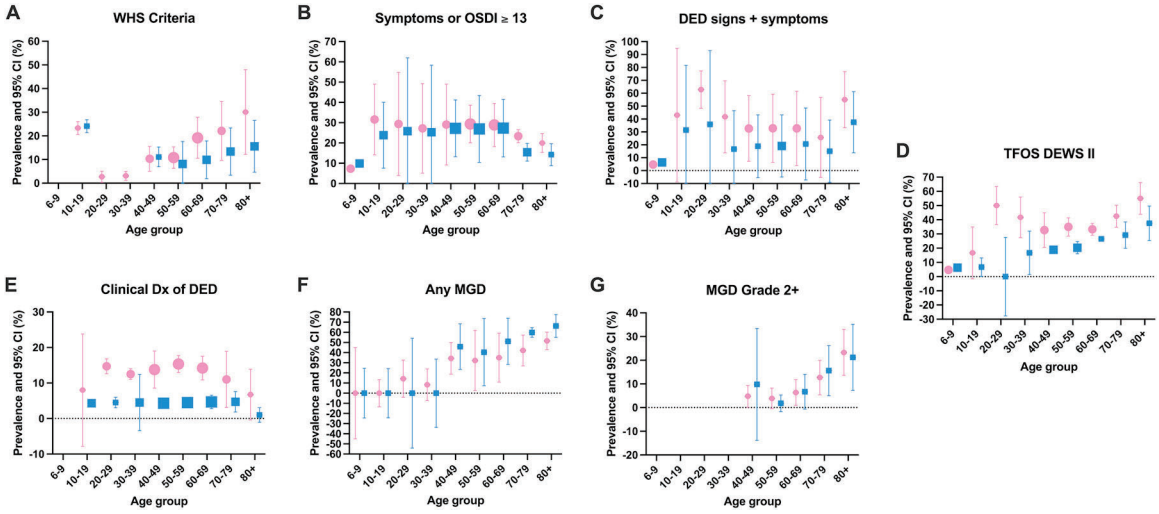

FIGURE 3. Prevalence of dry eye disease (DED) based on age and sex for different diagnostic criteria.

3.3.2. Prevalence of symptomatic DED

Figure 3, B, represents prevalence of symptomatic DED by age and sex. The prevalence ranges from 7.3% in those 6-9 years old to 31.6% in females aged 20-29 years; however, confidence intervals (CIs) are wide (n=33).[132-134,138-141,143-167] There were higher than expected rates of disease in the age groups <30 years, except a low rate in children aged 6-9 years. Rates were consistent in the adult groups aged 40-80 years, with rates in men in the order of 20% and, in women, 30%. Higher rates were observed in the ≥80-year age group, and in all ages >10 years, women had a consistently higher prevalence.

3.3.3. Prevalence of DED based on signs and symptoms

Figure 3, C, represents prevalence of DED based on the presence of signs and symptoms by age and sex (n=10).[97,131,132,141,145,151,157,161,162,164,168] The prevalence ranges from 4.7% in those 6-9 years old to 62.9% in females aged 20-29 years; however, the CIs are wide. The rates of disease are reasonably consistent across the adult age groups, with some reduction in symptomatic disease above the age of 70 years in both sexes. Sex differences were not pronounced in most age groups except for in those aged ≥70 years where women have a higher rate than men.

3.3.4. Prevalence of DED based on TFOS DEWS II criteria

The overall prevalence is broadly similar to diagnoses based on signs and symptoms of DED (n=3),[131,132,169] with prevalence ranging from 5.4% (in 6-9-year-olds) to 44.2% (Figure 3, D). Above the age of 30 years, the prevalence is higher among females than males, and males show a more obvious age-related effect.

3.3.5. Prevalence based on claims data

Overall prevalence based on claims, either identifying DED based on the *International Classification of Diseases* diagnosis code or using insurance claims data based on diagnosis or treatment code was generally low (n=1),[170] ranging from 2.8% to 8.5%. There were insufficient data to disaggregate prevalence by age and/or sex.

3.3.6. Prevalence of DED based on clinical diagnosis

Figure 3, E, represents prevalence of DED-based clinical diagnosis by age and sex (n = 8).[134,139,147,148,150,154,171,172]

The prevalence ranges from 1.0% in men aged ≥80 years, to 15.3% in women aged 50-59 years. Rates were reasonably consistent over age in adults with lower rates in those 10-15 and ≥80 years of age. Women had a higher rate of clinically diagnosed DED at all ages.

3.3.7. *Prevalence of any MGD*

Figure 3, F, represents prevalence of any MGD by age and sex (n=6).[97,131,173-176] The point estimates of prevalence ranges from 0% in those <20 years old to 66.3% men ≥80 years old. Rates appear to markedly increase above the age of 40 years and in each of the 40-plus age groups, MGD is significantly more prevalent in older men aged ≥70 years than in older women ($P < .05$). CIs in most age groups are wide.

3.3.8. *Prevalence of clinically significant MGD*

Figure 3, G, represents prevalence of clinically significant MGD (Grade 2 or above) by age and sex (n=2).[164,174] Rates appear to increase with age although sex differences are equivocal. There are no studies reporting rates of clinically significant MGD in younger individuals.

Thirty-three of 52 studies included in the systematic review from the 2024 data set did not contribute to the meta-analysis because of nonavailability of data stratified by age decade or by sex, or because of duplicate data.[20,23,97,139,140,160,161,163,165,166,168,176-197] The overall prevalence rates reported include all international cohort studies (24 and 52 from the 2015 and 2024 data sets, respectively). Meta-analysis includes only those studies publishing disaggregated age and sex data. Supplementary Table S1 shows the complete list of studies and data extraction for those identified in the recent data set. The 2015 data set was included previously as supplementary data.[15]

3.4. ANNUAL INCIDENCE OF DED: A limited number of population studies have attempted to assess the incidence of DED since the last TFOS DEWS II update. In a retrospective analysis of approximately 6.7 million medical claims from the United States Department of Defense Military Health System, the annual DED incidence in those aged ≥2 years was low but gradually increased over time from 0.55% in 2008 to 0.87% in 2012, respectively.[170] DED annual incidence was consistently higher in women than men and increased with age.[170]

A similar retrospective analysis was conducted of electronic medical records of 1,458,830 new patients presenting to Indian hospitals across 4 states, and the results revealed an annualized incidence (average across 8 years) of DED signs and symptoms of 1.46%.[198]

An analysis of the Taiwanese National Health Insurance Research Database between 2001 and 2015 (covering more than 23 million inhabitants) yielded an age-adjusted annual incidence of DED ranging from 0.15% (in 2001) to 0.37% (in 2015).[199]

An ancillary cohort study of the Vitamin D and Omega-3 Trial (VITAL), assessed the incidence of DED in 12,174 men aged ≥50 years and 11,349 women aged ≥55 years during a median (range) 5.3 (3.8-6.1) years of follow-up in the United States. All participants were initially disease-free, and 2% of participants experienced clinically incident DED.[200] The annualized incidence (average across 5 years) of clinically diagnosed DED and of clinically diagnosed DED plus incident reports of severe DED were 3.8 and 16 per thousand, respectively.[200] The Salnés Eye Study 2 re-examined a cohort of 264 Spanish individuals from the Salnés Eye Study 1 now aged 51 years and older, 11 years later.[201] The annualized incidence of DED signs and symptoms in these individuals was 2.3% (95% CI 1.8-2.8).[201] Participants (n=1682) from the Singapore Malay Eye Study (SiMES) were reexamined 6 years later, and symptoms of DED were evaluated using the Salisbury Eye Evaluation Study dry eye questionnaire.[202] The 6-year incidence of DED symptoms was 5.1% (95% CI 4.1-6.4).[202]

The incidence of DED is typically difficult to estimate as some with the disease will report resolution or a reduction in symptoms over multiple sampling periods. This may particularly occur for milder cases of DED where symptom report may vary over time. Incidence from claims data may be influenced by changes in reimbursement or the introduction of new therapies during the study.

3.5. NATURAL HISTORY OF DED: Longitudinal studies of DED are rare. A cohort of 784 patients from 1000 with DED from the Women's Health Study and Physicians' Health Study were surveyed about change in their disease 1 year after enrolment in the study, and medical records were obtained for 261 of the participants.[203] The mean disease duration was 10.5 years, and most participants reported no change to their disease status over time. Ocular surface symptoms were unchanged in 32% of participants and improved in 44%. Visual symptoms were unchanged in 52% and improved in 19%. Social impact was unchanged in 71% and improved in 19%. Risk factors associated with progression of ocular surface symptoms included a higher spend on treatment (more than $20 per month), history of more severe DED symptoms, and use of systemic beta-blockers. Worsening of visual symptoms was additionally associated with a history of ocular surgery, untreated depression, and blepharitis or MGD. Disease presentation and treatments varied by sex where women were more likely to present with corneal staining but worsening of symptoms was not predicted by corneal staining; however, treatments beyond level 1,[204] higher symptoms, and having a tear breakup time test performed were associated with progression of symptoms.

One retrospective study followed clinic-based participants with a diagnosis of either Sjögren (n=101) or non-Sjögren (n=101) DED over a 7-year period.[205] In this arguably more severe disease group, with escalation of treatment, predominantly with use of topical anti-

inflammatory/immunomodulatory therapies or in-office treatments, both groups experienced a significant reduction in ocular surface staining although no patient-reported outcomes were recorded.

In a small retrospective study of DED in the absence of treatment (n=73),[206] changes in signs were observed with an increased bulbar redness and reduced tear meniscus height and lipid layer thickness after 8 years. Change in symptoms over time were not reported.

In a registry-based study, signs of MGD and evaporative DED were apparent at an earlier age than aqueous-deficient DED,[207,208] although there may be some confounding because of ethnic background, where up to two-thirds of those with signs of MGD do not experience symptoms (asymptomatic disease).[174] There is indirect evidence to suggest that signs of MGD may precede other disease markers by up to 10 years,[207] which may have implications for the timing of treatment of asymptomatic MGD. In a small treatment trial of adults with symptomatic MGD who were treated for 12 months, one-third of participants had improvement in MGD signs and improvement was greater in younger participants (those aged <40 years) and with less ocular surface damage.[209]

In summary, there is reasonable evidence for development of signs before symptoms in untreated disease, which may have an impact on treatment considerations. There is evidence that treatment improves corneal staining but limited data on the sequence of improvement of signs and symptoms. Longitudinal natural history studies are much needed.

3.6. RISK FACTORS FOR DED: Conclusive and probable risk factors for DED grouped into major categories are described in Table 3 and have been divided into modifiable and nonmodifiable factors.

In clinical practice, administering a questionnaire to the patient (such as via paper or a digital application) for completion before the consultation is recommended to save time and to ensure that all commonly associated factors have been explored. The following section briefly highlights the most important risk / associated factors by category. Irrespective of a possible causal mechanism, all associated factors described may be useful in elucidating the etiology of DED and seeking to identify the possible driver(s) in an individual.

3.6.1. Systemic disorders

Numerous systemic disorders (Table 3) have been associated with an increased risk of DED, emphasizing the multifactorial origin of the disease.

Autoimmune disorders are well-established risk factors of DED. Up to 90% of patients with Sjögren's disease develop DED.[210,291] Rheumatoid arthritis, systemic lupus erythematosus, and scleroderma are also associated with a vastly increased risk of DED.[210,212,292] Other autoimmune diseases that have been consistently linked with an increased risk of DED are sarcoidosis, thyroid disease, inflammatory bowel disease (Crohn disease and ulcerative colitis), and psoriasis.[210,211,213,215,216,293,294] Less commonly investigated autoimmune disorders, also associated with DED in some studies, include lichen planus, lichen sclerosus, granulomatosis with polyangiitis, polyarteritis nodosa, primary biliary cirrhosis, mixed connective tissue disease, antiphospholipid syndrome, and dermatomyositis.[6,210,215,292,294-296] Infiltration of the lacrimal gland and accessory lacrimal glands by lymphocytes and other immune cells that leads to impaired tear secretion and aqueous-deficient DED has been suggested as a core mechanism. However, there is no peer-reviewed evidence for inflammation in the meibomian gland in obstructive MGD.[6,25]

The endocrine system plays an important role in maintaining ocular surface homeostasis. Hormone disorders such as androgen deficiency, polycystic ovarian syndrome, and thyroid disease have been linked to an increased risk of DED.[3,213,214,221] Sex hormones exert effects on all major components of the tear film.[3] In thyroid disease, immune-mediated lacrimal gland dysfunction, and exposure keratopathy due to orbitopathy (such as in TED), are important mechanisms.[3,214,297] Diabetes also has been consistently, although mildly, associated with DED in systematic reviews and meta-analyses (odds ratio [OR] of approximately 1.2).[211,213,218] Decreased corneal sensitivity and impaired reflex tear secretion have been suggested to be linked[298] alongside factors such as hyperglycemia, advanced glycated end product accumulation, oxidative stress, metabolic disease, and vascular disease.[102] Menopause also has been proposed as a risk factor for DED, but definitive evidence is lacking.[3,15,299]

In addition to psoriasis, other dermatologic disorders also may be associated with an increased risk of DED. Rosacea without systemic involvement may present with ocular symptoms alone and can lead to chronic blepharoconjunctivitis and MGD.[213,222,223] Acne vulgaris has been linked to DED, possibly by an associated risk of MGD or as a result of side effects of isotretinoin therapy.[215,224] Atopic disorders such as eczema, asthma, and virtually all types of allergy have been consistently linked with an increased risk of DED.[213,215,225,226,277] Allergic eye disease may aggravate DED, and suggested mechanisms include increased inflammation at the ocular surface, altered epithelial barrier and corneal innervation, and tear film instability. Antihistamine use, which may result in anticholinergic side effects, may also partly explain the association. Periocular eczema also has been linked to blepharitis. Alternatively, DED may aggravate allergic eye disease, because allergens may be less efficiently removed from the ocular surface in the presence of reduced tear turnover and DED is associated with an upregulation of inflammatory mediators, including some of those common to the allergic pathway, such as complement. Complement also plays a role in DED in the absence of allergy.[300] Complicating

TABLE 3. Risk Factors Associated With DED

Risk factor for DED	Evidence Level[a] (C = consistent, P = probable)	Modifiable? (M = possibly modifiable, N = nonmodifiable)
Systemic disorders		
Autoimmune disorders		
Sjögren's disease[210]	C	N
Rheumatoid arthritis[210,211]	C	N
Systemic sclerosis[210]	C	N
Systemic lupus erythematosus[210,212]	C	N
Sarcoidosis[210]	C	N
Thyroid disease[211,213,214]	C	N
Inflammatory bowel disease (Crohn disease and ulcerative colitis)[215,216]	C	N
Psoriasis[217]	C	N
Hormone disorders or status		
Diabetes[211,213,218]	C	N
Androgen deficiency[3,219,220]	C	M
Polycystic ovary syndrome[221]	P	M
Dermatologic and atopic disorders		
Acne rosacea[213,222,223]	C	M
Acne vulgaris[215,224]	P	M
Eczema[213]	C	M
Asthma[225]	C	N
Allergy[226]	C	M
Pain disorders		
Irritable bowel syndrome[4]	C	N
Fibromyalgia[4]	C	N
Chronic pelvic pain[215,227,228]	C	N
Migraine[4,229]	C	N
Other headache disorders[229]	C	N
Osteoarthritis[213,215]	C	N
Back pain[4]	C	N
Temporomandibular joint disorder[227]	P	N
Mental health or neurodevelopmental disorders		
Depression[211,213,230-232]	C	M
Anxiety[230,231]	C	M
Stress (including posttraumatic stress disorder)[213]	C	M
Burnout[215]	P	M
Autism[215]	P	N
Sleep disorders		
Obstructive sleep apnea syndrome[233]	C	M
Insomnia and poor sleep quality[140,234]	C	M
Other disorders		
Osteoporosis[213]	C	M
Parkinson disease[235]	C	M
Sinusitis[215]	P	M
Ophthalmic disorders		
Meibomian gland dysfunction[236,237]	C	M
Anterior blepharitis[6,238,239]	C	M
Allergic conjunctivitis[226]	C	M
Glaucoma[213]	C	M
Facial nerve paralysis (Bell's palsy)[215,240,241]	C	N
Ocular surface disorders interfering with the tear film or inducing inflammation (eg, pterygium, pinguecula, conjunctivochalasis)[159, 213,242-248]	C	M
Thyroid eye disease (including Graves disease)[106,113,249,250]	C	M
Ocular rosacea[222,223]	C	M

(*continued on next page*)

TABLE 3. (*continued*)

Risk factor for DED	Evidence Level[a] (C = consistent, P = probable)	Modifiable? (M = possibly modifiable, N = nonmodifiable)
Surgery or procedures		
Eye surgery (eg, refractive surgery, cataract surgery, intravitreal injections, glaucoma surgery, retinal surgery)[213, 215,251-257]	C	N
Eyelid and periorbital surgery[257,258]	C	N
Periocular botulinum toxin injections[257,258]	C	N
Cosmetic periocular and ocular procedures[257-259]	P	N
Hematopoietic stem cell transplantation (graft-vs-host disease)[260,261]	C	N
Medications		
Anticholinergic medications (antihistamine, antiarrhythmic, bronchodilator, antidepressant, anti-Parkinson, and antispasmodic medications)[257,258,262-264]	C	M
Eye drops (eg, preservative-containing, antiglaucoma, antihistamine, anesthetics, some NSAIDs)[258, 262,263,265]	C	M
Anticancer drugs (eg, chemotherapeutic agents and hormone therapy)[64,258,266-269]	C	M
Vitamin A derivatives (including isotretinoin)[270-272]	C	M
Dupilumab[273-275]	C	M
Hormone replacement therapy[3,214,258]	P	M
Antiandrogen therapy[3,46,276]	C	M
Proton pump inhibitors[215]	P	M
Psychostimulant agents used for ADHD[215]	P	M
Environment		
Air pollution from NO_2 and CO[277]	P	M
Low humidity[277]	C	M
High or low temperatures[277]	P	M
Air conditioning and wind[277]	P	M
Contact lens wear[213,258,278]	C	M
Screen use[213,279-281]	C	M
Cosmetics[259]	C	M
Demographic factors		
Increasing age[15,26,213,282]	C	N
Female sex and gender[15,26,213,282]	C	N
Asian race / non-White race[208,213]	C	N
Nutrient/gene-related		
Vitamin A deficiency[283]	C	M
Vitamin B_{12} deficiency[283]	P	M
Vitamin C deficiency[283]	C	M
Vitamin D deficiency[283-285]	P	M
ω-3 Fatty acids deficiency[283]	C	M
Altered gut microbiome[283,286-288]	P	M
Genetic predisposition[135,289,290]	P	N

ADHD = attention-deficit hyperactivity disorder, DED = dry eye disease.

[a]Consistent evidence implies the existence of multiple adequately powered and otherwise well-conducted studies published in a peer-reviewed journal, along with the existence of a plausible biological rationale and corroborating basic research or clinical data. Probable evidence implies the existence of at least 1 adequately powered and otherwise well-conducted study published in a peer-reviewed journal. Risk factors with mixed or inconclusive results from multiple studies or from a systematic review or meta-analysis are described in the Diagnostic Methodology report. The term nonmodifiable implies a lack of evidence that change will impact DED.

the association between DED and allergy is the overlap in symptoms such as burning, itching, and tearing.[225,226,301] It is therefore important to consider allergic conjunctivitis in patients with DED, and particularly in patients with a history of atopic disease. Specific questioning about eyelid margin itching may suggest the presence of Demodex blepharitis.[302]

Chronic pain disorders (the body's heightened sensitivity to non-noxious stimuli) can present with ocular discomfort symptoms similar to those of dry eye. Fibromyalgia, chronic

pelvic pain, and irritable bowel syndrome are among the most established risk factors.[4] Development of neuropathic pain within the trigeminal somatosensory system has been proposed to underlie this link.[4] A study of twins in the United Kingdom found evidence for shared genetic factors underlying these disorders and DED, suggesting a common chronic pain predisposition that accounts for clustering of pain disorders.[290] Other pain conditions that have been linked to DED are migraine and other headache types, osteoarthritis, back pain, and temporomandibular joint disorder.[4,215,227,303]

There is also strong evidence for an association between DED and depression, anxiety, burnout, and stress (including posttraumatic stress disorder).[4,213,215,230-232] A meta-analysis of studies in patients with DED found an overall prevalence of 40% for depression (OR of 1.8), and of 39% for anxiety (OR of 2.3).[230] These relationships are likely bidirectional, and studies show an association with psychiatric disorders with DED symptoms that is greater than with signs. Proposed mechanisms that link psychiatric disease to DED include altered pain perception, somatization, and increased serum inflammatory markers.[4] Clinicians may find it beneficial to inquire about mental health history in patients with DED, as appropriate, and consider referrals to mental health professionals when indicated.

Obstructive sleep apnea has been linked to DED.[213,234] It increases the risk of floppy eyelid syndrome, which may lead to exposure keratopathy at night and to increased conjunctival inflammation and MGD.[304] Leakage of air from a continuous positive airway pressure mask may also lead to irritation, conjunctivitis, and DED.[305,306] Insomnia and poor sleep quality may be a consequence of DED, but could also result in DED.[4,140,234] For example, obstructive sleep apnea is associated with a persistent low-intensity inflammatory state that may be pertinent to DED etiology.[307]

Other disorders that have been convincingly linked with DED are osteoporosis (which may reflect common underlying mechanisms such as sex hormone or vitamin D deficiency, or a role of purinergic signaling),[308] autism (which may reflect hypersensitivity to external stimuli),[215] Parkinson disease (which may be linked to decreased blink rates and lower tear secretion),[235,309] and sinusitis (which may sometimes involve the tear ducts, or reflect common mechanisms like allergy, or may represent referred pain).[215]

3.6.2. Ophthalmic disorders

MGD may be the most important factor associated with DED.[236] Its prevalence varies widely between studies, but has an estimated global prevalence of 35.8%, and the majority of patients with DED have underlying MGD that is considered to be a major cause of evaporative DED.[236,237,310] Signs of MGD occur earlier in the natural history of DED progression (typically between 24 and 29 years) than other clinical signs such as tear film instability, hyperosmolarity (31-38 years), and ocular surface staining (46-52 years).[207]

Anterior blepharitis is a common chronic inflammatory disorder of the eyelid margin located anterior to the gray line. This area includes the eyelid skin and the eyelashes and its follicles. Anterior blepharitis is traditionally subcategorized based on its etiology: bacterial, seborrheic, fungal, or parasitic. Anterior blepharitis is a risk factor for other forms of ocular surface disease including DED, MGD, chalazion, and keratitis.[238,311]

Other disorders of the ocular surface also have been associated with a disruption in the tear film or inflammation at the ocular surface, potentially leading to DED. Conditions that have been most associated with increased risk of DED are pterygia,[159,242,312] pingueculae,[243-245] conjunctivochalasis,[246-248] and allergic conjunctivitis.

Patients with glaucoma are also at increased risk of DED, most often associated with use of antihypertensive eye drops. Both active ingredients[265] and preservatives can affect the ocular surface. In addition, glaucoma surgery can affect the ocular surface.[213,313,314]

Facial nerve paralysis, including Bell palsy, can lead to exposure keratopathy through incomplete blinking, and also has been associated with impaired lacrimation and MGD.[215,240,241] Facial nerve dysfunction may lead to lagophthalmos and reduced blink force, compromising tear film distribution and postulated to compromise meibomian gland expression.[315]

3.6.3. Surgery and other procedures

Almost all eye surgery been linked to an increased risk of DED. The greatest amount of evidence is available for refractive surgery,[252-254,316] cataract surgery,[251,255] and intravitreal injections,[256] but it is likely that all eye surgery is associated with an increased risk of DED.[213,215,257,258] Mechanisms that lead to DED after eye surgery include incisional procedures leading to transsectional nerve damage, surgical trauma to the ocular surface during surgery, phototoxicity, toxicity from eyedrops (eg, from povidone iodine, anesthetics, or preservatives), and damage and stress from repeated drying, irrigation, and exposure to surgical illumination.[257,317,318]

Eyelid and periorbital surgery, such as blepharoplasty, ptosis surgery and brow surgery, also have been associated with an increased risk of DED.[257] Mechanisms include lacrimal gland injury and postoperative incomplete eyelid closure and incomplete blinking. Similarly, periocular botulinum toxin injections can lead to lagophthalmos. The TFOS Lifestyle Reports discuss the impact of eye, eyelid, and periocular procedures on the ocular surface in further detail.[257,259]

Allogeneic hematopoietic stem cell transplantation, mostly used in the treatment of various hematologic diseases, can lead to graft-vs-host disease. In this disease the graft's immune cells attack the host's body cells, and this can become chronic. It can affect multiple organs including the eyes and principally involves the ocular surface which can lead to severe DED. More than

50% of recipients develop DED, mostly 6-24 months after transplantation.[260]

3.6.4. *Medication use (see Section 7.2.2)*

A large population-based study in the Netherlands found that 52 of the 99 most commonly used medications were associated with an increased risk of DED.[262] Although not all these medications may be causally linked to DED, and some of the associations may reflect an association with underlying disease (severity), it highlights the importance of assessing medication status in patients with DED. This section discusses various medications that have been most frequently associated with DED, but it is recognized that medication-associated DED is not limited to these medications only.

Medications with anticholinergic (side) effects have been consistently and causally linked to DED, such as antihistamine, antiarrhythmic, bronchodilator, some antidepressant, anti-Parkinson, and antispasmodic medications.[257,258,262-264] These drugs affect the muscarinic receptors of the lacrimal glands and conjunctival goblet cells, causing decreased aqueous and mucous secretion.

Several medication-related pathophysiological mechanisms have been demonstrated: allergic, toxic, immune-inflammatory effects; chemical interaction with the tear film leading to a disrupted lipid layer; reduced aqueous secretion; damage to goblet cells and epithelium of the cornea and conjunctiva; and neurotoxic effects on the corneal nerves and eyelids including meibomian glands.[64,257,258,313,314]

Systemic chemotherapeutic and other anticancer agents may have cytotoxic effects at the ocular surface and affect tear film quality and reflex tear secretion.[64,258,266-269,319] Examples include alkylating agents such as cyclophosphamide, antimetabolites such as 5-fluorouracil and methotrexate, monoclonal antibodies such as rituximab, and the aromatase inhibitors.

Vitamin A derivatives or retinoic acids, including isotretinoin, are used for acne vulgaris and in antiaging regimens, and may be administered topically or orally. They are secreted into the tear film by the lacrimal gland[320] and are associated with tear film instability, lower Schirmer test scores, and atrophy of meibomian glands, leading to DED.[25,259,270-272,321]

Interleukin (IL)-3 and IL-4 receptor antagonists (such as dupilumab) are increasingly used in patients with atopic dermatitis with good effect but may cause several adverse effects at the ocular surface, including a mild to sometimes severe conjunctivitis, punctate keratitis, blepharitis, and loss of meibomian glands.[273-275]

Hormone replacement therapy has been associated with increased prevalence of DED.[3,214, 258]

In addition, proton pump inhibitors, antacids, and psychostimulant agents used for attention-deficit/hyperactivity disorder have been linked to a highly increased odds of having DED, but biological pathways are currently unclear.[262,263]

3.6.5. *Environmental factors*

Climatic risk factors may play an important role in the etiology of DED. The TFOS Lifestyle Environmental Conditions report has recently and comprehensively summarized evidence for environmental risk factors for DED.[277] Both low and high temperatures have been associated with DED, as has low humidity, in several experimental and population-based studies. Air-conditioning and wind are well-known risk factors that patients often report as triggering symptoms,[322] although limited evidence is available from research studies. Air pollution such as from NO_2 and CO was found to be probably associated with DED, and soil pollution from chromium is likely associated with DED and Sjögren's disease. Evidence for a risk of other air, soil, and water pollutants was not conclusive.[277]

Contact lens wear has been reported as a factor associated with DED in many cross-sectional population-based studies, but prospective studies are lacking. It has been postulated that mechanisms leading to DED may include thinning of the tear film after insertion of the contact lenses, increased friction between the lens and the ocular surface, leading to meibomian gland dropout, decreased tear film stability, ocular surface staining, and eyelid wiper epitheliopathy.[237] It is important to recognize that contact lens discomfort symptoms overlap with DED, and symptoms do not necessarily reflect underlying DED[213,258] (see Section 7.4, Contact lenses and DED).

Screen or visual display terminal use has been consistently linked to DED.[279-281,323] As few as 1-2 hours of screen use per day may even be associated with adverse ocular surface effects.[279] Important mechanisms include decreased blink rate and incomplete blinking that are presumed to lead to increased evaporation and decreased lipid release from meibomian glands.[324] It is also important to consider digital eye strain, independently of DED, in the differential diagnosis of persons who are heavily exposed to a digital environment.[280] This requires a full refractive correction to be determined for the distances required and a binocular vision assessment to ensure suboptimal visual input is not the cause.

Cosmetic products may be associated with adverse effects at the ocular surface and may aggravate or initiate DED symptoms. The TFOS Lifestyle cosmetics report identified 10 ingredients that are commonly present in cosmetics that particularly have significant adverse effects: benzalkonium chloride, chlorphenesin, formaldehyde-releasing compounds, parabens, phenoxyethanol, phthalates, prostaglandin analogues, retinoids, salicylic acid, and tea tree oil.[259] Finally, recent reviews have found impacts on the ocular surface from environmental endocrine disruptors in foods, packaging, and pesticides.[325] No clear or consistent link between smoking/vaping and DED has been identified, although effects on the tear film are evi-

dent.[4,326-329] Although a direct causal relationship between smoking and DED may not be certain, it is reasonable to suggest that smoking cessation should be encouraged as part of a comprehensive approach to promoting ocular surface and holistic health in patients with DED.

3.6.6. *Demographic factors*

Female sex and gender are strong risk factors for DED (see Section 3.3). As discussed in Section 2.2, sex-related differences in prevalence are underpinned by differences in the ocular surface and adnexa including anatomy and immunity, a higher prevalence of autoimmune disorders and psychiatric disease (and their related medications), higher general pain sensitivity in women, and reduced androgen levels (already lower than in men) after menopause.[3,24,26]

Increasing age is also a well-established risk factor for DED, but DED is prevalent in both the young and the old. Systematic reviews have found that signs show a stronger relationship with age than symptoms. MGD also increases in prevalence with age.[15,310,330-333]

Prevalence values for DED and MGD are higher in Asian countries than in Western countries,[26] and a pooled analysis of interethnic differences suggested differences in disease type and age at which signs manifest in Asian and White populations.[208] There is also evidence that White races are less affected than non-White races in Western studies.[213] It is difficult to unravel precise causes for these differences, which likely reflect socioeconomic, cultural, genetic, anatomic, lifestyle, and environmental differences between groups.

3.6.7. *Other factors*

Nutrition may play a role in the development of DED, but there is no strong evidence to support recommendation of an optimal diet. Poor nutrition that drives systemic disorders may be associated with risk factors for DED. Limited but increasing evidence links alterations in the gut microbiome to ocular surface health, possibly by altering the immune system.[283,287,288,295,295,334-336] The TFOS Lifestyle Nutrition report concluded that there is evidence that deficiencies of vitamins A and C and ω-3 fatty acids are risk factors for DED. Moderate evidence was also found for vitamin B_{12} and D deficiencies as risk factors.[283] There is no clear evidence that alcohol use,[4,283] a Mediterranean diet,[283,337] and water intake[283] represent risk or protective factors for DED.

Genetic factors likely contribute moderately to DED. A large twin study in the United Kingdom established that heritability accounted for 29% for DED symptoms and 41% for DED diagnosis. The remaining 60% to 70% was attributed to unique environmental factors.[135] A recent genome-wide association study in Taiwan with more than 14,000 DED cases and almost 26,000 controls found 11 independent risk loci, including *MUC16*, which encodes for a mucin protein that is expressed at the ocular surface. A polygenic risk score including 932 loci was able to detect individuals with a high risk of DED.[289] A limitation of this study was the use of self-reported DED only and that the findings were not replicated in an independent cohort. This lack of replication is also a major limitation for several, mostly small, candidate gene studies that have found a link between genetic variations and DED.[338-344] Further studies are warranted before genetic testing becomes clinically useful in DED.

3.6.8. *Risk factors for MGD*

There is debate about the impact of MGD on risk factors and prevalence of DED. It is recognized as a risk factor for the disease, and recent estimates of DED prevalence suggest that MGD may be present in 50% to 70% of cases of DED.[345,346] Nonetheless, there have been attempts to establish demographic risk factors for MGD, which may or may not be independent of those for DED more broadly. Age is frequently reported as a risk factor in MGD, [131] particularly in elderly populations[187] and where MGD is diagnosed using meibography to determine meibomian gland loss or dropout.[11,347] Ethnic background (Asian compared with other backgrounds) is consistently reported as a risk factor. The impact of sex in MGD is however equivocal, in contrast to its impact in DED more broadly where female sex predominates.

In examining population-based studies, there is a higher rate of asymptomatic MGD in White males[174] and a higher risk of MGD in males based on gland plugging and telangiectasis in the Singapore Malays study.[173] In a smaller population-based study from Japan where symptomatic disease was diagnosed using eyelid margin irregularities and gland plugging, males similarly had a higher risk of MGD compared with females.[131] Male sex was not an independent risk factor for MGD in a population-based study of adults in Iran.[187] There is wide variability in the impact of sex on MGD depending on how the disease is diagnosed using individual or combined eyelid signs (gland dropout, orifice plugging, altered meibum secretion, degree of gland expressibility, eyelid telangiectasia, either presence/absence or presence above a certain level), whether symptoms are present, study design, and whether the study is based on a clinical sample, specific disease group, or is population-based.

For the meta-analysis reported above (Section 3.3), large population-based studies only have been included and 2 analyses report either all MGD, irrespective of definition, or clinically significant MGD signs with symptoms, broadly based on the TFOS International Report on Meibomian Gland Dysfunction.[236] For population-based studies reporting any eyelid changes with or without symptoms (Any MGD: Figure 3, F), MGD is more prevalent in older men (≥70 years of age) than older women but there is no difference between sexes in the rates of clinically significant MGD (Figure 3, G).

3.7. MORBIDITY AND IMPACT: DED severely affects the lives of sufferers. It negatively affects quality of life (QoL), including physical, psychological, and emotional well-being, social functioning, daily living activities, and independence. General and mental health, social functioning, physical and emotional states, bodily pain, and vitality are significantly poorer in those with DED compared with those without[348-351] and that health status worsens in those with more severe disease.[350,351] In individuals with mild and moderate disease (n = 217), blurred vision, productivity loss, and visits to eye care practitioners were increased compared with age-matched normal individuals (n = 67).[352]

Adverse QoL effects appear to be consistent over time, irrespective of geography and with variations in ethnic background,[353,354] and will likely increase with population aging.

The economic cost of DED can be measured in direct resource use (service provision, medication costs), out-of-pocket costs, cost of lost productivity, and reduced QoL (quantified as a utility). There are limited studies that include an associated utility algorithm or that meaningfully evaluate productivity impacts of DED.[355-357]

3.8. SUMMARY AND OUTSTANDING QUESTIONS: This update has considered the prevalence of DED in studies that have shown disease rates by age and sex. Eight major diagnostic groups were identified, and meta-analyses reported here include DED diagnosed using the Women's Health Study criteria, symptom report; claims data from health or insurance databases; signs and symptoms; diagnosis according to the TFOS DEWS II criteria; clinical diagnosis; any MGD or clinically significant MGD (grade 2 or above). Prevalence varied with diagnostic criteria where not all disease increased with age or showed a female predominance. Broadly, using the Women's Health Study criteria, DED increased with age and was more common in women. A clinical diagnosis of DED showed a female preponderance but not an age-related effect. Symptoms and signs were more common in women with higher rates in younger and older adults. Any or severe MGD was age-related, with males more likely to show any MGD. Some studies included in the "Any MGD" analysis reported asymptomatic MGD, and the age and sex effects here have not been stratified. These findings are perhaps not unexpected given the multifactorial nature of DED, the specificity of ocular symptom measurements, and differences in the etiology of different subtypes of DED.[1] Prevalence data in some recent studies may be confounded by the impact of the COVID-19 pandemic. Previous meta-analysis showed that both the pandemic and mitigating factors (mask and screen use) were associated with greater ocular symptoms and signs of ocular surface disease.[26,144,190,196]

Studies reporting rates of DED in those aged <20 years are limited. Rates are lower than adults for clinically diagnosed DED, DED with signs and symptoms (<10 years), and any or significant MGD. High rates of symptom reporting are evident in those aged <20 years; however, although it is recognized that symptom report alone is not specific for DED and childhood anterior or posterior blepharitis, Demodex infestation, and allergy may be common comorbidities or contributors to ocular symptoms. Most studies reporting symptoms did not report signs although they may be present.

Risk factors are reported as consistent or probable, and potentially modifiable or nonmodifiable. New risk factors related to environment, climate, and lifestyle are included. There is some evidence for an increase in prevalence in DED over time that may conceivably be due to the impact of new risk factors including changes in the digital environment. Given the differences in prevalence and age or sex associations with different diagnostic criteria for DED, it may be important to disaggregate risk factors particularly for DED and MGD where possible. Given the high prevalence of symptom reporting in childhood, appropriate triaging for other conditions and hypothesis-driven and appropriately powered studies to explore risk factors in children would be valuable.

Outstanding questions include the following:

1. Disease severity. A limited number of studies explored the prevalence, risk factors, or natural history by disease severity, which could help to triage and manage those more likely to experience more severe DED.
2. Geographical mapping was not considered as part of this update.
3. A potential lack of generalizability of prevalence measures for DED in children and adults aged <40 years. These estimates mostly originate from studies in Asia, and there are limited studies in other regions.
4. The need for appropriately powered studies to determine risk factors in those aged <40 years.

4. PATHOPHYSIOLOGY

4.1. INTRODUCTION: The consensus view of the 2017 TFOS DEWS II report envisioned a tear film–centric model of the pathophysiology of DED,[6] broadly classified by compromised quantity, aqueous-deficient DED or quality, and evaporative DED of the tear film. In DED, tear hyperosmolarity is considered to set up a cascade of signaling events within surface epithelial cells that leads to the release of inflammatory mediators and proteases. Such mediators, together with the tear hyperosmolarity itself, are conceived to cause goblet cell and epithelial cell loss and damage to the epithelial glycocalyx. Damage is reinforced by inflammatory mediators from activated T cells, recruited to the ocular surface. The net result is the characteristic punctate epitheliopathy of DED and a tear film instability that leads at some point to early tear film breakup. This breakup exacerbates and amplifies tear hyperosmolarity and com-

pletes the vicious circle events that ultimately lead to ocular surface damage and self-perpetuation of the disease. Epithelial injury and a defective glycocalyx, loss of tear volume and of goblet cell mucin, lead to increased frictional damage and friction-related symptoms. The tear hyperosmolarity and epithelial injury caused by DED, stimulates corneal nerve endings, leading to symptoms of discomfort, increased blink rate and potentially, a compensatory, reflex increase in lacrimal tear secretion. The TFOS DEWS II report also highlighted causes of aqueous-deficient DED that included lacrimal gland infiltration and dysfunction, neurosecretory or reflex blocks, androgen deficiency or aging-related downregulation of secretion, obstruction of the lacrimal ducts in cicatricial disease, and iatrogenic causes such as prescription medication and surgical damage to trigeminal nerves. For evaporative DED, conditions that affected the ocular surface included MGD, anterior blepharitis, xerophthalmia, ocular allergy, androgen deficiency as well as iatrogenic causes such as topical preservative use, contact lens wear and certain antiglaucoma drugs.

Subsequent research has led to a more nuanced understanding of the disease. Given the inconsistent relationship between symptoms of DED and signs such as measurable inflammation, hyperosmolarity, ocular surface staining, low tear volume, low TBUT, or MGD,[346,358] the data suggest that the associated signs help identify subtypes of the disease, with symptoms providing no predictive ability as to which etiology is active in that patient.[346] The implications of the lack of correlation of signs and symptoms in DED are particularly important in relation to pathophysiology, because while most historical literature assumes that inflammation and inflammatory pathways are the common effector of DED, evaporative subsets show a muted if any increase in inflammatory mediators in the tear film,[358-362] and thus inflammation cannot be assumed to be active in every patient with DED. For example, one study based on mass spectrometry found that patients with evaporative DED exhibited no increase in proteins associated with the inflammatory response compared with normal controls, whereas 51% of differentially upregulated proteins in the aqueous cohort were associated with inflammation.[358] Similar data showed that although aqueous-deficient subjects (Schirmer test value $\leq$5 mm) had elevated lipid peroxides in tears compared with healthy controls, subjects with 6-10 mm wetting were not significantly different from normal controls.[359] Another study based on multiplex bead analysis found that evaporative patients tend to exhibit much higher levels of epidermal growth factor, but no difference in the IL-6 and IL-8 cytokines compared with healthy controls and essentially no detectable TNF-α in tears,[361] reinforcing the idea that not all forms of DED are driven by the same underlying pathways. In mouse models, bilateral lacrimal gland excision showed dramatic increases in IL-1β, IL-6, and TNF-α protein concentration in the tear film over 4 weeks, whereas topical benzalkonium chloride and environmental chamber–induced DED showed no significant difference from controls in those tear film cytokine proteins, despite concurrent increases in tear osmolarity and cytokines measured from corneal mRNA transcripts.[363] Immune-mediated etiologies, represented by immunologic diseases that include Sjögren's disease, Stevens-Johnson syndrome, and GVHD, are widely regarded as being more severe than evaporative counterparts.[364] At the extreme end, severe forms of dry eye found in ocular GVHD tend to be accompanied by fibrotic processes,[365,366] and Sjögren-related DED is associated with lymphocytic infiltration of the lacrimal gland, neither of which is evident in common evaporative DED.[366] In a rabbit model, cauterization of the meibomian glands results in only moderate increases in hyperosmolarity and loss of goblet cells compared with models in which lacrimal excretory ducts and accessory glands of rabbits are sealed.[367] These data may help explain why anti-inflammatory medications have a dichotomous effect, with some patients reporting improvement,[368,369] whereas others report high failure rates of cyclosporine A and lifitegrast in the general population.[370-373] Because many patients are still poorly served by the available therapeutic options, it may be that our understanding of the pathophysiology of DED is incomplete.

Note that a variety of the pathophysiological aspects of the disease are discussed in more detail elsewhere. For example, androgen deficiency is discussed in the Sex, Gender, and Hormones (Section 2.5.1), neuropathic damage is covered in the Pain and Sensation (Section 6.5), the tear proteome is outlined in the Tear Film (Section 5.4), and iatrogenic causes are described in detail in the TFOS Lifestyle Report[257] and in the Iatrogenic section of the digest report (Section 7).

4.2. INITIATING TRIGGERS OF DISEASE: Except in certain situations of injury,[175] surgery,[252,316] and therapy,[374] establishing causality is difficult when diagnosing DED. Metabolic disease induces mitochondrial stress[375-380] and advanced glycation end products in the lacrimal gland,[381,382] hormonal changes that alter glandular production and fatty acid metabolism,[3,42,69,383,384] and biophysical stresses of friction,[385-389] hyperosmolarity,[390-398] and swelling pressure.[399] These effects are compounded by the biological activity of dysregulated or self-reactive immune cells,[400-403] cellular stresses from cytokines,[363,404-410] proteases,[411-413] reactive aldehyde species,[414] extracellular deoxyribonucleic acid (DNA) and neutrophil extracellular traps,[415-417] exogenous toxins,[376,395] damage- or danger-associated molecular patterns,[392] gut dysbiosis,[418,419] as well as neurogenic inflammation.[420-425] Typical pathways of initiation of evaporative processes include phenotypic alterations in corneal epithelial cells that lead to a compromised glycocalyx,[426] keratinization of the meibomian gland that alters the lipid profile of the tear film,[427,428] and incomplete blinking or reduced blink rate during screen use that exposes the ocular surface to desiccating stress.[429] In aqueous deficiency, inflammatory ingress into

the lacrimal gland can be driven by androgen deficiency or autoimmunity, causing a cascade of protease release, cytokine expression, inflammatory cell recruitment, dendritic cell maturation, and an adaptive T cell–mediated response.[3,402,430] As disease severity increases, the evidence suggests there is a progressive accumulation of these mechanisms.[4,402,431,432]

4.3. HYPEROSMOLARITY: When exposed to hyperosmolar conditions, epithelial cells begin to change their morphology and lose their microplicae.[433] Increasing levels of hyperosmolarity (excess salt) on the ocular surface cause an increase in epithelial apoptosis,[434] transglutaminase-mediated cornification of epithelial cells,[435] and increased secretion of IL-1β, IL-6, IL-8, TNF-α, and matrix metalloproteinase 9 (MMP-9) from ocular surface epithelial cells.[45,436-438] Hyperosmolarity-induced epithelial cell stress results in desquamation, revealing the immature glycocalyx and microplicae-free cells beneath.[433] These immature cells are hydrophobic and contribute directly to tear film instability. When patches of hydrophobic cells are adjacent to normal hydrophilic cells, the differential surface tension causes the tear film to break up and evaporate within a few seconds, dramatically increasing the local osmolarity and exposing cells to a toxic environment.[439,440]

In addition to the hypothesis that inflammatory cells infiltrating either the lacrimal gland or conjunctiva are a source of oxidative damage at the ocular surface,[6,441] *in vitro* evidence suggests that hyperosmolarity directly induces reactive oxygen species (hydroxyl and peroxyl activity) in human corneal epithelial cells, along with reductions in the antioxidant superoxide dismutase-2, vitamin D, Notch ligands Dll3 and Jag 1, and a tripling of the proapoptotic Bax/Bcl2 ratio.[442] Reactive oxygen species undergo lipid peroxidation and cause reactive aldehyde species to be produced, causing a cascade of protease release, cytokine expression, inflammatory cell recruitment, dendritic cell maturation, and an adaptive T cell–mediated response.[414,443] *In vitro* data have shown that exposure to hyperosmolarity causes mitochondrial DNA to leak into the cytoplasm of human corneal epithelial cells, activating the cGAS-STING pathway and increasing cytokines such as CXCL10 and interferon beta (IFN-β),[444] whereas human conjunctival impression cytology samples from patients with evaporative, short TBUT dry eye (2.9 seconds) confirmed an increase in STING proteins compared with more normal controls.[445] Evaporative hyperosmolar DED patients (327 mOsm/L, 7.0 seconds TBUT, 10.4 mm average Schirmer test value) exhibit specific upregulation of IFN-γ, without concomitant increases in IL-2, IL-6, IL-10, TNF-α, or IL-17A.[446] IFN-γ, which is strongly associated with dose-dependent CD40, MICA, and MHC II expression along with epithelial cytotoxicity in corneal epithelial cells,[447-449] increases NLRP3 oxidative stress and contributes to pyroptosis in human corneal epithelial cells under hypertonic conditions.[450] These data mirror earlier findings that hyperosmolarity disrupts the balance of oxygenases and antioxidant enzymes such as SOD1 and PRDX4, stimulates lipid peroxidation (eg, 4-hydroxynonenal and malondialdehyde), increases COX_2, and damages corneal mitochondrial DNA.[451,452] Hyperosmolarity also potentiates the negative effects of the oxidizing blue light in corneal and conjunctival epithelial cells, increasing H_2O_2 production, phototoxicity, and altering the mitochondrial membrane potential in these cells compared with those in normal media.[453] Downstream of hyperosmolarity, a Toll-like receptor 4 (TLR-4)–dependent upregulation of dual oxidase 2, a member of the NADPH oxidase family that regulates the production of intracellular reactive oxygen species, increases alongside the high-mobility group box 1 production in human corneal epithelial cells.[392,454] This result was consistent with data that MyD88$^{-/-}$ mice lacking TLR-4 signaling exhibited significantly less corneal fluorescein staining, cytokine, and protease expression following 5 days of hyperosmolar stress and scopolamine administration,[455] implicating TLRs and damage- or danger-associated molecular patterns (eg, HSPs, HMGB1, S100A, tenascin-C) as essential agents in evaporative, hyperosmolar DED pathophysiology.[455-457] Although inflammatory ingress may be an eventual result of hyperosmolar exposure,[458] clinically, it is likely a matter of time and severity that governs which patients progress to the inflammatory phenotype as a result of hyperosmolarity.[367] For example, at low levels of hyperosmolarity, protective responses such as the increase in IGF binding protein-3 are observed, but at increasing levels of osmolarity and length of exposure, cell viability is challenged as respiration and glycolysis are decreased alongside endoplasmic reticulum stress and caspase-3 activation, leading to unchecked mitophagy and eventual cell death.[379,459,460] Similarly, short-term exposure to low-humidity environments showed an increase in damage or danger-associated molecular patterns such as HSP-60 and a small 2.4-fold increase in MMP-9 mRNA expression in patients with evaporative DED, but no statistically significant increase in IL-6 or IL-8 expression.[456] These data suggest that the severity of tear hyperosmolarity should help inform the underlying pathophysiology, wherein a strongly elevated osmolarity will lead to oxidative stress and inflammation, whereas a middling or low osmolarity is suggestive of a subclinical inflammatory state[461] and may mitigate against prescription of anti-inflammatory therapy.

4.4. PROTEASES: Another aspect of the pathophysiology of aqueous deficiency disease that separates it from evaporative disease is the disrupted balance of protease and anti-protease activity on the ocular surface. The normal tear film exhibits an equilibrium of protease and protease inhibitors.[411] Excess protease is observed in Sjögren's disease, where cathepsin S catabolizes anti-proteases such as cystatin C,[412] MMP-9 is highly expressed whereas thrombospondin-1 lags behind,[462] and plasmin activity is increased 10-fold in Sjögren compared with normal

tears.[463] In non-autoimmune DED, protease activity is stratified by disease severity and is strongly associated with aqueous deficiency. For example, mild evaporative DED–type subjects with low TBUT (5.0-7.2 seconds) and normal Schirmer test value (13-19 mm) exhibited only slightly elevated tear MMP-9 activity (35-66 ng/mL), compared with the tears of more severe aqueous-deficient cohorts (eg, Sjögren's disease and Stevens-Johnson syndrome, with 6 mm Schirmer test value) with levels in the 101- to 381-ng/mL range.[364] These data are consistent with observations that 11% to 14% of early stage, patients with EDE (4.9 second TBUT, 312 mOsm/L, 14.0 mm Schirmer value) presented with enzyme-linked immunosorbent assay–validated MMP-9 positivity.[464] Increases in neutrophil elastase, MMPs,[465] and neutrophil extracellular traps are seen in severe aqueous-deficient subjects (eg, chronic ocular GVHD, Sjögren's disease, and ocular cicatricial pemphigoid) that are not commonly observed in non-autoimmune DED and healthy controls.[415] Similarly, the anti-protease cystatin S was found to have a significant, inverse relationship with aqueous severity in non-autoimmune DED subjects, falling from about 2000 ng/mL in controls (16 mm Schirmer value) to 400 ng/mL in moderate aqueous-deficient DED (4 mm Schirmer test value), whereas MMP-9 was only mildly upregulated to the 20- to 40-ng/mL range in those subjects.[466] The increase in cathepsin S, a serine protease that is known to degrade a variety of essential components of the normal tear film and glycocalyx (ie, lactoferrin, sIgA, and proteoglycan 4),[412,467] is also stratified by inverse tear volume, increasing monotonically as tear volume decreases.[362] Of note, although cathepsin S is dramatically enhanced in Sjögren's disease compared with non-autoimmune subjects on average, non-autoimmune subjects with 0 to 5 mm wetting on the Schirmer strip exhibited equal or higher levels of cathepsin S than patients with Sjögren's disease with >15 mm of wetting.[362]

These data invoke the question of causality, whether aqueous deficiency leads to inflammation and protease release, or vice versa. In an animal model of severe aqueous deficiency, excision of the lacrimal gland resulted in a significant upregulation of serine proteases in corneal tissue including tryptase, urokinase plasminogen activator receptor, and protease activated receptor 2 expression, which on activation is able to induce mitogen-activated protein kinase (MAPK) / extracellular signal-related kinases 1 and 2 (ERK1/2) signaling with downstream nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), intercellular adhesion molecule 1 (ICAM-1), and cytokine expression,[413] suggesting that aqueous deficiency can precede a measurable protease burden at the ocular surface. In contrast, MMP-9 positivity was found in 84% of GVHD compared with 33% of non-autoimmune DED patients, even as the GVHD patients trended toward increased Schirmer test values, lower OSDI, and higher TBUT than the DED controls,[468] suggesting that immune infiltration into the lacrimal gland precedes the protease release in GVHD.

Ultimately, in non-autoimmune DED, the transition from low levels of protease release to inflammatory DED with excessive protease concentrations is likely related to progressive alterations in the lacrimal gland and ocular surface. These might result from androgen deficiency,[3,6] accumulation of advanced glycation end products in the lacrimal gland,[381] diabetic keratopathy and nerve degeneration,[469] or other factors that lead to significantly less aqueous flow, thereby tipping the homeostasis toward a severe hyperosmolar state and associated excessive, pathogenic protease release. It is recognized that in marked EDE or lipid deficiency, tear and ocular surface homeostasis may be sufficiently disrupted to cause inflammation and that in advanced disease, features of both are displayed.

4.5. SUBFUNCTIONAL OR ABSENT GLYCOCALYX: The glycocalyx lubricates,[470] retains water at the ocular surface,[471] provides wetting to the ocular surface,[472] acts as a barrier, and helps remove debris from the ocular surface.[473] The hydrophilic epithelial surface provides 82.5 seconds/cm of specific resistance compared with 12.9 seconds/cm for the lipid layer,[471] establishing the glycocalyx as one of the principal structures responsible for preventing evaporation. Electron micrographs of intracellular, pre-expression glycocalyx showed that "the increase in numbers of subsurface vesicles that occur in some external eye diseases may reflect an attempt to increase the binding of the mucus to the eye surface. In late keratoconjunctivitis sicca, however, the subsurface vesicles are absent."[474] These foundational observations identified the difference between evaporative DED and other ocular surface conditions, where exogenous stresses lead to a compromised epithelial glycocalyx-the proximal cause for an increase in evaporation, hyperosmolarity, and its downstream complications in EDE.

Evidence that the glycocalyx plays a central role in disease pathogenesis has begun to accumulate; for example, knockout of MUC4, the most abundant membrane-associated mucin in the conjunctiva with an ectodomain predicted to extend >2 μM above the apical cell surface, resulted in significantly reduced microplicae, tear film disruption, and increased rose bengal dye penetrance into deeper layers of the ocular surface.[475] *In vitro* evidence from corneal and conjunctival co-culture has shown that hyperosmolarity (caused by a decreased blink rate) directly downregulated components of the epithelial glycocalyx, increased TLR-4 expression, initiated release of cytokines and proteases including MMP-9, and eventually, cellular apoptosis.[429] The hyperosmolarity compromised glycocalyx led to significantly lowered TBUT, lower Schirmer test value, and persistently elevated fluorescein staining, whereas replenishment of the glycocalyx using human recombinant lubricin (recombinant human proteoglycan 4) was able to reverse these clinical indicators, as well as inhibit epithelial NF-κB translocation and nor-

malize IL-8, TNF-α, IL-1β, TLR-4, and MMP-9 expression within 1 day of supplementation.[429] A striking aspect of this study is that the common clinical expressions of DED were recapitulated without immune cell involvement, establishing the hyperosmolarity-compromised glycocalyx as a causal, initiating event in evaporative disease. A related study found that intraperitoneal injections of streptozotocin, used to induce metabolic hyperglycemia similar to type 1 diabetes in mice, caused dramatic reductions in the extent of the corneal glycocalyx, with associated reductions in tear film volume, number of goblet cells and upregulation of TLR-4, MAPK, IL-1, IL-6, and IFN-γ genes within as little as 1 week following injection.[476] In a C57BL/6 mouse model, scopolamine and desiccating stress induced an almost complete abrogation of secreted (MUC5AC, MUC2) and transmembrane mucins (MUC1, MUC4, MUC15, and MUC16) along with significant increases in conjunctival IFN-γ, leading to persistent epithelial defects.[477] Importantly, an *in vitro* study evaluating the impact of applied hyperglycemia in media (15 and 30 mM glucose vs 5 mM control) showed that elevated glucose exposure had no effect on the amount or distribution of membrane associated mucins in either corneal or conjunctival epithelial cells,[478] suggesting that the pathophysiology of streptozotocin-induced glycocalyx loss is more likely due to global metabolic disease than exposure to hyperglycemia alone. In support of these data, a study of vitreoretinal surgery found that conjunctival MUC4 and MUC16 gene expression increased postsurgery and tear osmolarity was reduced in normal individuals, likely as a protective response, but older individuals with diabetes exhibited a lower goblet cell density, lower MUC5AC and increased cytokine response by comparison, once again linking systemic metabolic disease to ocular surface glycocalyx impairment.[479] In a mouse model of GVHD, allogeneic transplantation of a mixture of spleen and bone marrow cells resulted in a significant reduction in the area and thickness of the corneal glycocalyx, reductions in MUC4 and MUC5AC, and a coincident reduction of tear film volume and an increase in fluorescein staining, which were partially abrogated after application of topical rebamipide.[480]

More recent data have shown that components of the glycocalyx also regulate immune cells,[481] transduce extracellular environments to intracellular signaling pathways,[482] and actively inhibit proteases such as MMP-9,[483] which would further implicate the catabolism or downregulation of the glycocalyx as an initiating event in DED pathogenesis. As the glycocalyx is altered in ocular surface pathology, large decreases in sialic acid[484] and increases in galectin-3 are observed in the tear film that strongly correlate with disease severity.[485,486] When released into the tear film, galectin-3 seems to amplify the IL-1β-mediated inflammatory response,[487] which is similar to how degraded low-molecular-weight hyaluronic acid becomes proinflammatory in other tissue systems.[488,489]

In summary, hyperosmolarity, metabolic disease, and inflammation impair the ocular surface glycocalyx and initiate the characteristic clinical signs of DED. The degree of impairment of the glycocalyx seems to be a fulcrum for transitioning from common evaporative DED to an inflammatory, aqueous-deficient state, as the balance of fluid output overwhelms the fluid input.

4.6. MEIBOMIAN GLAND DYSFUNCTION: MGD is a chronic, diffuse abnormality of the meibomian glands, commonly characterized by terminal duct obstruction and/or qualitative and quantitative changes in the glandular secretion.[236] Although it is a disease distinct from DED, when the severity of MGD is of a sufficient degree, it may give rise to EDE.[311] Furthermore, although a widely cited study is often misquoted to suggest that 86% of all DED subjects have MGD, the denominator of that estimate excluded patients with DED that could not be classified as either aqueous or evaporative, and when included, found that 60.7% of those with DED had evidence of MGD.[490] Larger, more recent estimates of the prevalence of MGD within those with DED estimate a number, from 51.3% to 70.3%.[345,346] Those with pure MGD exhibited the lowest severity of signs of ocular surface damage,[345] consistent with the assumption that evaporative DED is often a subclinical inflammatory state.[358] Indeed, in patients with MGD, significant negative correlations were found between inflammatory mediators such as IL-8, C5a, and Schirmer test scores.[416] One study, however, has suggested there may be inflammatory mediators and cytokines present in meibum in patients with MGD.[491] As a causative pathophysiology of DED, it is currently estimated that between 33% and 50% of DED patients have sufficiently impactful MGD to initiate a dry eye state.[346]

By leveraging novel models of primary human meibomian gland epithelial cells, ductal from human tissue,[492,493] 3D cultures and organotypic cultures,[444,494-499] researchers have further investigated the pathophysiology of MGD. Peroxisome proliferator–activated receptor gamma (PPARγ) agonists, such as rosiglitazone, may significantly promote cell differentiation, lipogenesis, and antiinflammation in human meibomian gland epithelial cells.[167,500-504]

The meibomian gland is located in a physiologically hypoxic environment[505] that is impaired in MGD.[506] An increased expression of hypoxia-inducible factor 1α (HIF1α) plays a role in the beneficial effect of hypoxia on the meibomian glands,[507] and as such, a low-oxygen environment may be important in imitating the *in vivo* condition in culture. Animal models include injection of complete Freund adjuvant in rabbits[508] and mice,[509] electrocauterization of meibomian gland orifices in rats,[510,511] transitory alkali exposure of the rat eyelid margin,[512] Soat1-null mice,[513,514] apolipoprotein E knockout (ApoE KO) mice,[515] and Elovl1-deficient mice.[516]

Recent animal studies have indicated that it may be possible to restore gland structure after atrophy. In mice, fibroblast growth factor receptor 2 gene (FGFR2) knockout could lead to significant gland acinar atrophy,[517] but this change is reversible if the knockout condition is removed. Moreover, this recovery relies on the extent of ductal atrophy, which indicates that ductal epithelia may serve as a reservoir for meibomian gland progenitor cells for regeneration.[518] The importance of FGFR2 and other FGFRs were also reported in humans, in which FGFR-inhibiting anticancer drugs could induce significant gland atrophy, and patients who use these drugs may develop MGD.[519]

The relationship between systemic lipids and MGD has been a focus of several recent studies. Patients with elevated serum total cholesterol, low-density lipoprotein, and triglyceride levels exhibited significantly higher levels of meibomian gland loss compared with healthy controls.[520] Mice fed with a high-fat diet developed hypertrophic meibomian glands,[521] decreased PPAR-γ expression, increased meibomian gland acini cell apoptosis and mitochondrial damage, and activation of MAPK and NF-κB signaling within the gland.[522] A high-fat diet significantly altered the rhythmicity of the meibomian gland, which may offer new insights into the regulation of the glands by dietary lipids.[523] Patients who have long-term dyslipidemia also showed significant meibomian gland atrophy and changes in meibum quality, even while undergoing statin treatment.[524] Dietary cholesterol has a direct impact on meibum components and meibomian gland pathophysiology.[525-527] In contrast, dyslipidemia and increased triglyceride levels were found to be protective factors for meibomian gland atrophy in an elderly female population.[14] A systematic review has suggested there is moderate evidence for a beneficial effect of ω-3 supplements on MGD.[528]

Meibum proteins also play an important role in ocular surface homeostasis. A discussion of the role of meibum proteins in MGD is beyond the scope of this review but these have been described more fully elsewhere.[529] Ectodysplasin A protein secreted from meibomian glands plays a significant role in regulation the proliferation of corneal epithelial cells through the epidermal growth factor receptor signaling pathway. This discovery connects meibomian gland function with corneal epithelial homeostasis,[530] and suggests future research pathways surrounding the feedback between the eyelid and ocular surface.

4.7. INFLAMMATORY CELL RECRUITMENT: By leveraging novel single-cell RNA sequencing (scRNA-seq) methods, $CD45^+$ lymphocyte populations were quantified in mouse corneas before and after short term oral scopolamine hydrobromide and desiccating stress. T cells (18.6%), resident macrophages (18.2%), B cells (12.8%), type 2 conventional dendritic cells (cDC2)/macrophage (9.9%), natural killer (NK) cells (9.3%), monocytes (8.9%), and neutrophils (7.9%) were the most prominent cell types in normal corneas, whereas a striking shift toward resident macrophages (55.2%), MMP12 and MMP13 high macrophages (11.5%), and type 2 conventional dendritic cells (cDC2)/macrophage (8.5%) were observed following the induced hyperosmolar aqueous-deficient DED eye.[531] In the mouse conjunctiva, pharmacologic suppression of tear secretion followed by desiccating stress recruited monocytes from the blood and promoted maturation of Ly6ChiMHClo monocytes to Ly6CloMHCIIhi macrophages.[532] In a hyperosmolar model that exposed mice to a low-humidity environment without the scopolamine administration, scRNA-seq revealed that conjunctival $CD4^+$ T cells, Mo/Mφs, and DCs were amplified during DED progression, $CD8^+$ T cells gradually decreased, and epithelial cells exhibited EMT-like characteristics that created a positive feedback loop of proinflammatory MφC3 activation.[533] Importantly, although mouse models tend to echo human DED in terms of innate or myeloid immune cells (macrophages, dendritic cells, neutrophils), lymphoid cells such as NK, natural killer T (NKT), or $\gamma\delta$, or adaptive $CD4^+$, $CD8^+$, or Th17 lymphocytes do not necessarily follow the same trajectories between species.[534] For example, brush cytology of human conjunctival tissues revealed an increase in IL-17A producing $\gamma\delta$ cells from $\approx$4% in mild evaporative disease (3.3 seconds TBUT, 26 OSDI) to 7% to 10% of the $CD45^+$ cells in more symptomatic human samples (2.0 seconds TBUT, 49 OSDI), which was far lower than the increase from 25% to 33% of $CD45^+$ cells in normal vs induced DED mouse conjunctivas.[535] Of note, conditional knockdown of lymphangiogenesis in a scopolamine, desiccating stress murine model resulted in the significant reduction of TNF-α, IL-1β, IFN-γ, and IL-8 within corneal tissues but did not lessen the release of those cytokines in the lacrimal gland, highlighting the importance of angiogenic immune cell trafficking in the progression of aqueous DED.[536]

4.8. IMMUNE CELL DYSFUNCTION: Dysfunctional regulatory T cells (Tregs) exhibiting reduced Foxp3, CD24, and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) expression lose the ability to suppress the chronic Th17 mediated inflammation in an IL-6 dependent manner.[537] Blocking the substance P / neurokinin-1 receptor interaction,[423] or the TLR4/MyD88 pathway,[538] both of which are upstream of NF-κB, were shown to normalize the Treg/Th17 balance. In mice colonized with intestinal microbiota from patients with Sjögren's disease, a reduced frequency of $CD4^+Foxp3^+$ T regulatory cells in cervical lymph nodes was observed,[539] whereas in aged mice, $CD4^+CD25^+Foxp3^+$ T cells were dysfunctional, lost suppressive ability, and produced significant amounts of inflammatory cytokines IL-17 and IFN-γ.[540]

4.9. FUTURE DIRECTIONS: Recent studies exploring the pathophysiology of DED have continued to define the etiologically distinct subpopulations within the disease, recognizing limited evidence for inflammation in evaporative DED, and expanding roles for metabolic, hormonal, phys-

ical, and molecular impacts on the ocular surface. Neural regulation of the epithelium is taking on increasing importance, as are factors from the ocular surface that damage nerves, such as hyperosmolarity, reactive oxygen species, and other potentially excitotoxic stressors. Yet major questions remain about how to best classify the subsets of disease within a single patient and to what extent observed pathways are causal to symptomatology. The natural history of the disease is unclear, when or if inflammation becomes involved, or when acute inflammation transitions into a chronic state. Although animal models of the pathophysiology have provided valuable mechanistic insights, they often fail to fully replicate human disease progression. If, as the existing data show, inflammation is most strongly associated with aqueous deficiency, whether this is due to a relative increase in hyperosmolarity compared with that seen in evaporative disease, or inflammatory mediators and proteases from the diseased lacrimal gland driving downstream changes in the epithelium is not known. In part due to these questions about pathophysiology, it is currently not possible to predict which patients will respond to anti-inflammatory or other types of therapy and which ones will show positive responses to lubricants. Although yet to be established, there may be value in evaluating human immune cell dynamics and cell populations using advanced imaging methods such as functional *in vivo* confocal microscopy.[541] As the diversity of drugs expands, it will be increasingly necessary to align etiology with mechanism of action. Hundreds of millions of dollars of development rests on predicting responders from nonresponders. Thus, it is becoming ever clearer that classical indices of DED such as corneal staining and TBUT are insufficient to support modern drug development efforts, and that more informative methods of determining disease subtype are critical for future research.

5. TEAR FILM

5.1. INTRODUCTION: The Tear Film Report of TFOS DEWS II described the biophysical and biochemical aspects of tears and how these change in DED.[542] The report noted that DED is characterized by loss of tear volume, more rapid breakup of the tear film, and increased evaporation of tears from the ocular surface. The tear film lipids, proteins, mucins, and electrolytes contribute to the integrity of the tear film, but exactly how they interact was an area of active research. Tear film osmolarity, proteins, and mucins could potentially be used as biomarkers for DED. Some of these themes have been explored by research since the publication of the TFOS DEWS II report, and these are highlighted in the current report.

For the current report, the authors undertook searches in PubMed, Scopus, and Web of Science databases for keywords *tear film* and *dry eye* since the publication of the TFOS DEWS II Tear Film Report in 2017. After reviewing the titles and abstracts of documents obtained, the authors have concentrated on those areas that have produced the greatest number of articles. This report focuses on concepts, and more detail on specific components can be found within the cited references.

5.2. CLINICAL MEASUREMENTS OF THE TEAR FILM: The reproducibility of noninvasive TBUT (NITBUT; measured without fluorescein) obtained with combinations of several commercially available instruments have been reported. Instruments generally had good agreement,[543-547] but measurements from different devices are not interchangeable. Reproducibility tended to worsen for those with DED.[545] Another study showed that NIBUT, whether measured by small-cone (E300) or large-bowl (K5M), had poor agreement with fluorescein TBUT, even when precise timing of blinking was taken into consideration, and Bland-Altman analysis showed limits of agreement that spanned the entire dynamic range of the instruments (31.4 seconds) with an average difference of 3.9 seconds between the methodologies, making the techniques neither interchangeable nor comparable to each other.[548] One of the challenges in interpreting the reported correlations of signs of DED is the influence of subjectivity and low interrater reproducibility.

Simple correlations between symptoms and tear film or ocular surface characteristics were sought. Although correlations do not imply causation, they can point the way to a greater understanding of the mechanisms involved in signs and symptoms of DED and identify areas for further investigation, recognizing that there may be confounders in individual studies and populations may differ. There was no correlation between tear evaporation and symptoms (OSDI score), nor between tear evaporation rate and tear production.[549] However, one study found significant positive correlations between OSDI and tear evaporation rates in subjects with refractive errors. Both OSDI and tear evaporation rate were lower in those without refractive errors.[550] These findings may have been confounded by the inclusion of participants with uncorrected refractive errors, smokers, or differences in other demographic factors, but it may be important to adjust for refractive error in studies of tear film and symptoms.

OSDI score has been negatively correlated with NITBUT,[544,551-555] tear meniscus height (TMH),[551,552] and meibomian gland area,[555] and positively correlated with meibomian gland loss.[554,555] NITBUT was positively correlated with TMH, meibomian gland dropout grade,[551,552] and corneal or eyelid margin staining.[544] Females with refractive errors had significantly lower tear film lipid layer (TFLL) thickness, TMH, and NITBUT scores than those without refractive errors.[551] A strong correlation was found between TMH and NITBUT, but no correlations between these parameters and OSDI scores.[556] These differences may be due to difference between populations and between techniques. TBUT has been correlated with meibo-

mian gland irregularity determined from meibography images taken with a keratograph.[557]

TMH was significantly correlated with the Schirmer test value and tear film breakup time and negatively correlated with ocular surface staining score, but there were no correlations with any MGD indicator.[552]

A study of normal subjects and those with MGD found a positive correlation between precorneal tear film thickness and thinning rate and negative correlations between precorneal tear film thickness and TFLL thickness and between precorneal tear film thinning rate and TFLL thickness.[558] There were no associations between TFLL thinning rate and any of precorneal tear film thinning rate, precorneal tear film thickness, or TFLL thickness.[558] TFLL thickness has been positively correlated with age and meibomian gland expressibility and negatively correlated with meibomian gland dropout[553,559] and OSDI score.[553]

Several studies have been conducted to determine whether tear film osmolarity correlated with clinical signs and symptoms of DED. Across population-based studies, tear osmolarity is generally not correlated with TBUT, corneal fluorescein staining score, eyelid hyperemia, tear production, blink interval, Ocular Protection Index, Schirmer test score, meibum expressibility, meibum quality, or MGD.[560-564] However, this may depend on how DED was classified, as other studies found correlations between tear osmolarity and OSDI discomfort subscore, corneal and conjunctival staining scores,[565] OSDI score, ocular surface staining, and Schirmer test score.[566] Data from the DREAM (Dry Eye Assessment and Management) study, while demonstrating some correlations between tear osmolarity and signs and symptoms, were interpreted as being not indicative of causation as changes in tear osmolarity were not associated with changes in signs and symptoms of DED.[567] Other recent longitudinal studies, however, have suggested a relationship between change in tear osmolarity with treatment and iatrogenic effects changes, suggesting a link in certain DED subtypes.[568-573] The Diagnostic Methodology report of TFOS DEWS III[1] gives more background on correlations and the limitations of this approach, and the hyperosmolarity section of the Pathophysiology section of the TFOS DEWS III Digest report (see above Section 4) discusses the role of osmolarity in more detail.

5.3. THE TEAR LIPIDS—COMPOSITION AND FUNCTION: Several studies have examined the TFLL. These have examined changes to the lipids in DED and/or MGD patients compared with controls, as well as which lipids contribute to tear film stability and reduced tear evaporation.

Broadly, meibum, combinations of meibum lipids, and a thicker TFLL were associated with a significantly slower evaporation flux[574-577] and significantly slower tear breakup.[578] Tear film proteins can also reduce evaporation of water, and this is enhanced when some of the proteins (human serum albumin and lactoferrin) but not others (human lysozyme and bovine mucin) are used in conjunction with a mixture of polar and nonpolar lipids found in the meibum.[579] Lysozyme can reduce the surface tension of lipids on water, by disrupting the order of lipid molecules.[580]

Patients with MGD have significantly lower peak height ratios of the CH_3/CH_2 bands in NMR studies than normal subjects, and this was hypothesized to be due to changes in branched hydrocarbon chains, which contain fewer CH_2 moieties, and straight-chain hydrocarbons, which contain more CH_3 moieties.[581] The authors further hypothesized that, as van der Waals interactions between CH_2 moieties were responsible for lipid ordering, the more CH_2 in meibum from MGD patients could contribute to a more ordered TFLL. A more ordered TFLL can contribute to a more patchy layer, resulting in a deterioration in spreading and decreased elasticity.[581] Another factor that is involved in TFLL stability is saturation of lipids, with increased saturation resulting in stiffer, thicker, and more elastic films at high surface pressures.[582] Lipid saturation is a major factor that contributes to lipid disorder and the phase transition temperature of lipid layers.[583] Meibum of MGD patients has lower surface pressures in Langmuir trough experiments compared with meibum in those without MGD, and formed more brittle unstable patchy layers.[584]

The cholesteryl ester to wax ester ratio decreases in patients with MGD.[585-587] An optimal mixture of wax and cholesterol esters in the TFLL may be necessary to disrupt the ordered packing of pure lipid species, leading to better lipid spreading,[588] a more stable tear film,[589] and thinner lipid layer patterns.[590] In a model system, mixtures of cholesteryl oleate (CE) and behenyl oleate (a wax ester mimic) plus phosphatidylcholine increased the surface pressure of films on phosphate-buffered saline.[591] A layer of behenyl oleate can form a crystalline state on water and reduce its evaporation.[592] Iso-branched wax esters help to reduce evaporation and increase surface pressure.[593] On the other hand, addition of cholesterol to cholesteryl esters increases film rigidity.[594] People with thin TFLLs (who tended to be those with EDE) and people with irregular TFLL patterns (who tended to be those with aqueous-deficient DED) had lower levels of cholesteryl esters and lysophospholipids, but higher levels of glycerolipids and phospholipids in their tears than those with normal TFLL patterns.[595]

Other meibum and tear film lipids that have been examined are the *O*-acyl-*ω*-hydroxy fatty acids (OAHFAs). Ultralong OAHFAs found in TLLF and meibum are capable of integrating other species in the polar lipid layer, thereby molding its properties and/or providing a base for the creation of hierarchical structures within the TFLL.[596] *In vitro*, OAHFAs can spread well on the surface of phosphate-buffered saline to form a solid monolayer with a crystalline structure.[597] Longer-chain OAHFAs prevented evaporation of water at a mean molecular area of approximately 18 $Å^2$/molecule.[597]

Mixtures of OAHFAs and wax esters may produce even more evaporation-resistant films.[597] Indeed, wax esters ex-

isting in the solid state under physiological conditions were capable of forming a mixed condensed monolayer with OAHFAs, and such a monolayer exhibited very high evaporation resistance *in vitro*.[598] Although OAHFAs can induce stable multilamellar cholesterol ester films on water or phosphate-buffered saline,[599] OAHFAs alone reduced evaporation, with cholesterol esters having no effect.[599] An additional double bond in the hydroxy fatty acid chain of OAHFAs was accompanied by more disordered molecular organization, which led to a loss of the evaporation resistance.[600] The ultralong chain lengths observed in naturally occurring OAHFAs may require the presence of double bonds to achieve an appropriate balance between spreadability and evaporation resistance.[600] The observations of ordered lipids resulting in deterioration of spreading and reduced elasticity, whereas a more disordered molecular organization leads to a loss of evaporation resistance, requiring further exploration of how the ordering of different types of or combinations of fatty acids can optimize the evaporative resistance, spreadability, and elasticity of the film.

Individuals with symptomatic MGD or symptomatic mixed MGD/ADDE had reduced abundance of several OAHFA species in tears, and to a lesser extent in meibum, compared with normal subjects.[601] Other meibum-derived OAHFAs had negative correlations with precorneal tear film thinning rate, and one meibum-derived OAHFA had a positive correlation with the precorneal tear film thinning rate.[602] Conversely, tear film–derived OAHFAs had no association with the precorneal tear thinning rate.[602]

There also have been several studies examining the contributions of sphingolipids to the TFLL and meibum. Meibomian gland loss has been associated with loss of some sphingosines from the TFLL.[590] Individuals with poor meibum quality had changes to their sphingolipids in tears and meibum.[603,604] The presence of both sphingomyelin and ceramide increases surface tension because of the change in their position in the TFLL under lateral pressure.[605] Sphingomyelin at the interface of the TFLL with the aqueous phase has a role in capturing the protein lysozyme and entrapping it in the TFLL.[606]

Finally, several experiments have shed important light on how meibum lipids are synthesized and the consequences of disturbance in their synthesis. Using mice with specific genes knocked out has shown that fatty acid ω-hydroxylase Cyp4f39, acyl-CoA reductase FAR2, the elongases of very long chain fatty acids -1i or -3, alcohol acyltransferases Awat1 and Awat2, and sterol O-acyltransferase 1 are all involved in various stages of the production of meibum lipids such as OAHFAs, wax esters, and cholesterol esters.[607-614] Some of the major findings when genes for these enzymes were knocked out was shortening of TBUT,[607] plugged meibomian gland orifices, tear film instability and increased tear evaporation rate,[608,612] increases in blink frequency and evaporation from the ocular surface,[609] shorter chain, branched and unsaturated cholesterol esters,[610] and changes in the melting temperature of their meibum.[610,611]

5.4. THE TEAR PROTEOME: Recent findings continue to emphasize the importance of the tear proteome as a noninvasive tool for trying to discriminate between types of DED[615,616] and also for disease monitoring, for instance revealing changes in the levels of proteoglycan 4, in tears of those with Sjögren's disease.[617]

One interesting study explored the potential of tear MUC5AC and IL-8 levels to distinguish between Sjögren and non-Sjögren DED, indicating that lower MUC5AC and higher IL-8 levels could serve as biomarkers for Sjögren syndrome, aiding in its diagnosis.[618] Similarly, the tear thrombospondin-1/MMP-9 ratio is significantly reduced in Sjögren's disease compared with non-Sjögren DED, offering another potential biomarker.[462] Additionally, the MMP-9/lactoferrin ratio was positively correlated with ocular inflammation and tear film stability in stable controlled patients with Sjögren's disease.[619]

Tear biomarkers related to ubiquitination (LMO7 and HUWE1) and the regulation of intracellular vesicle dynamics (TPD52) are significantly elevated in patients with Sjögren's disease, suggesting a role for these pathways in the underlying mechanisms of the disease.[620] Other robust protein biomarkers have been identified using liquid chromatography–tandem mass spectroscopy in multiple cohorts, highlighting several proteases and protease inhibitors plus noting the relevance of oxidoreductase proteins in Sjögren's disease.[621] Oxidative stress biomarkers serve as indicators for assessing the extent of ocular surface damage, and research using both laboratory models and individuals with DED underscores redox imbalance as one of the many pathophysiological factors driving the disease.[622] Sjögren's disease also may be characterized by reduced or absent levels of complement regulators (CD59, CD55, and CD46), alongside upregulation of C3. This imbalance may drive excessive complement activation in ocular tissues and may suggest a possible therapeutic pathway.[623]

Ongoing research continues to shed light on the role of tear cytokines in the context of DED. A recent meta-analysis of tear film cytokines in Sjögren's disease revealed elevated levels of certain cytokines, including IFN-γ, TNF-α, IL-1α, IL-1Ra, IL-4, IL-6, IL-8, IL-10, IL-17, IL-21, and IL-22. Interestingly, IL-23 levels were significantly lower compared with healthy controls.[624] Additionally, reduced levels of epidermal fatty acid–binding protein (E-FABP), which regulates inflammatory pathways on the ocular surface, further suggest its potential as a biomarker for epithelial damage and altered lipid metabolism in the disease.[625] However, despite these advances, a persistent challenge in the field is the lack of standardization in sample collection and analytical techniques,[626] which hinders the translation of these findings into clinical practice.

Tear proteomic profiling has also revealed altered biological pathways related to corneal sensitivity and nerve pa-

rameters in DED and neuropathic corneal pain[627,628] (see Section 6.5.2.

One emerging area of interest in this field involves examining the complex interplay between the tear proteome and the ocular surface microbiome, and its potential contribution to the onset of dry eye. Recent data indicate that the interaction between these 2 systems is beneficial to combat pathogens and maintain ocular health through modulation of the inflammatory response.[629] Moreover, the application of whole-metagenome sequencing has enabled researchers to pinpoint unique microbial compositions in individuals with DED, with the relative abundances of certain bacteria being correlated with specific tear proteins in the tear fluid.[630] Understanding the crosstalk between these 2 components of the tear fluid may provide opportunities for the development of personalized therapeutics tailored to individual patient profiles.

5.5. LIPID-PROTEIN-MUCIN INTERACTIONS: Interactions between the various components of the tear film are important for maintaining its stability and protecting the ocular surface and the expression of these components is interconnected. For instance, there is a compensatory mechanism where the absence of lipid secretion, caused by stearoyl-CoA desaturase-1 deficiency, leads to increased tear volume and enhanced expression of mucins, along with changes in the expression of lipid metabolism genes.[613] The knowledge gained from understanding the interactions between tear film components is also being used to develop dry eye treatment strategies. Two treatment studies suggest that mucomimetic polymers can improve the structure and functionality of the lipid layer.[631,632] Cross-linked hyaluronic acid is particularly effective in promoting tear ferning and the spreading of meibum *in vitro*, suggesting the therapeutic potential of these polymers for ocular surface health.[633]

5.6. MUCINS: The realm of ocular mucins has witnessed several scientific discoveries, shedding additional light on their important role in maintaining ocular surface health. Membrane-associated mucins act as regulators of transcellular barrier function, tear film stability, and apical epithelial cell architecture.[475,634] Membrane-associated mucins are distinctly distributed along the conjunctiva of those with and without DED, emphasizing the vital role of these mucins in minimizing eyelid friction during blinking.[635] Advanced modeling approaches have enabled researchers to establish a relationship between the loss of membrane-associated mucins and the premature rupture of the tear film, predicting quantitatively the shortening of NIBUT observed in DED.[636]

Through comprehensive genomics analyses, researchers have identified mucin variants lacking sialylation due to a point mutation in a sialyltransferase gene, St6galnac1, which plays an important role in synthesizing sialyl-Tn and serves to protect the conjunctival mucosa against foreign particles.[637] Other investigations have highlighted the rapid turnover of gel-forming mucins in the healthy ocular surface[638] and the potential benefits of thymosin β4, resolvin D2, and microRNA inhibition in their ability to stimulate the secretion of gel-forming mucins.[639-641] A randomized clinical trial comparing thymosin β4 to placebo in a 28-day study showed no difference to placebo in corneal fluorescein staining and symptoms.[642]

5.7. MICRO RNAS: The altered expression of microRNAs (miRNAs) in tear fluid has positioned them as promising candidates for noninvasive biomarkers in DED. As of this writing, approximately 300 distinct miRNAs have been identified in tear fluid, many of which are recently discovered and are poorly understood in terms of their regulatory functions.[643] RNA sequencing experiments has revealed that extracellular vesicles in the tear film in both non-Sjögren DED and healthy controls carry distinct miRNA profiles, with 126 differentially expressed miRNAs between the groups.[644] Among these, 9 miRNAs (miR-127-5p, miR-1273h-3p, miR-1288-5p, miR-130b-5p, miR-139-3p, miR-1910-5p, miR-203b-5p, miR-22-5p, and miR-4632-3p) were significantly upregulated in DED and were associated with inflammation, indicating a potential role in disease pathogenesis. Other studies, on the other hand, have shown an inverse relationship between miRNAs in tears and inflammation. For instance, miR-223 inhibits hyperosmolarity-induced inflammation through downregulating NLRP3 activation in human corneal epithelial cells and those with DED.[396] Comparison of the expression of 43 miRNAs in the tears of those with Sjögren's disease and healthy controls have revealed 14 significantly differentially expressed miRNAs that may be involved in the pathogenesis of Sjögren's disease, although none were correlated with ocular staining scores.[645] Because the precise molecular mechanisms by which these miRNAs contribute to DED remain largely unclear, and the lack of standardized methods hampers comparison across studies, further research is essential to clarify the specific roles of miRNAs within the ocular environment.

5.8. TRANSLATIONAL DRY EYE MODELS OF TEAR FILM:

5.8.1. In vitro models

Translational models of the tear film aim to bridge the gap between basic research and clinical applications. Significant advances in re-creating the ocular surface *in vitro* have emerged using organs-on-chips and stem cell-derived organoids. Organ-on-chip technology employs microfluidic devices to replicate the ocular surface environment, allowing for detailed study of tear film components such as mucins and inflammatory cytokines. By using microfluidic platforms with segmented channels, researchers can simulate dry eye conditions by exposing cultured human corneal epithelial cells to an air-liquid interface within a chip.[646] Additionally, induced pluripotent stem cells have

facilitated the creation of organoids, offering a new platform to study the molecular mechanisms involved in DED. These approaches have allowed the generation of functional conjunctival epithelial lineage cells, including goblet cells.[429,647,648]

Biomimetic models that rely on the enzymatic removal of mucins in cell culture have emerged to more accurately recapitulate the pathologic changes in lubrication, adhesion, and barrier function often observed in mucin-deficient DED.[649] In addition, hydrophilic and hydrophobic glass surfaces have been used to model the interactions of the tear film with a healthy cornea or a hydrophobic cornea in the absence of a glycocalyx.[650] Also noteworthy is the development of miniaturized analogs of a blinking human eye, which use a dome-shaped 3D cell culture scaffold to mimic the dynamic interface between the ocular surface and the external environment.[429] Other investigators have used molecular modeling of the tear film and machine learning models to better understand the contribution of proteins to tear film stability and proteomic changes in MGD, respectively.[651,652]

5.8.2. In vivo models

Compared with *in vitro* models, there have been relatively fewer innovations in the development of animal models of DED in recent years. The established models rely on surgical procedures, such as removal of the exocrine glands, exposure of the eyes to drugs like benzalkonium chloride, and placement in a dry environment, with or without scopolamine (see Section 5). Additionally, there are numerous genetically modified models targeting genes involved in tear production, inflammation, or autoimmune responses. These models continue to generate important results in the development and approval of therapeutic agents for DED.[653] Almost all current models mimic a severe aqueous-deficient, inflammatory form of DED (see Section 4) that is not reflective of EDE, and there is a need to develop alternative models for designing and testing therapies that better address different subtypes of DED. More recently, there has been a renewed emphasis on the use of canine models of evaporative and aqueous-deficient DED to better align with human disease.[654]

5.9. SUMMARY AND FUTURE DIRECTIONS: This review has identified several areas for future research that may help in the understanding of the pathogenesis and subclassification of DED, as well as identifying better biomarkers to help clinicians classify and monitor DED. Researchers should use lipids that more closely align with those in meibum, rather than mimics of meibum lipids for future *in vitro* experiments. Further research is needed to explore the relationship between disordered lipids that result in spreading and increased elasticity, compared with ordered lipids that lead to improved resistance to evaporation. Additionally, incorporating more accurate models of the muco-aqueous layer in laboratory experiments may help elucidate the roles of lipids, mucins, proteins, and other components in stability and evaporation. There is also a need for a more detailed understanding of whether tear biomarkers can be used to differentiate subtypes of DED as described in the TFOS DEWS III Diagnostic Methodology report.[1] Analyses of microbiome changes across individuals of different ethnicities and countries of residence may provide further insights into its potential role in DED pathogenesis or as a marker for the disease. Understanding the potential role of different microRNAs in DED pathogenesis, DED subtype, or as biomarkers could be a highly promising area for future investigation.

6. PAIN AND SENSATION

6.1. INTRODUCTION: As described in the TFOS DEWS II Pain and Sensation report,[40] the ocular surface, particularly the cornea, is densely innervated with sensory fibers that have important functions in the maintenance of ocular surface health. The dynamic nature of ocular surface nerves is often underappreciated, as this system continuously undergoes remodeling in adults, particularly nerve terminals in the corneal epithelium. Many common insults can amplify this remodeling, including accidental or surgical injury or disease processes that lead to chronic inflammation (eg, Sjögren's disease, Stevens-Johnson syndrome, herpes keratitis). Damage to ocular sensory nerves can result in corneal nerve loss, a change in nerve architecture, and altered sensitivity to stimulation. Although nerve regeneration can occur after such damage, it is typically gradual and incomplete. As a result, it often fails to fully restore the original density, architecture, and function of the corneal innervation, leading to persistent changes in neural excitability. This update will consider new evidence in corneal nerve remodeling during normal physiology and following trauma, surgery, or inflammation and recent evidence for the anatomic and functional status of the corneal nerves in diagnosing and managing DED.

6.2. CORNEAL NERVE REMODELING IN ADULTS: The development of corneal nerves begins in the fifth gestational month with the formation of sensory axons around the cornea, followed by their radial extension into the corneal tissue. Although the specific molecular signals controlling corneal nerve growth are still unknown, molecules such as nerve growth factor (NGF) and various neurotrophic factors released by corneal cells contribute to the development and survival of corneal nerves. The growth of the corneal nerves does not stop once development is complete but continues to occur continuously. In adults, corneal subbasal nerves and their terminals undergo continuous morphologic rearrangements throughout life whereas the stromal nerves present few morphologic changes.[655-657]

Observations from living human eyes using *in vivo* confocal microscopy reveal that subbasal nerves move centripetally at rates of 10 to 20 μm per day. These nerves elongate by adding new material near the site of nerve penetration into the epithelium from the Bowman layer. Distal nerve segments eventually degenerate or slough into the tear film as a result of the turnover of the corneal epithelium.[40,658] Additionally, intraepithelial nerve terminals undergo spontaneous morphologic changes through long-term reconfigurations and short-term reorganization in response to outward migrations of differentiating epithelial cells. Notably, corneal nerve remodeling is more prominent in the central regions of the cornea than in the periphery. Research in living transgenic and knock-in mice reveals that, over time, there are noticeable changes in the subbasal nerve fibers and intraepithelial nerve terminals. The presence of continuous remodeling is supported by the expression of growth-associated protein 43sub in the epithelial nerves of intact corneas.[656,658]

Continuous remodeling is insufficient to maintain lifelong corneal innervation. As mammals age, the density of corneal nerve terminals decreases, leading to reduced corneal sensitivity and changes in tearing regulation. In older individuals, there is a noticeable reduction in subbasal and intraepithelial nerve density and increased tortuosity and disorientation of subbasal nerves. These changes affect all corneal sensory nerves, whether nociceptive or cold thermosensitive, and result in an abundance of simple nerve terminals and a scarcity of complex nerve terminals in aging individuals. The reduced density and dysfunction of corneal nerves, especially cold thermoreceptors, may play a role in the altered tearing and sensitivity in the older population.[659]

6.3. CORNEAL NERVE REGENERATION AFTER SURGERY: Corneal nerves may be severed during corneal and anterior segment surgery, such as photorefractive keratectomy (PRK), laser-assisted *in situ* keratomileusis (LASIK), cataract surgery, iridectomy, trabeculectomy, and corneal transplantation. The survival of corneal nerves relies on transporting essential substances from their parent nerve cells in the trigeminal ganglion. Therefore, surgical procedures that disrupt corneal nerve fibers can lead to rapid degeneration of the distal axons, reduced corneal sensitivity, and impaired functional integrity of the ocular surface.

Although corneal nerves can regenerate, this process is slow and imperfect. After most corneal surgeries, the regeneration of nerves is characterized by decreased nerve density, changes in nerve structure, and diminished corneal sensitivity. Regeneration is more delayed and incomplete when nerves are cut closer to their origin.[660] Consequently, surgical disruption of the subbasal and subepithelial nerve plexuses typically results in less severe and short-term damage to corneal innervation than deep or penetrating incisions affecting major stromal nerve bundles.

Although corneal sensitivity typically returns to preoperative levels after LASIK within 6-12 months,[661] a significant proportion of patients may experience long-term dry eye symptoms because of impaired nerve regeneration.[662,663] Nerve damage in small-incision lenticule extraction surgery is less marked than in LASIK, resulting in a faster nerve regeneration 3 months postsurgery. However, no significant difference is observed at 6 months.[253] A meta-analysis of corneal sensitivity recovery has shown a more rapid early recovery of corneal sensitivity with small-incision lenticule surgery compared with femtosecond LASIK.[664] This is also described in the TFOS Lifestyle Elective Medications and Procedures report.[261]

In PRK, where the corneal epithelium is removed, and the corneal stroma is reshaped with an excimer laser without creating a flap, nerve regeneration and recovery of corneal sensitivity occur more rapidly than in LASIK.[665] Despite this, subbasal nerve density, architecture, and corneal sensitivity may remain reduced for up to 1-2 years post-PRK.[666]

In cataract surgery, small, perilimbal incisions have minimized the risk of significant injury to corneal innervation. However, in corneal transplantation procedures such as penetrating keratoplasty, a full-thickness incision cuts all corneal nerves, resulting in complete denervation of the transplanted cornea. Nerve regeneration following penetrating keratoplasty is slow, and even years later, the innervation density of the transplanted tissue remains lower than that of the host peripheral cornea.[667] Stromal nerves regenerate poorly, which may be attributed to the misalignment of Schwann cell channels in the donor cornea with the stromal nerve stumps in the host cornea. This contrasts with the perilimbal incisions used in cataract surgery, where stromal nerves on opposing sides of the incision remain closely aligned. Following penetrating keratoplasty, limited nerve regeneration occurs. A few subbasal nerve fibers elongate through the epithelium at the graft margin to enter the donor basal epithelium. Regenerated subbasal nerves may exhibit atypical orientations and morphologies.[667] The corneal nerve density and corneal sensitivity remain significantly reduced compared with healthy corneas even decades after surgery.[668]

Although theoretically expected not to alter corneal innervation, other corneal transplant techniques, such as Descemet membrane endothelial keratoplasty, show a temporary decrease in nerve density early after transplantation. However, complete recovery of corneal nerve density and function to preoperative values typically occurs within 6-10 months postsurgery.[669] Studies have also reported similar results with Descemet stripping automated endothelial keratoplasty, where the corneal sensations were noted to be normal within 6 months following surgery.[670] Another study comparing corneal sensation between Descemet membrane endothelial keratoplasty and penetrating keratoplasty showed that corneal sensation improved significantly following Descemet membrane endothelial keratoplasty but

was slightly but not significantly decreased after penetrating keratoplasty.[671] These findings suggest better preserved corneal sensations following endothelial transplants compared with penetrating keratoplasty.

6.4. NERVE REGENERATION IN PATHOLOGIC CONDITIONS: The peripheral nervous system has impressive regenerative capabilities following injury. However, injuries to the afferent axons of trigeminal neurons can lead to significant morphologic and functional changes, which depend on the magnitude and location of the damage.[660,672] The regeneration of neurons after injury is influenced by a supportive environment for axon growth and the involvement of non-neuronal cells like Schwann cells.[673]

As described in the TFOS DEWS II Pain and Sensation report,[40] mechanical trauma or inflammatory damage to the peripheral axons of corneal trigeminal neurons causes a complex cellular response and changes to their spontaneous and stimulus evoked firing rates. The expression, distribution, and activation thresholds of the transduction ion channels changes. These disturbances lead to increased responsiveness to normal stimuli (allodynia), spontaneous firing without intended stimulation, and increased abnormal or unpleasant sensations from a stimulus that would normally elicit a response (hyperalgesia).

The mechanisms that stimulate and direct neurite outgrowth from injured and intact areas of corneal innervation following local nerve injury still need to be fully understood. Corneal epithelial cells release several growth factors following an injury, which may play essential roles. Nerve growth factor is upregulated after corneal epithelial wounding, and topical recombinant human nerve growth factor has been used to stimulate corneal nerve regeneration and recovery of corneal sensitivity[674] in the treatment of neurotrophic keratitis, where sensitivity is reduced.[675] The role of nerve growth factor in the mechanisms resulting in allodynia or hyperalgesia, however, has not been established, although restoration of nerve function may be advantageous.

Ocular and systemic diseases, including herpes virus keratitis, diabetes, and aqueous-deficient DED, can negatively impact corneal nerves.[676] Diabetes significantly alters corneal nerve morphology and function, reducing nerve density and sensitivity. In diabetic patients, the appearance of subbasal nerves resemble intraepidermal small fiber neuropathy, making *in vivo* confocal microscopy of corneal nerves a valuable biomarker for monitoring diabetic neuropathy.[677]

In summary, in healthy adult corneas, the subbasal nerve fibers and intraepithelial nerve endings undergo continuous remodeling and regenerate rapidly following damage, whereas stromal nerves maintain their structure over time. The dynamic nature of intraepithelial nerve endings accounts for their rapid regeneration after injury, whereas subbasal nerve fibers regenerate more slowly, and stromal nerve trunks may not fully regenerate. Corneal nerve morphology is affected by trauma, ocular surgery, infections, chronic tear deficiency, and various systemic diseases. Although damaged corneal innervation can regenerate like other peripheral nerves, their morphology and function are often incompletely restored, leading to reduced sensitivity, abnormal sensations, and pain.

6.5. NERVE ABNORMALITIES AND DED: Many manifestations placed under the heading of "dry eye" occur because of morphologic and functional changes in ocular innervation. Changes in ocular sensory nerves induce symptoms such as unpleasant sensations of different intensities, ranging from dryness or ocular discomfort to lacerating and burning pain.[678] Conversely, abnormal nerve functioning can lead to alterations in tissue trophism and in the regulation of tear production and blinking, which in turn contributes to both symptoms and signs.[679] In other words, sensory innervation can be altered by chronic eye dryness and can also contribute to DED pathogenesis.

Reduction of the ocular surface moistness either by reduced tearing or increased tear evaporation is a stressful situation for the corneal epithelium exposed to a hyperosmotic tear film and the environment (see Section 4.3). Tear hyperosmolarity can independently affect subbasal corneal nerves as shown in an animal model.[680] As a response to this hyperosmolar and desiccating stress, corneal epithelial cells and immune resident cells produce local inflammatory mediators, primarily IL-1 and TNF-α, which stimulate the production of matrix metalloproteases, activate dendritic cells, and local inflammation occurs,[6] leading to sensitization of nociceptive nerve terminals and development of discomfort and pain sensations. When dryness becomes chronic, it also leads to corneal nerve damage and, consequently, the morphofunctional changes of the sensory nerves during chronic eye dryness resemble both those produced during inflammation and those produced by nerve injury. The eyelid movement causes mechanical friction at the ocular surface when the tear film is thin and does not lubricate well. Together with the chronic inflammation, this movement damages the epithelium and the intraepithelial nerve terminals, and eventually the subbasal nerve fibers, triggering the mechanisms of nerve degeneration and regeneration.[681]

In clinical practice, these degeneration and regeneration processes are evidenced by signs such as a reduction in the density and branching of the subbasal plexus nerve fibers, and an increase in their tortuosity when explored by *in vivo* confocal microscopy, as well as by a reduction in corneal sensitivity to stimulation (shown by increased sensation thresholds) that often occurs simultaneously with hyperalgesia and spontaneous pain sensations and is aggravated by tear film instability.[682] A recent *in vivo* confocal microscopy study of corneal nerves confirmed these nerve and branch density, and length reductions in 23 participants with DED and showed that osmolarity exhibited a weak negative correlation to these nerve parameters, whereas other ocular surface signs were not associated with any nerve parameters.[683] Despite the nerve changes reported, it does ap-

pear however that not all sensory modalities are equally affected and corneal hypersensitivity to cold stimuli has been reported in DED,[684] in computer vision syndrome,[685] and contact lens discomfort.[686] Repeated evaporative cooling at the ocular surface may change the excitability of corneal receptors and their subsequent responsiveness.[40,682,687] Tear film instability can cause changes in suprathreshold scaling by both cold thermoreceptors and polymodal nociceptors.[688]

Most of the alterations of ocular surface sensitivity in chronic eye dryness are due to functional changes in corneal nerves that resemble those seen in injured nerves. There is an increased excitability of corneal nerves consecutive to the increased activity and expression of sodium channels,[689-691] resulting in an increased spontaneous firing of cold thermoreceptors that not only leads to dryness sensations but also to dysregulation of protective mechanisms driven by thermal sensory input such as tearing and blinking, contributing to increase and perpetuate the ocular surface disturbances.

In contrast to the pathophysiology of neuropathic pain, defined by pain or altered sensation due to nerve damage, nociplastic pain occurs in the context of no discernible tissue or nerve damage. Instead, nociplastic pain is believed to be related to dysfunctional central sensory processing of pain through a process called sensory sensitization. Either neuropathic or nociplastic pain can cause chronic ocular pain and are particularly associated with a subset of DED patients who report pain of a magnitude that is disproportionate to ocular surface findings (Table 4).[692] Quantitative sensory testing and functional magnetic resonance imaging (fMRI) studies remain the primary methods of assessing nociplastic pain, but the underlying pathophysiology remains poorly understood.

6.5.1. Impact of DED on nerve structure and function in animal models

Animal models have been developed as correlates of human DED. Although their initial focus was on immune abnormalities, recent studies have found that various insults that create tear and ocular surface abnormalities also affect the corneal nerves.

Several mouse models mimic DED in Sjögren's disease, including an IL-2 receptor α-chain knockout model.[693] In one study, intraepithelial corneal nerve density in knockout mice was compared to that in mice using confocal microscopy at 4 weeks, 6 weeks, 8 weeks, and 10-11 weeks after birth. After combining data from each time point, the overall density was significantly decreased in the knockout mice compared with wildtype. These results suggest that corneal nerve alterations coincide with the onset of DED, with reduced nerve fiber density commonly noted.

Nerve alterations also have been examined in a GVHD murine model,[694] using an allogeneic bone marrow transplant (case) group (strain B10.D2 to strain BALB/c) and a syngeneic bone marrow transplant group (strain BALB/c to strain BALB/c, control group) at 1, 2, 3, and 4 weeks posttransplant. Corneal nerve tortuosity and branching were increased in the allogeneic bone marrow transplant group at 4 weeks posttransplant compared with 1 week posttransplant. Conversely, no significant differences were observed in branching or tortuosity in the syngeneic group at any time point. These findings support the induction of morphologic changes in corneal nerves associated with the progression of GVHD-associated DED.

Other protocols have been developed to model DED following ocular surgery, such as photorefractive keratectomy (PRK).[695] PRK (−9D) was performed monocularly in New Zealand rabbits while the contralateral cornea was used as a control. Corneal nerve fiber density was evaluated in post-PRK corneas with acetylcholinesterase histochemistry staining at 1 day (n = 3), 1 month (n = 3), 2 months (n = 3), 3 months (n = 3), and 6 months (n = 4) after surgery. Compared with controls (n = 3), nerve density was significantly decreased in post-PRK corneas after 1 day, 1 month, and 2 months but was not different at 3 and 6 months postsurgery. Nerve morphology alterations (increased tortuosity and aberrant innervation) persisted, however, until the final time point at 6 months. Findings indicate surgery may result in corneal nerve alterations that do not fully return to baseline by 6 months postsurgery, consistent with the effects of refractive surgery in human.[661]

Other studies focused on DED prompted by iatrogenic challenges. One murine study used confocal microscopy with staining using an anti-β3 tubulin antibody to examine corneal nerve fiber density after repeated topical administration of benzalkonium chloride for 14 days.[696] There was a significant reduction in density in benzalkonium chloride–treated mice compared with controls.

Partial or complete lacrimal gland excision also has been used to model DED.[420] In one study, the number of corneal nerve terminals in the surgical group was assessed qualitatively by confocal microscopy and compared to a sham-surgery and control group. The surgical group demonstrated fewer corneal nerve terminals compared with both sham-surgery and control groups. This study also examined nerve function with electrophysiological studies and found that postsurgical animals developed time-dependent corneal mechanical hypersensitivity accompanied by increased spontaneous ciliary nerve fiber electrical activity. Other investigators have found similar functional changes after gland excision, with increased activity and expression of sodium channels that lead to increased excitability of corneal nerves. This results in a greater spontaneous firing of corneal cold thermoreceptors, causing sensations of dryness, and an enhanced excitability of corneal nociceptors that results in low-frequency spontaneous firing and increased response to stimulation, resulting in sustained discomfort and hyperalgesia.[690,697,698] These findings suggest that beyond nerve density and morphology, corneal

TABLE 4. Mechanistic Characterization of Pain, Reproduced From De Lott and Associates[692]

Type	Neurobiology	Clinical Signs and Symptoms	Treatment	Non-ocular and Ocular-Related Examples
Nociceptive	Actual or threatened tissue damage via activation of nociceptors	Localized to the site of injury and surrounding area	• Remove source of nociception • Promote tissue healing • Topical medications • Short-term systemic medications: NSAIDs, acetaminophen, anti-inflammatory drugs, opioids	Non-ocular: burn Ocular: corneal abrasion
Neuropathic	Damaged somatosensory system, either peripheral or central	Localized to a dermatome (peripheral) or site of injury (eg, CNS demyelinating lesion in the spinal cord causing leg paresthesias and pain)	• Peripheral neuropathic: nerve blocks, topical medications (eg, capsaicin) • Systemic medications: gabapentinoids, tricyclic antidepressants, SNRIs, sodium channel blockers	Nonocular: diabetic neuropathy Ocular: Post-herpetic neuralgia
Nociplastic	Altered pain perception and processing in the central nervous system	Regional and diffuse pain Multisensory sensitivity COPCs	• Lifestyle: education, sleep, exercise • Behavioral/Integrative: self-management, psychotherapy, etc • Medications: SNRIs	Non-ocular: fibromyalgia Ocular: referred pain from chronic tension-type headache

CNS = central nervous system, COPCs = chronic overlapping pain conditions, NSAIDs = nonsteroidal anti-inflammatory drugs, SNRIs = serotonin-norepinephrine re-uptake inhibitors.

nerve function can be impacted differentially in DED subtypes.

Desiccating stress is often induced using a combination of scopolamine and low-humidity conditions (eg, humidity: 25%, constant airflow). In one study, desiccation reduced corneal sensitivity over time,[699] linking dry eye onset with corneal functional abnormalities. In summary, animal models can be used to study ocular surface nerve anatomy and sensitivity (and their upstream connection) as they relate to various insults that lead to chronic dry eye. Changes in morphology and sensitivity have been noted across many models, both in terms of reduced abilities to detect stimuli (further aggravating ocular surface disturbances) and the development of spontaneous discomfort and pain, that primarily stem from functional alterations in corneal nerves, resembling those observed in peripheral nerves after injury. These data highlight the intricate relationship between corneal nerves and dry eye.

6.5.2. Structural and functional nerve alterations in DED in human

In DED, alteration of corneal nerve structure and function is frequently implicated in the onset, progression, and severity of symptoms. Disruption of the tear film in DED can result in nerve fiber damage that stimulates regenerative processes that are often improper or incomplete. The TFOS DEWS II Pain and Sensation report in 2017[40] determined that although IVCM is a reliable method for detecting abnormal corneal nerve morphology, nerve density was a somewhat unreliable marker for nerve fiber damage, but that other morphologic parameters were more useful, including increased tortuosity, reflectivity, and increased beading. More recently, a higher frequency of microneuromas has been documented.[682] These structural aberrations, in turn, may lead to further impair tear film production, and the blinking reflex resulting in additional nerve damage and conceivably neuropathic dysfunction.[682] Typical manifestations of neuropathic dysfunction include ocular pain, loss of normal sensation, and tear hyperosmolarity. Damage to nerve fibers in DED can also result in physiologic disruption of the corneal nerve's usual neurotrophic functions, including regulation of nerve growth factor and substance P, that help mediate epithelial growth, local immunoregulation, and nerve fiber regeneration.[682,700] Together, these structural and functional changes likely play a significant role in the pathophysiology of DED.

Interestingly, some individuals with DED report symptoms that are disproportionate to ocular surface alterations, and others are found to have signs that are more severe than their symptoms suggest.[157] One possible explanation for the symptom-to-sign discordance is the presence of central abnormalities, with inappropriate processing of somatosen-

TABLE 5. Corneal Nerve Tortuosity in DED Subtypes

Author(s)/year	Location	Sample Size	Methodology	Findings
He et al (2017)[706]	Japan	GVHD (n = 12) Controls (n = 10)	Imaging: multi-image (3), central cornea + inferior limbal epithelia Analysis: ImageJ/NeuronJ, OSE grading scale	↑ tortuosity in GVHD compared with controls
Tepelus et al (2017)[701]	United States	SDED (n = 22) NSDED (n = 12) Controls (n = 5)	Imaging: multi-image (3), central cornea Analysis: ImageJ/NeuronJ, OSE grading scale	↑ tortuosity in SDED and NSDED compared with controls
Guerrero-Moreno et al (2021)[704]	France	AIDED-NCP (n = 7) MGD-NCP (n = 11) AIDED (n = 8) MGD (n = 8) Controls (n = 10)	Imaging: single image, central cornea Analysis: ImageJ/NeuronJ, study-designed tortuosity scale	↑ tortuosity in AIDED-NCP compared with controls. Tortuosity not different in MGD-NCP, painless AIDED, or painless MGD compared with controls.
Li et al (2021)[702]	China	SDED (n = 22) NSDED (n = 20)	Imaging: multi-image (5), central cornea Analysis: ImageJ/NeuronJ, OSE grading	↑ tortuosity in SDED compared with NSDED.
Luzu et al (2022)[705]	France	SDED (n = 71) MGD (n = 20) Control (n = 20)	Imaging: multi-image (5), central cornea Analysis: ImageJ/NeuronJ, OSE grading	↑ tortuosity in SDED compared with MGD and controls.

AIDED = autoimmune DED, DED = dry eye disease, GVHD = graft-vs-host disease, MGD = meibomian gland dysfunction, NCP = neuropathic corneal pain, NSDED = non-Sjögren DED, OSE = Oliveira-Soto and Efron Scale, SDED = Sjögren DED.

sory signals as a result of changes in the central nervous system referred to as central sensitization. Central sensitization is associated with generalized somatosensory dysfunction and cutaneous hypersensitivity in addition to excessive ocular pain.

6.5.2.1. Corneal nerve anatomy in DED. Different manifestations of corneal nerve fiber abnormalities likely have clinical significance for patients with various subtypes of DED but are not yet fully understood. In recent years, several studies have improved the understanding of IVCM in DED. Table 5 summarizes the key findings from studies exploring corneal nerve tortuosity.

Nerve tortuosity appears to be more extensive in Sjögren DED compared with non-Sjögren DED[701,702] based on the Oliveira-Soto and Efron grading scale.[703] However, greater tortuosity was seen in corneal neuropathic pain associated with autoimmune disease compared with corneal neuropathic disease in non-autoimmune DED, MGD, or MGD without pain and controls.[704,705]

Corneal nerve fiber density (CNFD) also has been studied in various DED subtypes (Table 6), and in most studies it appears to be significantly decreased in Sjögren DED, GVHD, non-Sjögren DED, and neuropathic corneal pain compared with controls,[701,707-710] although within studies, density is similar between DED types.[711] One small study has evaluated MGD and found no reduction compared with controls[704]; however, a larger study found a reduction in both ADDE and EDE compared with controls.[712] The reduction in density is greater with increased symptoms. Those with normal to mild symptoms have similar CNFD to controls.[713] These studies reinforce that CNFD values are generally lower in some DED subtypes, most notably in autoimmune-associated DED, and that CNFD may be affected by degree of symptoms severity/ocular pain.

Newer nerve parameters, such as microneuromas, defined as irregular expansions of subbasal nerve endings that suggest nerve damage underlying ocular symptoms, have been included in some studies (Table 7). Conceivably, because of either the subjective determination of microneuromas or the difficulty of discriminating a turn in the nerve from an ending, there are inconsistencies in the literature.[719,720] Some studies found an increase in microneuromas among DED subtypes compared with controls, most notably in autoimmune DED and MGD,[704,721] with a greater increase where there was coexisting neuropathic corneal pain.[704] Although these studies suggest that microneuroma frequency or number is increased among several DED subtypes compared with controls, other studies have not replicated these findings in those with symptoms of DED.[722] These findings point to the need for additional studies that standardize microneuroma determination and further explore their utility as a biomarker for DED.

TABLE 6. Corneal Nerve Fiber Density in DED Subtypes

Author/Year	Location	Sample Size	Methodology	Findings
Shetty et al (2016)[713]	India	Normal-mild EDE (n = 29) Moderate-severe EDE (n = 23) Control (n=43)	Imaging: single image, central cornea Analysis: ACCMetrics	↓ CNFD in EDE with moderate-to-severe symptoms compared with controls. CNFD not different in EDE with normal-to-mild symptoms compared with controls.
He et al (2017)[706]	Japan	GVHD (n = 12) Control (n = 10)	Imaging: multi-image (3), central cornea + inferior limbal epithelia Analysis: ImageJ/NeuronJ	CNFD not different in GVHD compared with controls.
Tepelus et al (2017)[701]	United States	SDED (n = 22) NSDED (n = 12) Control (n = 5)	Imaging: multi-image (3), central cornea Analysis: ImageJ/NeuronJ	↓ CNFD in SDED and NSDED compared with controls.
Kobashi et al (2018)[709]	Japan	NSDED (n = 25) Control (n = 25)	Imaging: multi-image (5), central cornea Analysis: ImageJ/NeuronJ	↓ CNFD in NSDED compared with controls.
Nicolle et al (2018)[714]	France	DED (n = 32) Control (n = 15)	Imaging: multi-image (5), central cornea Analysis: ImageJ/NeuronJ	↓ CNFD in DED compared with controls.
Giannaccare et al (2019)[711]	Italy	SDED (n = 20) GVHD (n = 19) Control (n = 30)	Imaging: multi-image (3), central cornea Analysis: ACCMetrics	↓ CNFD in SDED and GVHD compared with controls.
Dikmetas et al (2020)[715]	Turkey	GVHD (n = 22) Control (n = 28)	Imaging: multi-image (3), central cornea Analysis: ImageJ/NeuronJ	↓ CNFD decreased in GVHD compared with controls.
Moein et al (2020)[708]	United States	NCP (n = 25) DED (n = 30) Control (n = 16)	Imaging: multi-image (3), central cornea Analysis: ImageJ/NeuronJ	↓ CNFD in the NCP and DED compared with controls.
Cox et al (2021)[710]	United States	ADDE (n = 24) EDE (n = 46) Control (n = 45)	Imaging: multi-image (3), central cornea Analysis: ImageJ/NeuronJ	↓ CNFD in ADDE and EDE groups to controls.
Guerrero-Moreno et al (2021)[704]	France	AIDED-NCP (n = 7) MGD-NCP (n = 11) AIDED (n = 8) MGD (n = 8) Control (n = 10)	Imaging: single image, central cornea Analysis: ImageJ/NeuronJ	↓ CNFD in AIDED and MGD-NCP compared with controls. CNFD not different in AIDED-NCP or MGD compared with controls.
Li et al (2021)[702]	China	SDED (n = 22) Control (n = 20)	Imaging: multi-image (5), central cornea Analysis: ImageJ/NeuronJ,	↓ CNFD decreased in SDED compared with NSDED.
D'Souza et al (2022)[716]	India	ADDE/EDE + symptoms (n = 10) Control (n = 15) ADDE ± symptoms (n = 57) EDE ± symptoms (n = 16) Control (n = 15)	Imaging: single image, central cornea Analysis: ACCMetrics	↓ CNFD in those with DE symptoms compared with controls. CNFD not different in ADDE (± symptoms) or EDE (± symptoms) compared with controls.

(*continued on next page*)

TABLE 6. (*continued*)

Author/Year	Location	Sample Size	Methodology	Findings
Jing et al (2022)[717]	China	DED (n = 155) Control (n = 20)	Imaging: multi-image (≥10), central Analysis: CS-Net	↓ CNFD in DED compared with controls.
Luzu et al (2022)[705]	France	SDED (n = 71) MGD (n = 20) Control (n = 20)	Imaging: multi-image (5), central cornea Analysis: ImageJ/NeuronJ	↓ CNFD in SDED compared with controls. CNFD not different in SDED compared with MGD.
Zhang et al (2022)[718]	China	DED (n = 25) Control (n = 20)	Imaging: single image, central cornea Analysis: ImageJ/NeuronJ, ACCMetrics	↓ CNFD in DED compared with controls.
Kasikci et al (2023)[712]	Turkey	ADDE (n = 22) + EDE (n = 21) Control (n = 20)	Imaging: multi-image (3), central cornea Analysis: ImageJ/NeuronJ	↓ CNFD in ADDE and EDE compared with controls.

ADDE = aqueous-deficient dry eye, AIDED = autoimmune dry eye disease, CNFD = corneal nerve fiber density, DED = dry eye disease, EDE = evaporative dry eye, GVHD = graft-vs-host disease, MGD = meibomian gland dysfunction, NSDED = non-Sjögren dry eye disease, NCP = neuropathic corneal pain, SDED = Sjögren dry eye disease.

TABLE 7. Corneal Nerve Microneuromas in DED Subtypes.

Author(s)/Year	Location	Sample Size	Methodology	Findings
Moein et al (2020)[708]	United States	NCP (n = 25) DED (n = 30) Control (n = 16)	Imaging: multi-image (3), central cornea Analysis: ImageJ/NeuronJ	MN frequency ↑ in NCP compared with controls. MN frequency not different in DED compared with controls.
Guerrero-Moreno et al (2021)[704]	France	AIDED-NCP (n = 7) MGD-NCP (n = 11) AIDED (n = 8) MGD (n = 8) Control (n = 10)	Imaging: single image, central cornea Analysis: ImageJ/NeuronJ	MN frequency ↑ in AIDED, AIDED-NCP, MGD, and MGD-NCP compared with controls.
Dermer et al (2022)[722]	United States	DED (n = 119) DED + refractive surgery (n = 19) Control (n = 18)	Imaging: multi-image (3), central cornea Analysis: ACCMetrics	MN frequency not different in DED or DED + refractive surgery compared with controls.
D'Souza et al (2022)[721]	India	ADDE/EDE + symptoms (n = 14) Control (n = 27) ADDE ± symptoms (n = 24) EDE ± symptoms (n = 65) Control (n = 27)	Imaging: single image, central cornea Analysis: ACCMetrics	MN frequency ↑ in those with DE symptoms compared with controls. MN frequency not different in ADDE (± symptoms) or EDE (± symptoms) compared with controls.
Luzu et al (2022)[705]	France	SDED (n = 71) MGD (n = 20) Control (n = 20)	Imaging: multi-image (5), central cornea Analysis: ImageJ/NeuronJ	MN frequency ↑ in SDED compared with controls but not compared with MGD.[705]

ADDE = aqueous-deficient dry eye, AIDED = autoimmune dry eye disease, DED = dry eye disease, EDE = evaporative dry eye, MGD = meibomian gland dysfunction, MN = microneuroma, NCP = neuropathic corneal pain, SDED = Sjögren dry eye disease.

Recent work has explored the use of AI technology in nerve analysis, particularly the use of deep learning AI models to automatically segment corneal nerves.[723] Using the TFOS DEWS II diagnostic criteria,[130] individuals with DED had reduced CNFD compared with controls, and values were similar to those derived from manual annotation.[717] Applying new technologies to IVCM will allow for more consistent and faster quantification of images that can be applied across centers, facilitating comparisons across different populations.

6.5.2.2. Corneal sensitivity in DED. Corneal sensitivity testing is an important diagnostic tool that can provide valuable insights into underlying somatosensory abnormalities in DED. The TFOS DEWS II Pain and Sensation report highlights that corneal sensitivity to mechanical stimuli tends to be reduced in patients with aqueous-deficient DED.[40] However, studies using air/gas esthesiometers have shown equivocal findings, with increased,[724,725] decreased,[726,727] and similar[728] corneal sensitivity in various DED subtypes. This could be due to differences in stimulus parameters (eg, cold, chemical stimulus included in some testing paradigms) or due to differences in neural responses across various DED subtypes (eg, aqueous-deficient DED vs more evaporative forms of DED).[729] Thus, consideration of the type of stimulus, the subtype of DED, and patient factors is critical to interpreting corneal sensitivity results. Table 7 summarizes studies evaluating sensitivity or pain responses in DED.

In a study of corneal mechanical sensitivity in those with DED symptoms tested using an air jet esthesiometer, 13% showed hypersensitivity and 11% had hyposensitivity.[728] When grouped by DED subtype, there was an association between aqueous-deficient DED and reduced sensitivity.[705] There is growing awareness that some individuals with symptoms of DED likely have neuropathic contributors to symptoms and this group, as a whole, display corneal hypersensitivity. Individuals with hypersensitivity had more severe ocular pain complaints whereas those with hyposensitivity had more severe epithelial disruption.[730] These data highlight that differences in corneal sensation and symptom reporting may align with differences in DED profiles.[730] In a study using the Cochet-Bonnet esthesiometer, mechanical corneal and pain sensitivity were assessed in individuals with short tear film breakup DED, defined as TBUT <5 seconds and Schirmer value >5 mm, and controls (n = 46). Pain sensitivity threshold but not mechanical sensitivity threshold was higher in the DED group.[731] Corneal hyperalgesia was present in 37% of the DED group and there was a strong relationship between pain sensitivity and subjective pain scores.[731] Cold sensitivity measured using cooling scores was assessed in a DED and control group and was compared with mechanical sensitivity threshold using a Cochet-Bonnet esthesiometer. Treatment with a lubricant containing TRPM8 agonist (0.01% menthol, a compound that activates cold thermoreceptors) or lubricant alone was applied bilaterally in a crossover design. Although there was no difference in mechanical sensitivity, the DED group reported a higher cooling response and score compared with controls, suggesting that mechanical and cold sensitivity may be differentially affected in DED.[684] The duration of disease was associated with a greater cooling response, suggesting that nociceptor activity may change over disease duration. Taken together, these results suggest abnormal pain and conceivably cold stimulus processing in different DED subtypes and that different qualities of stimulus processing and other ocular surface sites may help to understand neurogenic changes in different subtypes of DED.

Increased sensitivity (reduced mechanical threshold) has been reported after treatment of DED or GVHD with autologous serum tears,[732] Sjögren DED with cyclosporine,[733] and short TBUT DED with diquafosol (Table 8).[734] This underscores the dynamic nature of corneal sensitivity, highlighting changes in the short term, with disease, and with treatment, the implications of which need further study.

The relationship between corneal nerve structure and function is equivocal. Nerve structure was not consistently associated with gross corneal sensitivity using a cotton wisp test, where a significant relationship was only noted between corneal sensitivity and corneal nerve fiber area.[735] In a diabetic population, there was a stronger relationship between anatomy and function.[736] These data highlight that relationships between corneal nerve anatomy and function may vary with disease type and that relying on structural findings alone may not suffice in evaluating nerve functional health.

Given the importance of understanding nerve function in DED, there have been efforts to develop new esthesiometers aimed at improving precision, range, stimulus type and portability, to facilitate routine clinical use. These are reviewed in the TFOS DEWS III Diagnostic Methodology report.[1] In brief, these have included noncontact air jet esthesiometers,[737,738] liquid jet esthesiometers,[685,739,740] and a single-use filament mechanical esthesiometer (Kerasense; Dompè farmaceutici SPA).[738,741] Although the initial reports on these esthesiometers are encouraging,[685] further research is necessary to explore their clinical utility, particularly in individuals with DED.

In conclusion, sensitivity measurements can examine certain functional qualities of corneal nerves in DED. There is heterogeneity across and within DED subtypes, with conditions such as aqueous-deficient DED generally showing reduced corneal sensitivity, and with symptom severity overall relating to increased sensitivity. Variability in results also can be attributed to the type of nociceptors stimulated, nature of the stimulus, threshold vs pain sensitivity, esthesiometer type, and factors such as disease duration and severity, underscoring the complexity of DED. As research progresses, the integration of sensitivity assessment into routine clinical practice could enhance the man-

TABLE 8. Corneal sensitivity in DED

Author/Year	Location	Sample population	Methodology	Findings
Variation in testing paradigms and DED subpopulations				
Spierer et al (2016)[730]	USA	DE symptoms (none-severe) (n=129)	Corneal detection and pain thresholds and relationship with symptoms and signs of DED (Belmonte esthesiometer)	Those with hypersensitivity had more severe ocular pain complaints while those with hyposensitivity had more severe epithelial disruption
Corcoran et al (2017)[684]	USA	DED (TBUT ≤5 s, and corneal staining ≥4; n = 33) Control (n=15)	Cold sensitivity score by comparing responses to lubricants with or without a cold receptor stimulator (TRPM8 agonist: 0.01% menthol) (Cochet-Bonnet esthesiometer)	Mechanical sensitivity was similar between groups. Cold sensitivity reflected by cooling scores was greater in DED than controls (24.6 vs 12.1, $P = .0005$) and shorter disease duration (<10 y) had higher cooling scores than longer disease duration (>10 y)
Tagawa et al (2019)[731]	Japan	Short TBUT DED (TBUT < 5 s, Schirmer test value > 5 mm; (n = 60)) Control (n = 46)	Mechanical corneal sensitivity and pain sensitivity (fiber length that elicited pain) thresholds (Cochet-Bonnet esthesiometer)	Detection thresholds were similar between the groups Short TBUT DED had higher pain thresholds (26.3 ± 23.1 vs 6.9 ± 16.4 mm, $P < .01$), which correlated with subjective pain scores (scale 0-4; $r = 0.24$, $P < .05$)
Galor et al (2021)[728]	USA	DED (DEQ-5 ≥6) N = 403	Corneal detection thresholds in DED (Belmonte esthesiometer)	Mean corneal detection threshold of 87 ± 46 mL/min. 13% of participants with hypersensitivity and 11% with hyposensitivity
Alterations in corneal sensitivity in response to therapy:				
Levy et al (2017)[733]	France	SSDED (primary/ secondary; n=30) Control (n=15)	Corneal sensitivity tested after 6 mo of 0.05% cyclosporine use (Cochet-Bonnet)	Increased corneal sensitivity over 6 mo of treatment (5.1-5.6 mm, $P = .03$)
Kaido et al (2018)[734]	Japan	Short-TBUT DED (TBUT <5 s) DQS group (n = 12) AT (n = 15)	Corneal detection and pain thresholds measured after 5 wk of DQS or AT therapy. (Cochet-Bonnet)	DQS treatment significantly lowered pain sensitivity compared with AT after 5 wk of therapy. Neither lowered detection thresholds
Carreno-Galeano et al (2023)[732]	USA	DED (Ocular GVHD; n = 20)	Corneal sensitivity tested after 12 wk of 20% AST use (Cochet-Bonnet)	Mean corneal sensitivity increased at 12 wk of 20% AST in 17 patients with ocular GVHD (31.1±23.8 to 51.6±12.6 mm, $P = .001$)

AST = autologous serum tears, AT = artificial tears, DED = dry eye disease, DEQ-5 = 5-item Dry Eye Questionnaire, DQS = Diquafasol, GVHD = graft-vs-host disease, NCP = neuropathic corneal pain, TBUT = tear film breakup time.

agement and therapeutic outcomes for patients with DED. Most studies have focused on corneal sensitivity only, and there is limited evidence for other ocular surface regions including the bulbar and palpebral conjunctiva and eyelid margin, which may also contribute to ocular symptoms. A commercial, quantitative, and suitably sensitive, noncontact esthesiometer is urgently needed along with evidence-based normative and reference values for application in different subtypes of DED.

6.6. ANESTHETIC CHALLENGE IN DED: The TFOS DEWS II report emphasized the role of neurosensory abnormali-

ties in the etiology of DED and the need to evaluate somatosensory functions in its assessment.[40] The topical anesthetic challenge has emerged as an important tool for assessing neurosensory abnormalities. This simple and rapid test can differentiate between pain originating from peripheral nociceptor activation vs central (or nonocular surface) mediated pain arising from proximal sensory pathways or the central nervous system.[40] The test involves applying a drop of topical anesthetic (eg, 0.5% proparacaine hydrochloride) to the ocular surface and evaluating subjective pain relief. Pain relief typically indicates peripheral neuropathic or nociceptive causes of pain, as proparacaine stabilizes nociceptor neuronal membranes, impeding initiation and conduction of nerve impulses.[742] In contrast, the absence of relief suggests a central or nonocular surface etiology of pain, whereas partial improvement may indicate a mixed component to pain.[743] However, it is important to note that in patients with complete pain relief, the test cannot differentiate between pain due to peripheral nerve abnormalities or nociceptive causes like DED.[742] It is further not informative if no pain is present prior to anesthetic placement (in individuals with pain that waxes and wanes).

Persistent pain after anesthesia can indicate central nervous system abnormalities in pain-processing pathways.[744] Specifically, individuals with persistent pain after topical anesthesia often exhibit other features of somatosensory dysfunction. A study of veterans with DED symptoms were categorized based on anesthetic challenge response. Those with persistent pain had greater discordance between signs and symptoms and lower nonocular cutaneous cold and hot thresholds (see Section 6.7) compared with those where ocular pain was reduced following topical anesthesia.[745] These data suggest increased cutaneous sensitivity both at a site innervated by the trigeminal nerve (forehead) and at a distant site (forearm), supporting the contribution of central mechanisms in individuals with persistent ocular pain following anesthesia. Similarly, individuals with DED who experience central-dominant pain had higher ocular and nonocular pain scores compared with those who had greater reduction in pain with topical anesthesia (peripheral-dominant).[746] These findings have therapeutic implications as treatments for peripheral vs centrally mediated pain vary. For instance, patients with peripheral dominant pain may benefit more from anti-inflammatory and topical neuromodulation, whereas those with central pain may benefit from systemic neuromodulation.[742] Further studies are needed, however, that examine whether results of diagnostic tests can be used to predict therapeutic responses.

The anesthetic challenge is a simple and accessible test that can aid in the evaluation of somatosensory (dys)function in DED. When used appropriately, it can help identify the location of pain generation, which may assist in formulating a personalized treatment plan for individuals with ocular pain.

6.7. QUANTITATIVE SENSORY TESTING IN DED: Somatosensory function, including central abnormalities, can be investigated using quantitative sensory testing, a method of assessing an individual's response to various stimuli (thermal, mechanical, vibratory). Similar to ocular sensory testing, nonocular function can be evaluated using detection and pain thresholds, measuring degree of pain to a fixed stimulus, temporal summation (a phenomenon where repeated stimuli of the same intensity cause a gradual increase in pain intensity), and aftersensations (a sensation that persists after the external stimulus that caused it has stopped). Abnormalities in the latter 2 tests (temporal summation and aftersensations) have been linked to central abnormalities. Several studies have applied these protocols to the study of DED (Table 9).

Hot and cold thermal stimuli are frequently used to evaluate somatosensory (dys)function. Despite variation in quantitative sensory testing protocols, increased responsiveness to hot pain has been noted in individuals with DED where there are increased symptoms disproportionate to the signs (measured as a discordance score).[747] DED discordance score positively correlated with hot pain aftersensation intensity at the forehead.[749] These studies support the finding that abnormal perception of hot pain at sites near and distant to the eye correlate with DED symptoms in the absence of ocular surface signs and that the altered perception may arise from central abnormalities.

Mechanical pain also can be used as a stimulus to evaluate somatosensory (dys)function. Before LASIK surgery, mechanical cutaneous pain thresholds at the forehead negatively correlated with ocular pain intensity, indicating higher sensitivity in individuals with more severe pain. The presence of aftersensations to a cutaneous mechanical stimulus prior to surgery predicted symptoms of DED 6 month after surgery.[750] Using a thumbnail pressure test, no significant differences were found in mechanical pain testing or auditory testing in discordant DED compared with concordant DED or controls, although the sample size was relatively small.[769] These discrepancies highlight the need for more standardization in protocols when applied to the study of DED and perhaps better definition of DED subtypes.

6.8. BRAIN IMAGING IN DED: Advanced neuroimaging techniques, such as fMRI, have been applied to the study of individuals with chronic ocular pain.[752,753] fMRI is a neuroimaging technique that uses a light stimulus and measures regional changes in brain metabolism over time and has been extensively used in various studies.[754] In a small study of individuals with and without ocular pain, the chronic ocular pain group exhibited greater activation in brain regions like the primary somatosensory, insular, and anterior midcingulate cortices compared with controls. Instillation of topical anesthetic reduced activation in the primary somatosensory and anterior midcingulate cortices. These results suggest different activations patterns to light in individuals with chronic pain and photophobia, supporting the

TABLE 9. Quantitative Sensory Testing in DED

Author/Year	Location	Sample and Size	QST Metrics	Results
Galor et al (2016)[747]	United States	DED (n = 118)	Detection threshold for vibration, cool, warm, cold pain, and hot pain at the forearm Pain, aftersensation, and temporal summation of cold pain and hot pain stimuli at the forearm	HTPS positively correlated with burning pain, wind sensitivity, and ocular pain.
Shtein et al (2016)[748]	United States	Concordant DED (n = 25) Discordant DED (n = 23) Total DED (n =48) Controls (n = 26)	Intensity rating of pressure pain at the thumbnail Intensity rating of auditory tones in both ears	Intensity rating for pressure pain and auditory tones were not statistically different in discordant DED. Pressure pain and auditory sensitivity were not correlated with DED symptoms.
Ong et al (2018)[749]	United States	DED (n = 326)	Detection threshold for vibration, cool, warm, cold pain, and hot pain at the forearm Pain, aftersensation, and temporal summation of cold pain and hot pain stimuli at the forearm	Aftersensations of cold pain and hot pain at the forehead and forearm positively correlated with DED discordance scores. Intensity ratings at threshold of cold pain and hot pain positively correlated with DED discordance scores.
Levitt et al (2021)[750]	United States	LASIK (n = 43)	MPT at the forehead and forearm Temporal summation and aftersensation intensity of mechanical pain at the forearm Conditioned pain modulation at the forearm	MPT at the forehead negatively correlated with baseline ocular pain. MPTS positively correlated with baseline DE symptoms. Aftersensations were associated with increased baseline DED symptoms and ocular pain. Presence of aftersensations pre-LASIK was a predictor for chronic DE symptoms at 6 mo post-LASIK.
Rodriguez et al (2022)[751]	United States	DED (n = 235)	Aftersensation intensity of cold pain and hot pain at the forearm	Intensity of ocular pain due to light was a predictor for the presence of aftersensations.

DED = dry eye disease, HTPS = hot pain temporal stimulation, LASIK = laser in situ keratomileusis, MPT = mechanical pain threshold, MPTS = mechanical pain temporal summation

contribution of central mechanisms in driving pain in this group.[752] fMRI studies have also explored the neural impact of various therapies used to treat ocular pain. In a study examining the impact of botulinum toxin A administered to the forehead, frontalis, procerus, and corrugators showed that in those individuals who reported reduced unpleasantness scores when viewing the light stimulus during the post-injection scan exhibited activation in the spinal trigeminal nucleus in response to light stimuli prior to injections and this was not evident in those who did not show an improved response post-injection.[755] This suggests that photophobia may be driven by different neural pathways, and that individuals with activity within the spinal trigeminal nucleus during light stimulation may be the ones more likely to respond to botulinum toxin. Use of the FL-41 spectacle tint to reduce photophobia led to significant reductions in light-

evoked blood oxygen level–dependent signals (used as an indirect measure of neural activity) in the bilateral primary and secondary somatosensory, bilateral insular, right temporal pole, precuneus, anterior cingulate cortex, and paracingulate cortices as well as bilateral cerebellar hemispheric lobule VI, although the responses in regions associated with pain processing were not eliminated completely.[756] These findings indicate that fMRI may play a role in predicting and monitoring responses to therapy and suggest that more studies are needed to examine central mechanisms to pain in various DED subtypes.

6.9. FUTURE DIRECTIONS AND CONCLUSIONS: Ocular pain and sensation are intimately associated with the structural anatomy of corneal nerves. This structure can be altered by trauma, surgery, DED, other systemic diseases, natural aging, and incomplete or improper neural regeneration. These alterations are pleomorphic but most commonly manifest as reduced nerve fiber density and increased nerve tortuosity. Aberrant corneal nerve anatomy generally correlates with altered sensation, increased ocular pain, and symptoms typical of DED. Functional abnormalities of corneal nerves are also implicated in altered ocular sensation and include changes in growth factor activity and increased expression of sodium channels. Ocular pain, in some individuals can be driven by generalized dysfunction of peripheral and central nervous systems, with studies supporting activation of the primary somatosensory, insular, and anterior mid-cingulate cortices in individuals with chronic ocular pain, which may be mitigated with certain therapies.

Research into this field is ongoing and future developments may include the elucidation of specific pathways controlling corneal nerve development and regeneration, exploration of neuroimmune crosstalk in DED, greater implementation of artificial intelligence networks in processing large bodies of data, more sophisticated assessments of peripheral and central nerve function, and targeted treatments to address dysfunction in an individual patient.

7. IATROGENIC

7.1. INTRODUCTION: Iatrogenic disease is an adverse clinical condition resulting from diagnosis or medical treatment performed by a health professional. It affects many patients worldwide in all fields of medicine, including ophthalmology.[262] In the eye, the ocular surface and the tear film are commonly the most affected. This assumption is related to the fact that this anterior interface represents the first target for the effect or penetration of the first line of treatment for most eye diseases and is involved directly or indirectly in the most common surgical and nonsurgical procedures for the eye. Among all ocular surface disorders, DED is the most prevalent.[757] In addition to topical medications and surgical and nonsurgical procedures, systemic drugs and the use of contact lenses are major causes of iatrogenic DED (Table 10).

TABLE 10. Classification of Iatrogenic DED (Reproduced From Gomes et al[262])

- I. Drug-induced
  - A. Topical
  - B. Systemic
- II. Contact lens-induced
- III. Ophthalmic surgery*
  - A. Refractive surgery
  - B. Keratoplasty (Penetrating, lamellar and endothelial)
  - C. Cataract surgery
  - D. Eyelid surgery
  - E. Other surgeries
    1. Conjunctival surgery
    2. Glaucoma surgery
    3. Vitreoretinal surgery
    4. Strabismus surgery
    5. Intrastromal corneal ring segment implantation
    6. Others
- IV. Nonsurgical ophthalmic procedures
  - A. Botulinum toxin
  - B. Crosslinking (CXL)
  - C. Cosmetic procedures
  - D. Others
- V. Nonophthalmic conditions
  - A. Graft-vs-host disease (GVHD)
  - B. B. Others

*Ophthalmic surgeries were extensively reviewed previously and are not included in this update.[261,262]

The TFOS DEWS II recognized the importance of this topic, and a specific report about Iatrogenic DED was included.[262] The impact of elective medications and procedures was also highlighted in the TFOS Workshop: A Lifestyle Epidemic: Ocular Surface Disease.[261] The current review presents an update since these reports of the most common iatrogenic causes of DED, including a summary of pathophysiology and recommendations for management (Table 11).

7.2. TOPICAL DRUG-INDUCED DED

7.2.1. Prevalence

As mentioned in the TFOS DEWS II report, evaluating DED caused by topical medications is challenging, as clinical trials often exclude patients with ocular surface diseases, which may lead to an underestimation of symptoms of DED.[262] Most epidemiologic studies evaluate the effect of antiglaucoma topical medications on the ocular surface. Given glaucoma and DED are 2 common conditions that can occur concurrently in the same individual, these results may be confounded. Furthermore, patients undergoing

treatment for glaucoma frequently show signs of deterioration in the ocular surface.[758]

In a multicenter study conducted across 4 European countries with 9658 patients, more than 40% of individuals treated for glaucoma, reported symptoms of DED, such as pain or discomfort during the application of eye drops, foreign body sensation, dry eye, and burning.[759] Furthermore, more than 20% of patients exhibited signs of blepharitis, conjunctival hyperemia, or keratitis. These findings were more prevalent in patients treated with preservative-containing eye drops. Similarly, a recent cross-sectional comparative study with 320 patients concluded that glaucoma patients are more affected by DED than nonglaucoma patients, showing a lower TBUT and greater corneal staining in eyes with glaucoma using multiple eyedrops and daily doses.[760] Another cross-sectional study with 101 patients undergoing antiglaucoma treatment detected signs and symptoms of DED in more than 50% of the patients and advanced changes in the ocular surface in 27% of the individuals examined.[761] Reduced values on the Schirmer test were observed in 61%, decreased TBUT in 78%, and staining of the ocular surface in 22% of the subjects.

In a German study involving 20,506 glaucoma patients from 900 centers, the prevalence of DED was higher in women (56.9%) than in men (45.7%), and this difference was more pronounced in patients aged ≥50 years. The prevalence increases with age and duration of glaucoma, and it occurs more frequently when 3 or more medications are used.[762] In another epidemiologic study including 4107 glaucomatous patients, the most frequently reported dry eye symptoms were excessive discomfort after applying antiglaucoma eye drops (43%), pressure behind the eyelids (40%), foreign body sensation (31%), dry eye sensation (23%), excessive reflex tearing (21%), and eyelid itching (18%) of cases. These symptoms were significantly more prevalent when using preservative-containing than preservative-free eye drops.[763,764]

7.2.2. Topical drugs contributing to DED

According to the TFOS DEWS II report, various topical medications and excipients have been implicated in DED (Table 11).[262] Of particular concern is the use of chronic medications, such as antiglaucoma, antiallergic, and anti-inflammatory eye drops. However, there needs to be more specific data on active compounds, as ophthalmic formulations are often evaluated with preservatives, making it difficult to isolate the impact of the medications, preservatives, and excipients. Also, common excipients in ophthalmic preparations, such as solutions, ointments, suspensions, and emulsions, may cause dry eye symptoms.[262] The chemical properties of the formulation, including isotonicity/hypotonicity and pH, can influence the tear film and local tolerance after application.[262]

Topical drug-induced DED has mainly been studied in individuals in long-term treatment for glaucoma and ocular hypertension.[262] Other than the presence of benzalkonium chloride, the main risk factors for iatrogenic DED in antihypertensive topical drug users are the treatment duration, higher intraocular pressure, and glaucoma severity.[765,766] The frequency of mild or greater DED symptoms tended to increase with an increasing number of antiglaucoma medications.[766] Interestingly, patients on brimonidine performed the worst. Patients on timolol reported pain induced by light, and those on latanoprost complained of stinging.[766]

TABLE 11. Topical Drugs Implicated in DED[262]

Agents used to treat glaucoma
Beta-blocking agents (eg, betaxolol, timolol)
Adrenergic agonist drugs (eg, apraclonidine, brimonidine)
Carbonic anhydrase inhibitors (eg, brinzolamide, dorzolamide)
Cholinergic agents (eg, pilocarpine)
Prostaglandin analogs (eg, bimatoprost, latanoprost, travoprost)
Agents used to treat allergies (eg, emedastine olopatadine)
Antiviral agents (eg, acyclovir, idoxuridine, trifluridine)
Decongestants (eg, naphazoline, tetryzoline)
Miotics (eg, dapiprazole, pilocarpine)
Mydriatics and cycloplegics (eg, cyclopentolate tropicamide)
Preservatives (eg, benzalkonium chloride)
Topical and local anesthetics (eg, proxymetacaine, tetracaine)
Topical ocular nonsteroidal anti-inflammatory drugs (eg, bromfenac, diclofenac, ketorolac, nepafenac)

7.2.3. Mechanism

Topical medications can affect the ocular surface through various mechanisms, causing allergic, toxic, and/or immune-inflammatory effects or through chemical interactions with different components of the ocular surface.[262] These effects can result from disruption of the lipid layer of the tear film because of the detergent properties of the compounds, reduced aqueous secretion, damage to the ocular surface epithelium, neurotoxic effects on the corneal nerves, and injury to the eyelids, including the skin or meibomian glands.[326,767-769]

Indirect effects may also arise if chronic inflammation induced by the topical medication stimulates the precursors of the keratinized envelope, leading to the entrapment of mucous cell contents and squamous metaplasia.[767] Keratinization of the eyelid margins can further worsen MGD.[326] Additionally, destruction and/or dysfunction of goblet cells, which are increasingly recognized as having an important role in immunomodulation of the ocular surface, can exacerbate chronic inflammation.[770]

As reported in the TFOS DEWS II report, distinguishing between spontaneous changes caused by ocular surface disease and medication-induced effects presents a clinical challenge.[262] Conjunctival allergic reactions caused by eye drop use may be indicative, but both conjunctival congestion and papillary conjunctivitis can occur with or without atopy. Additionally, delayed allergic reactions may arise, of-

ten mimicking blepharitis with mild inflammation.[262] Similarly, determining whether corneal staining is due to pre-existing or induced DED, or caused by toxic epithelial damage and corneal melting, such as that induced by overuse of anesthetics or nonsteroidal anti-inflammatory agents, can be complicated. With many factors to consider, the relationship between eye drop use and ocular inflammation, tear film instability, or ocular surface staining is often difficult to establish, especially when treatment is essential for severe or vision-threatening conditions. This is particularly true in glaucoma, where treatments are usually prolonged.

7.2.4. Role of preservatives and excipients

Preservatives used in topical ophthalmic medications are derived from different chemical families, such as mercury derivatives, alcohols, parabens, ethylenediaminetetraacetic acid, chlorhexidine, and quaternary ammonium. They act as a surfactant to solubilize ionic components, facilitating stabilization of medications, and inhibit microbial activity.[769] Benzalkonium chloride is a quaternary ammonium compound widely used as a preservative in eye drops that has its toxicity to the ocular surface well-documented. Benzalkonium chloride concentrations as low as 0.0001% can cause damage to corneal epithelial cells, with more severe toxic effects observed at higher concentrations, such as 0.01% and 0.2%.[769,771]

The impact of benzalkonium chloride on the ocular surface involves multiple mechanisms of action. One main effect is through mitochondrial dysfunction, which leads to cellular dysfunction and subsequent cell apoptosis.[376,767] Additionally, exposure increases the production of reactive oxygen species, inducing oxidative stress in corneal epithelial cells.[376,767] This stress directly contributes to the apoptosis and damage to ocular surface and trabecular cells.

Benzalkonium chloride can activate various inflammatory pathways, increasing the production of the inflammation marker HLA-DR and the expression of pro-inflammatory cytokines such as IL-6, IL-8, and CCL2, resulting in inflammation of the ocular surface and worsening of DED symptoms.[767-769] Furthermore, benzalkonium chloride causes DNA damage, contributing to cytotoxicity and reduced cell viability. It also affects gene expression in trabecular and ocular surface cells, affecting genes related to apoptosis and inflammation, such as Fas and caspase-3.[772,773]

Corneal neurotoxicity is another harmful effect of benzalkonium chloride, which decreases corneal nerve fiber density and reduces tear production while also causing nerve inflammation and degeneration.[767,774] These combined effects result in significant damage to ocular surface cells, exacerbating conditions such as DED.[775] Recent research suggests that benzalkonium chloride may affect both the mucin and lipid layers of the tear film, as damage to goblet cells and meibomian glands has already been documented, leading to tear film instability.[15,314,764]

Increased tear osmolarity also has been observed in patients using preserved eye drops compared with preservative-free topical medications.[258] Once the tear film loses its protective properties, the compromised tear film not only leads to symptoms of DED and corneal damage but can also spread cytotoxic inflammatory mediators across the ocular surface. Consequently, increased corneal epithelial permeability has been observed in DED with more significant deterioration when using artificial tears containing benzalkonium chloride compared with preservative-free drops.[776] Changes in the tear film may, therefore, initiate a series of biological alterations on the ocular surface, leading to subsequent neurogenic inflammation and further tear film impairment, creating a vicious cycle. New preservatives, such as Polyquad®; Purite® and SofZia®, have significantly reduced cytotoxic effects compared with benzalkonium chloride. However, their impact on DED patients needs to be further investigated.[258,261]

Preservative-free eye drops are associated with better ocular surface health and higher tolerability.[776] A systematic review of randomized clinical trials comparing beta-blockers with and without preservatives in patients with glaucoma or ocular hypertension found that, although the difference in intraocular pressure reduction was not clinically relevant, preservative-free eye drops performed better in TBUT and Schirmer tests.[776] Additionally, in patients with DED, preservative-free eye drops demonstrated a significant reduction in ocular inflammation symptoms and increased antioxidants in the tear film compared with preserved eye drops.[777] Another observational study in patients with DED who switched from preserved drops to preservative-free drops containing hyaluronate showed significant improvement in the OSDI and reduced frequency of superficial punctate keratitis.[778]

The use of preservative-free prostaglandins has also shown significant benefits for ocular surface health compared with preservative-containing versions. The PRAMOS study demonstrated that patients using preservative-free prostaglandin analogs had a lower prevalence of conjunctival hyperemia and corneal staining compared with those using preserved eye drops.[779] A prospective study showed that switching from preservative-containing prostaglandin-timolol fixed combinations to a preservative-free formulation resulted in a significant improvement in ocular surface disease symptoms, and a reduction in conjunctival hyperemia.[780] Another randomized controlled trial revealed that transitioning from preservative-containing glaucoma therapies to preservative-free formulations improved both ocular surface health and intraocular pressure control.[570] These findings are supported by studies indicating that the absence of preservatives, such as benzalkonium chloride, prevents chronic ocular surface toxicity, leading to improved treatment adherence and long-term efficacy.[781,782]

7.2.5. Recommendations for management

The first step is to investigate which medication is causing DED and try to stop its use. This subtraction can be challenging when discontinuing the treatment, which as it can present a risk to the eye's health. Sometimes, multiple drugs and components are involved, or adverse effects appear long after treatment initiation, making identification of which is causing DED even more difficult.[262] In some cases, eye drops may be necessary to treat dry eye symptoms, but adding preserved drops to eyes already suffering from dryness caused by other drops may be ineffective and worsen the condition. Once the responsible drug is identified, efforts should be made to discontinue using the preservative or medication. Considering that toxicity is dose-dependent, reducing the number of preserved eye drops can minimize adverse outcomes.[262] In glaucoma patients, more invasive and definitive options such as laser trabeculoplasty or surgery may replace or diminish the need for topical medication when the ocular surface and QoL are very compromised.[262]

Conceivably the adverse effects of toxic preservatives may reduce in years to come. There is a significant shift in first-line primary open angle glaucoma therapy in most countries, from topical therapies toward laser therapies.[783] Preservative-free therapies are increasingly available and there is good evidence for their ocular surface benefits. A significant issue related to preservative-free eye drops is their cost, as they are generally more expensive than preserved drops. This is primarily due to higher production costs and patent fees associated with preservative-free drops. Allocation of health care and out-of-pocket costs for patients vary with region and insurers or health system payers, and preservative-free alternatives may not be available in certain jurisdictions.

7.3. SYSTEMIC DRUG-INDUCED DED: Systemic medications can contribute to DED through different mechanisms, including reduction of tear production, disruption of nerve input and reflex secretion, inducing inflammatory responses in secretory glands, or directly irritating the ocular surface through their presence in tears.[258,784] Some systemic medications can exacerbate immune responses, causing further harm to the ocular surface and its innervation, decreasing sensitivity or increasing pain, which worsens DED symptoms.[258,784] This section reviews how some of the most common systemic medications can negatively affect the ocular surface.

7.3.3. Prevalence

At least one-fifth of the best-selling systemic drugs in the United States have been associated with the development of DED. In the elderly population, 62% of DED cases are associated with the use of systemic medications, including nonsteroidal anti-inflammatory drugs, diuretics, vasodilators, analgesics/antipyretics, antiulcer agents, sulfonylureas, cardiac glycosides, anxiolytics/benzodiazepines, antiinfectives, antidepressants/antipsychotics, hypotensive agents, and antihistamines.[785]

In a secondary analysis of the Dry Eye Assessment and Management Study to evaluate whether systemic medication use is associated with DED severity, the authors found that 160 (30%) of the 535 participants used medications for hypertension, 129 (24%) used statins, 118 (22%) used antidepressant medications, 117 (22%) used antihistamines, and 16 (3%) used systemic corticosteroids.[786] A multivariable analysis demonstrated that antihistamines and corticosteroids were associated with the highest OSDI score among these systemic medications. Users of seizure medications had a higher composite signs severity score compared with non-users. Compared with non-users, antihistamine, aspirin, and vitamin D_3 users had significantly worse average TBUT. Unexpectedly, MGD scores were worse in users of vitamin D_3, although this could be confounded if those users of vitamin D_3 had vitamin D deficiency. Users of diuretics had significantly better scores for MGD compared with non-users.[786]

In a Military Health System (MHS) beneficiaries database, newly diagnosed DED (≅40,000 beneficiaries) and prevalent DED (>285,000 beneficiaries) patients were compared with matched non-DED patients.[787] In both the newly diagnosed and prevalent DED patients, comorbidities were significantly higher in the DED vs non-DED groups. Systemic medication use was also significantly higher in the DED than in the non-DED groups. The most commonly prescribed medications were narcotic analgesics/strong pain killers, decongestants/vasoconstrictors, β-blockers, antidepressants, diuretics, and anxiolytics.[787] Risk factors are described in Section 3.6.4 above.

7.3.2. Medications and mechanisms

7.3.2.1. Tamsulosin. Tamsulosin, a medication that blocks alpha-1 receptors, is widely prescribed for treating benign prostatic hyperplasia, but is also used in managing ureteral stones, prostatitis, and female voiding dysfunction. Epidemiologic research suggests a link between DED and benign prostatic hyperplasia itself, as well as the medications used to treat it, including tamsulosin (see Section 2.5.1).

7.3.2.2. Antihistamines/anticholinergic drugs. A large class of systemic medications that lead to the signs and symptoms of DED are those with anticholinergic activity. The anticholinergic class covers a wide range of therapeutic drug categories, including antidepressants, antipsychotics or neuroleptics, antiparkinsonians, H_1 antihistamines, decongestants, and antispasmodics.[788]

Anticholinergic medications, such as those used for overactive bladder, have been linked to a reduction in TBUT and an increase in DED symptoms, including burning and foreign body sensation. Studies have shown that medications like solifenacin can significantly worsen dry eye symptoms and signs compared with placebo.[789,790]

Oral antihistamines and anticholinergic drugs are commonly used to treat allergies and rhinitis. These medications, especially in combinations like pseudoephedrine and cetirizine, can increase the sensation of dryness in the eyes and mouth.[791]

Results of the comparison of DED symptoms, measured by the OSDI, between users and non-users of medications showed that patients who used antihistamines had worse TBUT and OSDI scores compared with non-users. Anticholinergic drugs, such as antihistamines, contribute to DED by acting on the G-protein–coupled muscarinic receptors of the lacrimal gland acini and mucus-producing conjunctival goblet cells, affecting the production of the aqueous and mucin components of the tear film. Furthermore, functional cholinergic receptors have been identified in the epithelial cells of the meibomian glands, leading to decreased tear film stability. Thus, despite being commonly used to treat allergy-related eye symptoms, antihistamines confer a high anticholinergic burden, contributing to the development and worsening of DED signs and symptoms.[264,786,792]

7.3.2.3. Isotretinoin. 13-*cis*-Retinoic acid, or isotretinoin, is a vitamin A derivative widely used in treating moderate-severe acne because of its atrophic effects on the sebaceous glands. This medication acts as a pro-drug that, on conversion, induces apoptosis in various cell types, including those of the sebaceous and meibomian glands. Toxicity may manifest as blepharoconjunctivitis, presenting symptoms such as crusting at the eyelid margins and conjunctival redness, and is often associated with dry eyes, light sensitivity, and contact lens intolerance.[257,267,793]

Recent studies using standardized tools, such as the OSDI, indicate that DED symptoms are more commonly found in patients treated with isotretinoin. The drug can lead to glandular atrophy, ductal keratinization, and small fiber neuropathy, which may reduce corneal sensitivity.[794,795] Other studies have reported severe DED symptoms and decreased tear film stability among patients who start using isotretinoin, associated with reduced TBUT, lower Schirmer test value, and decreased central corneal thickness.[270,796] Additionally, a few studies show that even after discontinuation of the medication, these signs and symptoms can persist for months posttreatment.[270,796] The adverse effect of isotretinoin on meibomian gland lipid production is likely to be through suppression of the peroxisome proliferator–activated receptor γ pathway, which inhibits meibocyte differentiation and meibum characteristics.[797] Topical isotretinoin is also recognized as a risk factor for iatrogenic DED (see Section 3.6.4).[798]

7.3.2.4. Chloroquine and hydroxychloroquine. Hydroxychloroquine is a medication commonly used to treat Sjögren's disease and other rheumatologic diseases, primarily aimed at relieving symptoms related to joint pain and fatigue, with a well-recognized anti-inflammatory effect. Research on the ophthalmologic effects of hydroxychloroquine is highly controversial, as studies from the early 21st century have shown that the substance can be secreted into the tear film, potentially worsening DED symptoms.[799] On the other hand, other studies indicate that patients with Sjögren's disease did not experience significant clinical improvement in xerophthalmia.[800,801] Likewise, other studies have shown no improvement in symptoms, tear production, corneal staining, or inflammatory markers after up to 24 weeks of treatment.[800,802] The divergent results regarding its efficacy may stem from the common use of hydroxychloroquine in the treatment of autoimmune disorders, which are often associated with DED, highlighting the need for further studies on this medication.

7.3.2.5. Corticosteroids and nonsteroidal anti-inflammatory drugs. Research on the impact of nonsteroidal anti-inflammatory drugs on the ocular surface is less extensive, and more studies should be conducted. However, selective cyclooxygenase-2 inhibitors, such as celecoxib and rofecoxib, have been associated with ocular side effects, primarily conjunctivitis and blurred vision. These effects disappear within 72 hours of discontinuation. Although other ocular side effects are less common and lack clear evidence of a direct link, the consistent findings of blurred vision and conjunctivitis suggest a potential connection. The occurrence of these ocular symptoms should lead physicians to consider discontinuing the medication, as this action generally resolves the symptoms without long-term effects.[803]

Aspirin, a nonselective and irreversible inhibitor of cyclooxygenase-1 and -2, is expected to reduce the inflammatory component of DED, leading to an improvement in signs and symptoms.[804] However, the findings of previous studies on the effects of aspirin on DED are contradictory. For instance, a 2018 prospective cross-sectional study involving 106 individuals without a prior diagnosis of DED showed that aspirin users had lower tear osmolarity, increased TBUT, and lower OSDI scores.[804] However, no significant difference in corneal staining was found despite greater symptomatic relief. In line with these results, other older studies were unable to find an association between aspirin and a higher prevalence of DED.[805] This underscores the need for future studies to provide a clearer understanding of aspirin's effects on DED.

The use of corticosteroids is a common practice in medicine for treating inflammatory disorders and has been associated with worsening DED severity, affecting both signs and symptoms.[786] However, it is important to consider the route of administration, as it can significantly impact the efficacy and potential side effects of these drugs. For instance, when administered topically, corticosteroids are effective in treating DED.[806] Additionally, many users of systemic corticosteroids have underlying autoimmune conditions that are independently associated with DED, making it challenging to isolate whether the worsening of DED signs and symptoms is due to medication use or the progres-

sion of the underlying disease.[96] More prospective studies are needed to determine the effects of systemic corticosteroid use on DED.[807]

7.3.2.6. Antibiotics. Antibiotics are commonly used to treat various infections, but few studies have been conducted regarding their impact on the ocular surface. Antimicrobials, particularly tetracyclines and macrolides, are generally used in the treatment of MGD and have shown promising results.[808,809]

The correlation between DED and antimicrobial use is sometimes indirect, as antibiotics can cause Stevens-Johnson syndrome. Some studies have shown that antibiotics are notable risk factors for this condition, potentially leading to severe damage to the ocular surface.[810,811]

7.3.2.7. Antidepressants, anxiolytics, and mood stabilizers. The use of antidepressants and antipsychotics is strongly associated with an increased risk of DED. A systematic literature review suggests that both depression and antidepressant use independently contribute to the development of DED. Various clinical and population-based studies have linked the diagnosis and severity of depression to DED and its symptoms. Additionally, other extensive population studies have also found an association between antidepressant use and DED.[812]

Possible mechanisms for the effects of antidepressants on DED might be related to their anticholinergic properties. Another plausible theory is that antidepressants disrupt the corneal epithelial barrier and promote an inflammatory response on the ocular surface by increasing serotonin levels in the tears, which in turn induces an inflammatory response and apoptosis of corneal epithelial cells by activating NF-κB signaling.[813,814]

The prevalence of DED in patients using antipsychotics was higher than in the general population, with higher prevalence also noted in those on multiple medications compared with those on a single medication. Patients treated with clozapine (monotherapy) and those on a combination of clozapine and quetiapine (polytherapy) showed the highest prevalence of DED.[815]

Selective serotonin reuptake inhibitors can affect the ocular surface by impairing tear film stability.[816] Conversely, serotonin-norepinephrine reuptake inhibitors, effective in treating chronic pain syndromes, may help alleviate DED symptoms. Patients using these inhibitors often have lower OSDI scores, suggesting they may be safer for the ocular surface compared with other antidepressants. Thus, although DED is common among antidepressant users, it may be better managed with serotonin-norepinephrine reuptake inhibitors.[817]

Lithium, widely used for the maintenance and acute treatment of bipolar disorders, has a narrow therapeutic index and various side effects, making prescribing considerations challenging challenging. Although the systemic adverse effects of lithium are well-documented, its ocular surface impacts are less well understood, which may affect patient compliance. Ocular side effects associated with lithium use include exophthalmos, abnormal eye movements, ocular myasthenia gravis, papilledema, photophobia, and alterations in the tear film, which can lead to DED.[818]

Given the complexity of these interactions, comanagement of individuals with DED and medicated for mental health conditions is advisable.

7.3.2.8. Hormone replacement therapy. DED is common among postmenopausal women, with estrogen hormone imbalance being identified as a potential causative factor (see Section 3.6.6).[299,819] Hormone replacement therapy may be used to relieve menopausal symptoms, using either estrogen alone or in combination with progesterone or progestin. However, estrogen replacement therapy after menopause has been associated with an increased prevalence of DED (see Section 3.6.4).[15] Each additional 3 years of hormone replacement therapy resulted in a significant 15% increase in the occurrence of dry eye.[819] The same study showed that the onset of DED was linked to initiating estrogen therapy.[819] Conversely, the use of androgens, both topically and systemically, have been reported to improve the signs and symptoms of MGD and DED.[3,48,820,821]

7.4. CONTACT LENSES AND DED: The relationship between contact lens wear and DED is complex. Contact lens wear is recognized as a consistent risk factor for DED (see section 3.6.5). Ocular symptoms occur more commonly in contact lens wearers than in nonwearers, and many large epidemiologic studies have used ocular symptom reporting as a surrogate for DED.[148] Signs such as corneal staining and tear film instability, due to the partitioning of the tear film during wear occur.[822] Signs of meibomian gland alterations are also more common in contact lens wearers than in age-matched nonwearers.[823] Contact lens discomfort is characterized by episodic ocular symptoms of discomfort and dryness that resolve when the contact lens is removed, as distinct from those in DED[278]; however, this distinction is not consistently considered when reporting symptoms in contact lens wearers. A further complexity is that other complications of contact lens wear may also lead to very similar ocular symptom-reporting.[823] Contact lens wearers may also have existing, or subsequently develop, DED. This has led to the use of series of descriptors that are often used interchangeably, including contact lens discomfort, contact lens–induced dry eye and contact lens–associated dry eye. With these caveats, this section will endeavor to summarize the evidence for prevalence of, mechanisms underlying, and remedial strategies for DED associated with contact lens wear.

7.4.1. Prevalence of DED in contact lens wear

The prevalence of symptoms of DED in soft contact lens wearers in a series of recent prospective and retrospective

TABLE 12. Effects of Contact Lens Wear on the Tear Film and Ocular Surface

Biophysical Tear Film Alterations	Effect
Division of the tear film into pre- and post-lens layers	
Tear meniscus volume	Reduced
Lipid layer spreading	Reduced
Tear stability	Reduced
Structural ocular surface alterations	
Conjunctival goblet cell density	Reduced
MG expressibility ↓	Reduced
MG obstruction and atrophy	Increased
Basal corneal nerve density or nerve tortuosity	Reduced
Ocular surface sensitivity	Corneal sensitivity unchanged in SCL use; altered sensory processing; conjunctival sensitivity increased Corneal sensitivity reduced in OK use
Ocular surface friction and eye lid wiper epitheliopathy	Increased
Biochemical tear film alterations	
Tear cholesterol	Increased
Malondialdehyde and 4-hydroxy 2-nonenal	Increased
Beta-2 microglobulin	Reduced
Proline rich protein 4	Reduced
Lacritin	Reduced
Lipocalin 1D1	Reduced
Secretory IgA	Effects equivocal, may depend on duration of wear and wear modality
Albumin	Increased
Deleted in Malignant Brain Tumors-1	Increased
Prolactin inducible protein	Increased
MUC5AC	Reduced
Inflammatory tear film/ocular surface changes	
Epithelial immune cells	Increased at ocular surface in SCL wear, increased antigen capture capacity. Density reduced at central cornea in OK
Tear cytokines IL-7, 8,13,15	Increased

MG = meibomian glands, OK = orthokeratology, SCL = soft contact lens.

studies in select population groups ranged between 31% and 77%.[824-826] A clinical diagnosis of DED by an eyecare professional was made in 24% of office workers in a Chinese sample[826] and in 14% of University students wearing contact lenses in Thailand.[827] The frequency of DED in scleral lens wearers has been more difficult to determine. These are mostly used for medical and therapeutic indications, and an estimate of 56% of scleral lens wearers had severe DED symptoms based on OSDI.[828,829] There is a lack of large-scale epidemiologic studies on the prevalence of DED associated with orthokeratology lenses. In a small retrospective study of contact lens complications conducted at a tertiary hospital in China, 24% of orthokeratology lens wearers presented with DED.[830] Understanding the pathogenesis of DED in contact lens wearers, and provision of mechanism-oriented treatment, are important.

7.4.2. Mechanism

Contact lens wear causes a variety of biophysical and biochemical changes in the tear film, as well as alterations in the ocular surface structure and function that are summarized in Table 12. These mechanisms collectively lead to reduced tear film stability, increased tear evaporation, and ocular surface damage,[822,823] and likely contribute to DED in contact lens wearers.

Rigid and soft contact lens wear increase tear film evaporation,[831] although they each interact differently with the ocular surface. Mechanical interactions are different between rigid and soft contact lenses because of material and fitting characteristics.[823,832,833] Upper eyelid wiper epitheliopathy is more common in rigid than soft lens wear.[834] There is a higher frequency of incomplete blinking,[835] leading to uneven tear distribution and exacerbation of dry eye symptoms. Corneal staining in the 3- to 9-o'clock positions is common among rigid lens wearers,[836,837] attributed to thinning of the tear film[838] adjacent to the lens edge, whereas soft contact lens wearers more often exhibit inferior arcuate cornea staining.[837]

It is conceivable that mechanisms in contemporary scleral lens use might resemble those observed with rigid corneal contact lenses, including friction between scleral lenses and ocular tissues,[832] increased mechanical stimulation of the ocular surface,[831] and tear film instability.[839]

Orthokeratology lenses worn overnight share some mechanisms for DED with soft and rigid contact lenses, but the unique mechanism of these lenses is related to their reverse geometry design,[830] which not only alters corneal shape but also significantly affects the density and distribution of corneal nerve fibers,[840,841] which may not fully recover shortly after cessation of wear.[842,843]

The evidence for changes in corneal sensitivity underpinning subjective sensation report in contact lens wear is limited. Contemporary rigid corneal and soft lenses do not appear to alter corneal sensitivity, although a reduction in central corneal mechanical sensitivity is observed in orthokeratology lens use.[842,844] Reduced corneal sensitivity may reduce tear secretion and blink frequency, thereby exacerbating symptoms of DED.[843] There is a substantial augmentation in tear inflammatory mediators in overnight orthokeratology lens wear, such as IL-17A, IL-6, and prostaglandin E2.[840] The TFOS Contact Lens Discomfort Workshop neurobiology report described the multifactorial effects of contact lens wear, include mechanical, cooling, drying, change in osmolarity, and chemical impacts on ocular surface neurosensory processes.[845] Altered sensory processing of cooling stimuli delivered to the cornea has recently been reported in symptomatic soft contact lens wearers suggesting nerve sensitization or maladaptation in the absence of sensitivity changes,[846] which is intriguing but the causality for DED has not been demonstrated.

Subclinical inflammation at the ocular surface is reported in several modalities of contact lens wear, evidenced by recruitment and activation of inflammatory cells to the cornea, conjunctiva, and eyelid margin and the presence of mediators in tears[822] for review). In naïve wearers, epithelial immune cells are recruited to the central and peripheral cornea within 2 hours of soft contact lens wear and morphologic changes suggest enhanced antigen capture capacity.[847] Increased density of inflammatory cells is seen at the eyelid margin in reusable hydrogel and silicone hydrogel wearers but not in daily disposable wear.[848] The link between subclinical inflammation and DED in contact lens wearers, however, has not been confirmed.

7.4.3. Recommendations for management of DED in contact lens wearers

Therapeutic and device-related management of DED in contact lens wearers broadly follows that described in the TFOS DEWS III Management and Therapy report[2] with caveats about the use of preserved medications during contact lens wear and noting that many products are not specifically registered for use in contact lens wearers. Given the potential interdependencies between DED, MGD, and contact lens discomfort, nonspecific strategies might include switching to daily disposable contact lenses, attention to the lens fitting relationship, avoiding preserved care systems with reusable contact lenses, improving blink completeness, attention to environmental triggers, ruling out ocular or systemic comorbidities, and recommending the use of unpreserved lubricants, with or without lipid additives.[823,849,850] Discontinuing from lens wear or reducing wear time may be successful[831] unless there is underlying DED.

In contact lens wearers with MGD and consequential evaporative DED, there is evidence for the benefits of treatments including IPL,[851] microblepharoexfoliation exfoliation,[852] or thermal pulsation,[853] including improved TBUT, and symptoms of DED (see TFOS DEWS III: Management and Therapy report). Low-level light therapy or photobiomodulation therapy has been used in the treatment of MGD and evaporative DED,[2] but there is no high-level evidence for benefit in contact lens wearers, although an observational study without a control group exists in wearers with discomfort.[854] Although there are no pharmacologic treatments approved for MGD in contact lens wearers, a phase 1[855] and phase 2[856] clinical trial both demonstrated benefit in improving comfortable wear time and meibomian gland signs in a population of symptomatic wearers with MGD, using a topical selenium sulfide treatment.

7.5. PROCEDURES

7.5.1. Botulinum toxin

Paradoxically, botulinum toxin A has been used in treating DED.[857] BTX-A is injected into the periorbital area, including the medial portion of the orbicularis muscle and not solely into the lateral canthal region, for the treatment of existing DED. The mechanism of action of botulinum toxin on lacrimal drainage in such instances has been suggested to be due to a paralysis of the orbicularis oculi muscle around the canaliculi with a decreased compression as well as weakness of apposition of the puncta during blinking.[858,859]

7.5.1.1. Mechanism. Dry eye due to BTX-A injection for treatment of blepharospasm or after blepharoplasty and periorbital surgery has been previously reported. The mechanisms by which DED is induced are multifactorial.

7.5.1.2. Direct inhibition of tear secretion. BTX-A blocks acetylcholine release within the lacrimal gland, disrupts neuromuscular junctions, and inhibits parasympathetic nerves, thereby suppressing both basal and reflex tear secretion.[860] This leads to a significant decrease in TBUT and Schirmer test results.[858,859,861]

7.5.1.3. Impact on meibomian gland function. BTX-A can also diminish lipid production by affecting neuromuscular transmission around the muscles of the meibomian glands.[862] Additionally, botulinum toxin-induced paralysis of the preseptal orbicularis oculi and Riolan's muscle weakens eyelid closure and reduces meibum secretion, thereby decreasing lipid layer thickness and tear film stability.[863] However, further studies are needed to examine the long-term effects on the meibomian glands.

7.5.1.4. Regulation of inflammatory responses. BTX-A injections may trigger inflammatory responses at the ocular surface and in the lacrimal glands, such as increased release of cytokines (eg, TNF-α and IL-1β),[864] which may further impact tear stability and tear secretion.

7.5.1.5. Chemodenervation of orbicularis oculi. BTX-A induces chemodenervation of the orbicularis oculi muscle, which can lead to poor blinking, lagophthalmos, and ectropion that may result in corneal dryness with symptoms of irritation, foreign body sensation, and epiphora. Epiphora is likely due to a combination of factors including DED causing reflex tearing, hypotonicity of the medial pretarsal fibers causing decreased outflow of tears, and malposition of the eyelids, causing impaired retention of tears.[860]

7.5.1.6. Recommendations for management.

1. For patients experiencing DED due to BTX-A treatment for other conditions, individualized management plans should be developed based on specific symptoms and tear volume.[860]
2. Patients undergoing BTX-A treatment should be regularly monitored for tear secretion levels, ocular surface condition, inflammatory responses, and changes in orbicularis muscle function. Patients should be educated about symptoms of DED and management, including maintaining good eye lid hygiene practices and avoiding excessive use of electronic screens.
3. Grading eyelid muscle tone and eyelid laxity and documenting any eye symptoms or findings prior to injection is of importance. It is recommended that patients who are to undergo BTX-A injections should undergo the snap-back test that measures muscle tone and the lower eyelid distraction test that measures eyelid laxity, resulting primarily from the stretching of canthal ligaments. Patients with severe test results are more prone to dry eye development after injection of the toxin into the lateral canthal region. Patients should be asked about any early symptoms including eye irritation, foreign body sensation, or tearing that were not present previously and/or by noticing any change in snapback and distraction tests during the treatment course. In the presence of positive test findings and dry eye symptomatology, one should temporarily discontinue repeated BTX-A injections into the lateral canthal rhytids.[865]

7.5.2. Corneal collagen crosslinking

7.5.2.1. Mechanism. Corneal collagen cross-linking (CXL) is a treatment that uses ultraviolet A light and riboflavin (vitamin B_2) as a photosensitizer to strengthen the cornea. This technique reinforces the chemical bonds within the cornea, thereby halting or delaying the progression of corneal ectatic diseases such as keratoconus.[866,867] Both epithelium-on and epithelium-off CXL have been reported to have a positive effect on DED and may improve tear film homeostasis and reduce DED symptoms in patients with keratoconus.[868]

Paradoxically, CXL can also induce DED symptoms through multiple mechanisms.

First, epithelial removal and delayed healing may induce DED.[869-871] This delay can affect the uniform distribution of tears and the corneal defences,[869,872,873] by compromising tear film stability[688,874] and making the ocular surface more prone to DED symptoms.

CXL may also reduce corneal nerve density,[875] impairing corneal sensory nerve function and decreasing corneal sensitivity, which in turn affects the tear secretion.[875] However, studies indicate that in most patients subbasal nerve regeneration occurs within 2-3 months after CXL,[875] reaching preoperative levels in 6-12 months.[876,877]

Additionally, although CXL does not directly alter meibomian gland morphology,[878] the effects on corneal sensory nerves,[878] the use of eyelid speculums during surgery, post-CXL inflammatory responses with elevation of IL-6, and medications used postsurgery[878] can indirectly affect gland function and secretion quality.

7.5.2.2. Recommendations for management. To effectively manage DED that may arise after CXL, a comprehensive approach is essential. First, bandage contact lenses, because of their material and characteristics, help protect exposed nerve endings and the corneal epithelium, reducing frictional damage from blinking and thereby alleviating dry eye symptoms.[879]

Second, during the postoperative phase, the use of corneal protectants, tear substitutes, and tear secretagogues is important. These medications should be selected based on their ability to replace the glycocalyx lost from the corneal epithelial cells, promote the restoration of the mucin gel on the epithelial cell surface, enhance the adhesion of the tear film to the corneal epithelium, and aid in the recovery of the lipid layer of the precorneal tear film.[879,880]

Lastly, advancements in modern CXL technology also help reduce the risk of postoperative complications. These improvements include transepithelial cross-linking, localized and personalized techniques, and accelerated procedures, which aim to enhance surgical outcomes and minimize corneal manipulation time.[874,881-883]

7.5.3. Other procedures

7.5.3.1. Eye cosmetics and beauty treatments. The use of eye cosmetic products and procedures represent a lifestyle challenge that may exacerbate or promote the development of ocular surface and adnexal disease. This topic has been recently explored in detail.[259]

7.6. NONOPHTHALMIC CONDITIONS:

7.6.1. Radiotherapy

7.6.1.1 Mechanism. DED is associated with radiation therapy for head and neck cancer,[884,885] temporal tumors, breast cancer, and Graves eye disease. This condition often results from direct damage to tissues around the eye.[885-887] The incidence of radiation-related dry eye depends on the type

of radiation therapy, tumor location,[888,889] and radiation dose.[885,890,891]

Mechanisms of radiation-induced dry eye are multifactorial:

1. Damage to the Lacrimal Glands and Tear Drainage System: Radiation therapy can cause inflammation of the tear ducts or obstruction of tear duct openings,[892,893] significantly reducing lacrimal gland function and leading to DED symptoms. Ultrastructural analysis of postradiation lacrimal glands reveals extensive tissue damage, including membrane rupture, loose cell junctions, and nuclear fragmentation.[894] Additionally, the expression of markers such as lysozyme, S-100, and CD117 is reduced in the lacrimal tissues.[894]
2. Corneal and Conjunctival Damage: Radiation therapy can cause direct damage to corneal epithelial cells and loss of conjunctival goblet cells,[890] which can destabilize the tear film. Additionally, inadequate conjunctival closure during brachytherapy can lead to conjunctival and scleral necrosis, further affecting ocular surface health and tear film stability.[895]
3. Eyelid Damage: The meibomian glands exhibit acute inflammatory responses to radiation therapy characterized by cystic dilatation of ducts containing keratin and atrophic decrease of the glands and ducts or their complete loss.[896]

7.6.1.2. Recommendations for management. The management of radiation-related DED can be divided into 3 key approaches:

1. Preventive measures to minimize the risk such as limiting the radiation dose to the lacrimal glands, ideally keeping the dose below 30 Gy[890,891,897,898]
2. Proper patient positioning, eye shielding, and adequate patient immobilization during radiotherapy sessions to reduce radiation-related toxicity[888]
3. Therapeutic interventions for DED[2]

Long-term management is essential for maintaining good ocular health and QoL. Regular ophthalmologic examinations and assessments to monitor changes in DED allow for timely adjustments in treatment plans and interventions. A multidisciplinary team, including ophthalmologists and oncologists, can provide comprehensive care and personalized treatment plans, thereby maximizing QoL.

7.6.2. Bariatric surgery

This topic has been reviewed in detail in 2 recent reports.[261,283]

7.6.3. Stem cell or bone marrow transplant

7.6.3.1. Mechanism. GVHD is a common complication following human leukocyte antigen (HLA)–matched allogeneic hematopoietic stem cell transplantation. GVHD can affect various organs, including the skin, gastrointestinal tract, liver, lungs, and eyes, with dry eye being the most common manifestation of ocular GVHD.[899,900] Ocular GVHD is seen in 60% to 90% of transplant recipients, primarily manifesting as secondary inflammation and fibrosis of the lacrimal and meibomian glands.[901,902] The mechanisms of DED in GVHD include the following aspects:

1. Lacrimal Gland Fibrosis: Fibrosis of the lacrimal gland is a major cause of GVHD-related dry eye. Studies have shown the presence of activated $CD34^+$ fibroblasts in the lacrimal glands of GVHD patients, with T-cell infiltration around the ducts, thickening of blood vessels, and duct basement membranes correlating with the severity of fibrosis.[903,904]
2. Renin-Angiotensin-Aldosterone: Angiotensin 1 type 1 receptor antagonists, such as valsartan, can reduce fibrosis, decrease inflammatory cell density, and increase tear secretion.[905] Angiotensin-mediated fibroblast activation and upregulation of TGF-β expression via the angiotensin 1 type 1 receptor are the primary mechanisms of fibrosis.[906]
3. Immune-Mediated Inflammation: In GVHD patients, increased density of T cells and dendritic cells[907] in the lacrimal glands, conjunctiva, and cornea contribute to inflammation and fibrotic responses.[908]
4. Cellular Senescence: Chemotherapy and radiotherapy-induced DNA damage and the inflammatory environment activate senescence-related molecular pathways.[909] Stress-induced cellular senescence promotes the secretion of various cytokines (eg, IL-1β, IL-6, IL-8), forming the "senescence-associated secretory phenotype," which exacerbates inflammation.[910]

GVHD patients often exhibit significant abnormalities in the meibomian glands,[911] leading to alterations in the tear film lipid layer and increased tear evaporation.[912] Corneal and conjunctival changes commonly include punctate keratopathy, filamentary keratitis, and epithelial defects,[913] accompanied by decreased corneal nerve fiber density, increased dendritic cell density, conjunctival epithelial squamous metaplasia, and reduced goblet cell density. These changes collectively contribute to the onset and progression of DED.[907,912]

7.6.3.2. Recommendations for management. GVHD-related DED can be potentially managed through a combination of preventive and treatment strategies. Preventive measures primarily include ensuring high-quality HLA matching between the donor and recipient, and the use of immunosuppressive agents such as cyclosporine, tacrolimus, and methotrexate to inhibit donor T cells.[914,915] Preservative-free and phosphate-free eye drops are recommended for tear retention and lubrication.[916]

Topical steroids,[917,918] and systemic oral immunosuppressants such as cyclosporine[919] are recommended to control inflammation. Biological tear substitutes like autologous serum eye drops provide significant anti-inflammatory and nutritional benefits.[920-922] In severe cases of DED, tem-

porary tarsorrhaphy may be necessary to limit ocular surface exposure, and surgical interventions may be required to treat cicatricial eyelid diseases.[923] These comprehensive measures aim to maximize symptom relief and enhance QoL for patients suffering from GVHD-associated DED.[2]

7.7. FUTURE DIRECTIONS AND CONCLUSIONS: Iatrogenic causes underpin a significant proportion of DED cases and may arise from topical and systemic medications and a range of ophthalmic and nonophthalmic surgeries. Lifestyle choices such as contact lens wear, cosmetics, and cosmetic procedures also contribute to iatrogenic DED. Improved understanding of the range of causes and how these contribute to different subtypes of DED and risk factors for more severe disease is likely to help reduce their impact. Evaluating and managing underlying DED pre- and postintervention will also better support these patients. A challenge in iatrogenic disease is understanding the impact of an intervention compared with progression of the primary condition. This report has also identified the need to better disaggregate the effects of topical medications from their preservatives and other excipients. In DED following use of systemic medications, there is greater certainty surrounding the adverse effects of isotretinoin in DED and the persistence of effect once the medication ceases. Although depression and antidepressants broadly are independent risk factors for DED, it is recognized that some classes, such as serotonin-norepinephrine re-uptake inhibitors may improve ocular symptoms and there should be further exploration of the impact of systemic corticosteroids on the ocular surface. The natural history of DED associated with lifestyle choices such as contact lens wear and particularly orthokeratology remains unclear.

8. CLINICAL TRIALS DESIGN

8.1. INTRODUCTION: The major international markets for medicines are the United States, European Union, and Japan. These 3 regions have consistent regulatory frameworks for approval of medicines following the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use guidelines.[924] There are some variations in evidentiary standards between these jurisdictions in clinical trials designs and features. As an example, for new DED treatments, the European Union requires a 6-month time frame for primary efficacy studies, rather than the 3 months required by the FDA. In addition, the European Union typically requires standard of care as one of the trial arms, in addition to vehicle as control, while using artificial tears as standard of care. Artificial tears are considered devices in the European Union and in the United States but not in Japan. As far as possible, this report will provide examples of treatments approved from these 3 regions.

The development of novel treatments for DED accelerated in the United States and European Union following release of both the TFOS DEWS II report in July 2017[204] and the FDA draft guidance for industry in December 2020.[925] Four new treatments for DED were approved between August 2018 and June 2024; three in the United States and one in Japan. Eight treatments were approved before July 2017, 2 in the United States, and 6 in Japan.[926-928] Although prior TFOS DEWS reports focused on challenges hampering the design and success of trials in DED,[929] this report will focus on how recommendations from the TFOS DEWS II report translated into innovations in trial design, an independent analysis of trial failures released in 2012, across 10 therapeutic areas,[197] and how flexibility in application of the FDA draft guidance have improved success rates for bringing novel treatments to patients. Innovations in trial design include better matching of treatment mechanism of action to patient subpopulations and more stringent control on concomitant treatments and sources of trial error.[927,928,930-934]

These innovations in trial design require a deeper understanding by both researchers and clinicians of their potential impact to support both continued approvals and to promote the continued introduction of novel treatments for patient subpopulations who may be underserved by currently approved treatments. The second point could impact the clinical utility of new market entrants as the potential consequence of designing approval programs for novel DED treatments that exclude patients with signs of DED that do not align with a drug/device mechanism of action may limit efficacy across the spectrum of patients who present with DED. For example, limiting the inclusion of patients with MGD in drug trials supporting currently approved products, knowing MGD is present in 51.3% to 70.3% of patients with DED,[345,346] may partially explain the apparent dissociation between the regulatory approval success of compounds in enriched populations and the subsequent high dropout/discontinuation rates for treatments in clinical practice.[929,935,936] Peer-reviewed literature reports discontinuation rates up to 40% within the first year of a filled prescription whereas certain ophthalmologic practices can see estimated discontinuation rates greater than 60% with some antiinflammatory treatments.[935] Newer treatments and associated clinical trial designs with enriched populations of patients will likely only expand as treatments tailored to newly identified subpopulations (eg, Ocular Neuropathic Pain) continue to be identified over time.[204,929]

In addition to searching the published, peer-reviewed literature for information on existing methodology, detailed reviews on development programs and outcomes that are publicly available were used as a primary source of information for this report. The FDA website (accessdata.fda.gov) and ClinicalTrials.gov which is a publicly available registry of clinical trials maintained by the US National Library of Medicine both list publicly available information, includ-

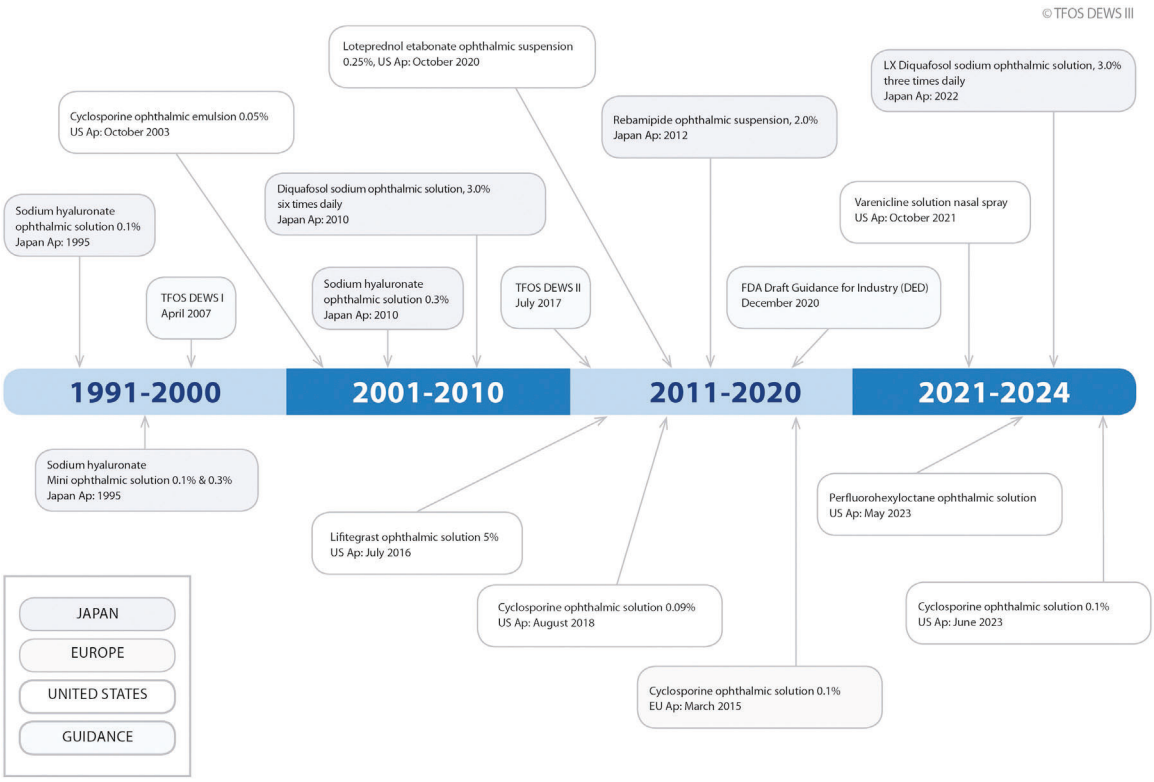

FIGURE 4. Treatments for dry eye disease (DED).

ing clinical protocols, statistical reports, and outcomes. The European Medicines Agency (EMA) also issues assessment reports on products that were accepted or not approved (withdrawal assessment report) for use. These can be found at https://www.ema.europa.eu/en/documents. The Pharmaceutical and Medical Device Agency (PMDA) publishes review reports on approved drugs and other products in English (https://www.pmda.go.jp/english/review-services/reviews/approved-information/0001.html). Beyond the major markets, global regulatory agencies generally publish assessment reports on products submitted for their review that are also readily available online (eg, the Australian Therapeutic Good Administration [TGA] published a report on a lifitegrast ophthalmic preparation: https://www.tga.gov.au/sites/default/files/auspar-lifitegrast-191107.pdf).

8.2. APPROVALS FOR DED: The recommendations for trial design from TFOS were first published in the TFOS DEWS report[937] and were followed by the TFOS DEWS II report in 2017.[929] A cyclosporine ophthalmic emulsion 0.05% was approved in 2003 in the United States and is indicated for an increase in tear production in patients whose tear production is presumed to be suppressed because of ocular inflammation associated with keratoconjunctivitis sicca. Sodium hyaluronate 0.1% and Mini 0.1% and 0.3% were approved in 1995 in Japan for conjunctival epithelial disorders including sicca syndrome. The TFOS DEWS report described the approval period followed by interest in the development of additional antiinflammatory and immunomodulatory drugs for the treatment of DED. This time was marked by the failure of numerous follow-on compounds and exploration of how the better control of trial design, end points, and operational excellence could potentially lead to a greater success rate in DED trials.

Lifitegrast ophthalmic solution (5%) was approved in the United States in 2016 and is indicated for treating the signs and symptoms of DED in adults older than 17 years. In Japan, a sodium hyaluronate ophthalmic solution (0.3%), diquafosol ophthalmic solution (3%), and rebamipide ophthalmic suspension (2%) were approved between 2010 and 2012 for conjunctival epithelial disorders including sicca syndrome (dry eye) and subsequently just dry eye. The TFOS DEWS II report described the approval followed by interest in exploration of how matching the mechanism of action of a proposed therapy to a potentially responsive population in which the treatment is likely to demonstrate efficacy; the inclusion of biomarkers and/or surrogate markers of disease; and recognition of strong placebo/vehicle effects that if properly controlled could potentially lead to greater success rates in DED trials.[929] These concepts were captured in the TFOS DEWS II report, trials for novel compounds, and the FDA's guidance document on trial design for DED, following which a period of successive approvals

for additional DED treatments was observed (Figure 4 and Table 13).

Globally, the availability of treatments for DED targeted at a subpopulation of patients who present predominantly with signs of inflammation or its impact (eg, hyperemia, corneal staining, and conjunctival staining) and aqueous-deficient DED (eg, decreased tear production) speaks to a potential pathway for approval of additional products.[938-942]

At the time of writing, compounds with immunomodulatory, antiinflammatory, tear stimulatory, and tear conservation mechanisms of action have all targeted DED patients. Approvals for these products in the major markets are generally followed by approvals in significant rest of world markets. However, a number of these compounds have failed repeatedly in clinical trials in other countries.

8.3. DEFINING A PATHWAY TOWARD APPROVAL:

8.3.1. Solving industry-wide failure rates

Pfizer completed a review of its portfolio and determined that in the 2010s the pharmaceutical industry, in general, faced declining R&D success rates that reached a low in 2016 when only 10% of phase 2 trials succeeded whereas phase 3 success rates were only 30%.[197,943] By 2020, this had improved to a phase 2 trial success rate of 53% and a phase 3 trial success rate of 80%.[197]

Improvements in phase II success rates were enabled by better understanding of disease heterogeneity, which led to better selection of patients for clinical trials and longitudinal sampling of mechanistic biomarkers (eg, advanced imaging techniques and blood-borne biopsies).[197,943]

Three fundamental elements were identified to significantly improve trial success rates: (1) exposure at the site of action (pillar 1); (2) binding to the pharmacologic target (pillar 2); and (3) expression of pharmacologic activity from the site of action (pillar 3). Successful trials could achieve all 3 pillars, achieve pillars 1 and 3, or achieve pillars 2 and 3. This was often combined with the inclusion of biomarkers and/or surrogate markers of disease to achieve an early proof of mechanism and/or early signal of efficacy.[197,943]

8.3.2. Overcoming disease heterogeneity with targeted subpopulations

Ocular surface diseases including evaporative DED,[929,937,944] aqueous-deficient DED,[929,937,944] MGD,[236,945] and contact lens discomfort (CLD)[278,946] represent ocular surface disease heterogeneity that has been accounted for in more recent DED trial designs by matching the mechanism of action of a proposed therapy to a potentially responsive population in which the treatment is likely to demonstrate efficacy.[82,927,930-932,938,939,941,942,947-955]

Trials targeting patients presenting with aqueous-deficient DED and inflammation have consistently linked decreased tear production with more severe disease that disrupts the ocular surface, exemplified by ocular surface staining, through the concept of the lacrimal functional unit.[927,930-933,938,939,941,942,947,948,955] The lacrimal functional unit is defined as an integrated system comprising the lacrimal glands, ocular surface (cornea, conjunctiva and meibomian glands), lids, and the sensory and motor nerves that connect them. These trials have inclusion criteria and associated primary end points focusing on increased ocular surface staining, decreased tear production, more severe symptoms (eg, VAS score $\geq$40 at screening and baseline and an OSDI score of $\geq$23), and inflammation.[927,928,930-934,938,939,941,942,947,948,950,952,953,955-957] As a group, many of these trials also excluded patients with signs of MGD, evaporative DED, or CLD.[927,931-933,938-942,947,948]

The products targeting patients presenting with aqueous-deficient DED and inflammation can be broken down into 2 main categories based on their mechanism of action. The first group of compounds includes immunomodulatory and antiinflammatory agents,[927,931-933,938-942,947,948] and the second can be broadly categorized as tear stimulatory or conservation agents for the various layers of the tear film (ie, lipid layer, aqueous layer, and mucin layer) (see Table 13).[928,930,934,949,950,952,953,955-957] Studies involving 3 of these compounds further restricted their inclusion/exclusion criteria and primary end points to closely match their products' proposed mechanism of action.[930,931,949,950,952,953,955]

Studies using a semifluorinated alkane to target the lipid layer included patients presenting with aqueous-deficient DED, inflammation, and MGD defined as a total MGD score $\geq$3 (range 0-15) at screening and baseline (secretion of 5 central glands on the lower eyelid was evaluated, and each was scored from 0 to 3: 0 = normal; 1 = thick/yellow, whitish, particulate; 2 = paste; 3 = none/occluded) and a TBUT $\leq$5 seconds at screening and baseline. Thus, the inclusion criteria were tailored toward the mechanism of action of the compound even though the primary end points were consistent with patients presenting with aqueous-deficient DED and inflammation.[949,950,952,953]

Studies involving a cholinergic agonist additionally targeted patients presenting with a baseline Schirmer score response to cotton swab nasal stimulation of at least 7 mm greater in the same eye that qualified as aqueous-deficient DED. Thus, patients with a marked response to mechanical activation of the trigeminal parasympathetic pathway in the nose were subsequently tested to determine if nicotinic acetylcholine (nACh) receptor activation of the same pathway results in increased basal tear production.[930,955]

A new topical corticosteroid additionally targeted patients presenting with bulbar conjunctival hyperemia at screening and day 1 of $\geq$2 as assessed using the Cornea and Contact Lens Research Unit Grading Scale. Corticosteroids cause adrenergically mediated vasoconstriction and noncompetitive antagonism of vasodilation due to prostaglandin E and bradykinin.[958] Thus, targeting patients presenting with aqueous-deficient DED, inflammation, and hyperemia would be consistent with a mechanism of action for a corticosteroid to reduce dilation of conjunctival blood

TABLE 13. Approved Products in Major Markets

Generic Name	Brand Name	Year Approved	Country	MOA	Approved Indication
Cyclosporine ophthalmic emulsion, 0.05%	RESTASIS	2003	USA	Calcineurin inhibitor immunosuppressant	Indicated to increase tear production in patients whose tear production is presumed to be suppressed because of ocular inflammation associated with keratoconjunctivitis sicca.
Lifitegrast ophthalmic solution, 5%	XIIDRA	2016	USA	LFA-1 antagonist	Indicated for the treatment of the signs and symptoms of DED
Cyclosporine ophthalmic solution, 0.09%	CEQUA	2018	USA	Calcineurin inhibitor immunosuppressant	Indicated to increase tear production in patients with keratoconjunctivitis sicca (dry eye)
Loteprednol etabonate ophthalmic suspension, 0.25%	EYSUVIS	2020	USA	Corticosteroid	Indicated for the short-term (up to 2 wk) treatment of the signs and symptoms of DED
Varenicline solution, 0.03 mg nasal spray	TYRVAYA	2021	USA	Nicotinic acetylcholine receptor agonist	Indicated for the treatment of the signs and symptoms of DED
Perfluorohexyloctane ophthalmic solution	MIEBO	2023	USA	Semifluorinated alkane	Indicated for the treatment of the signs and symptoms of DED
Cyclosporine ophthalmic solution, 0.1%	VEVYE	2023	USA	Calcineurin inhibitor immunosuppressant	Indicated for the treatment of moderate to severe DED (Vevizye in Europe)
Cyclosporine ophthalmic emulsion, 0.1%	IKERVIS	2015	European Union	Calcineurin inhibitor immunosuppressant	Treatment of severe keratitis in adult patients with DED, which has not improved despite treatment with tear substitutes
Purified sodium hyaluronate, 0.1%	HYALEIN ophthalmic solution 0.1%	1995	Japan	Increasing tear film stability / corneal healing	Indicated for the treatment of corneal and conjunctival epithelial disorders (keratoconjunctival epithelial disorder resulting from the following diseases: intrinsic diseases such as Sjögren syndrome, Stevens-Johnson syndrome and sicca syndrome [dry eye]. Extrinsic diseases caused by surgery, drugs, trauma, contact lens wearing, etc)
Purified sodium hyaluronate, 0.3%	HYALEIN ophthalmic solution 0.3%	2010	Japan	Increasing tear film stability / corneal healing	As above for 0.1% solution
Purified sodium hyaluronate, 0.1% (preservative free)	HYALEIN Mini ophthalmic solution 0.1%	1995	Japan	Increasing tear film stability / corneal healing	As above for 0.1% solution
Purified sodium hyaluronate, 0.3% (preservative free)	HYALEIN Mini ophthalmic solution 0.3%	1995	Japan	Increasing tear film stability / corneal healing	As above for 0.1% solution

(*continued on next page*)

TABLE 13. (*continued*)

Generic Name	Brand Name	Year Approved	Country	MOA	Approved Indication
Diquafosol sodium ophthalmic solution, 3.0% (6x Daily)	DIQUAS	2010	Japan	$P2Y_2$ purinergic receptor agonist	Treatment of DED
Rebamipide ophthalmic suspension, 2.0%	MUCOSTA	2012	Japan	Upregulates the gene expression of MUC1, MUC4, and MUC16	Treatment of DED
Diquafosol sodium ophthalmic solution, 3.0% (× Daily TID)	DIQUAS LX	2022	Japan	$P2Y_2$ purinergic receptor agonist	Treatment of DED
Cyclosporine ophthalmic emulsion, 0.05%	RESTASIS	2010	India	Calcineurin inhibitor immunosuppressant	Not known
Cyclosporine ophthalmic solution, 0.09%	CEQUA	2021	India	Calcineurin inhibitor immunosuppressant	Not known
Lifitegrast ophthalmic solution, 5%	SECA	2023	India	LFA-1 antagonist	Not known
Diquafosol sodium ophthalmic solution, 3.0% (6× Daily)	DIQUAS	2017	China	$P2Y_2$ purinergic receptor agonist	Indicated for DED diagnosed with corneal and conjunctival epithelial injury accompanied by tear abnormalities
Cyclosporine ophthalmic nanoemulsion, 0.05%	Zirun Cycloome	2020	China	Calcineurin inhibitor immunosuppressant	Indicated to increase tear production in DED where tear production is presumed to be suppressed because of ocular inflammation associated with keratoconjunctivitis sicca
Diquafosol sodium ophthalmic solution, 3.0% (0.4 mL:12 mg)	RUNLIMING	2022	China	$P2Y_2$ purinergic receptor agonist	Indicated for DED diagnosed with corneal and conjunctival epithelial injury accompanied by tear abnormalities
Varenicline solution, 0.03 mg nasal spray	TYRVAYA	2024	China	Nicotinic acetylcholine receptor agonist	Indicated for the treatment of the signs and symptoms of DED
Perfluorohexyloctane	HENGQIN	2024	China	Semifluorinated alkane	Indicated for the treatment of the signs and symptoms of DED

DED = dry eye disease, EU = European Union, LFA-1 = lymphocyte function-associated antigen 1.
Disclaimer: This is not necessarily a complete list of approved prescription medications approved in China and India.

vessels that can present as hyperemia associated with inflammation (see Table 15).[931,959]

Obstructive MGD presents as eyelid telangiectasia, gland orifice capping, gland dropout, altered gland expressibility, and low TBUT.[6,960,961] This distinction from aqueous-deficient DED is important because newer products in development such as selenium sulfide ophthalmic ointment[951,954] and devices for treating eyelid glandular abnormalities[962] have inclusion criteria and associated primary end points focusing on glandular morphology and associated evaporative DED.[963-969] Such trials may exclude patients with more severe symptoms, signs of inflammation, and significant ocular surface staining.[951] The inclusion and exclusion criteria and end points were modeled on the clinical studies performed using a thermal pulsation system.[970] These trial designs targeted at obstructive MGD and associated evaporative DED, illustrate a population-targeted trial design aimed toward a group of patients who may be underserved by some of the existing approved drug treatments.

8.3.3. US FDA clear guidance for industry and flexibility for dry eye approvals

The US FDA Draft Guidance for Industry on Dry Eye: Developing Drugs for Treatment[925] recommends the sponsor

of a new DED treatment demonstrate efficacy and safety in at least 2 adequate and well-controlled, multicenter independent studies. The guidance covers trial design, comparator(s), trial population, demonstration of efficacy, and safety database requirements in detail. The following section summarizes the key guidelines issued by the FDA.[925]

8.3.3.1. Trial design. The FDA will consider both traditional environmental exposure trials and an environmental challenge-model trial (using a controlled chamber with regulated temperature, air flow, and humidity) to support approval of a new drug. The FDA recommends parallel, randomized by patient, double-masked trials in which the investigational drug group demonstrates superiority over the control group (control agent can be the vehicle). Equivalence and noninferiority trials are generally discouraged because of difficulty in defining limits for equivalence, and crossover trials will only be evaluable for the first treatment period if any carryover from one period to another is detected. Future work to establish good assay validation (sensitivity) methods (inclusion of both a positive and negative concurrent control) could change the recommendation on equivalence or noninferiority trials.

The FDA guidelines are applicable to trials designed to demonstrate the efficacy and safety of a new product in support of a new drug application and do not preclude the use of such trial designs by companies or sponsors for the purposes of supporting internal decisions related to a compound's ability to progress in development based on measures of safety or efficacy such as proof of mechanism end points, biomarkers, and alternative thresholds for significance (eg, 0.1% not 0.05%).

Daily exposure to environmental factors can impact DED end points: cigarette smoke, smoke from wildfires, air pollution, dust, topically instilled drugs, and allergens are just a few examples.[971-973] A model has been developed to standardize climatic, air quality, and visual conditions in a controlled environment.[972,974,975] Aspects of the environment such as humidity, air flow, and temperature can be manipulated to impact the ocular surface, to identify individuals who may be more responsive to external environmental factors, and to create a reproducible environmental challenge.[975] This model is an attempt to reduce the number of patients, sites, and time to demonstrate the efficacy of an intervention.[972,974,975] Effects can be tested over 1 day in a controlled environment or longer-term natural exposure trials of 2 weeks or longer. The FDA recommends that safety trials be conducted for at least 6 weeks if efficacy trials are of shorter duration. Trials in which the investigational drug is used as an add-on to a standardized treatment regimen are also acceptable.[925]

Although confirmatory efficacy trials have used controlled adverse environment exposure to enrich study populations with patients whose condition can change in severity secondary to environmental stress, the prespecified primary end points supporting approval were environmental.[940] Trials have also used the controlled adverse environment to test an intervention's protection against the effects of an environmental challenge.[976]

8.3.3.2. Comparator(s). The comparators and methods for controlling variance have evolved from the first studies of cyclosporine in DED,[927] to the recent introduction of newer formulations and concentrations of the active.[927,932,933,947,948] The first studies of cyclosporine in DED evaluated cyclosporine plus artificial tears as needed to month 4 and then less than 8 times daily after month 4 to month 6.[939] Numerous trials tried to replicate this methodology and failed because of large placebo effects, lack of efficacy, and regression of signal, which could have resulted from a therapeutic effect for the artificial tears.[939,977] The subsequent approval of a novel integrin antagonist (lifitegrast) used only the vehicle and excluded the use of artificial tears during the treatment period. (See Supplementary Figure S1 for the 0.05% cyclosporine trial design, Figure S2 for the lifitegrast controlled environmental trial design, and Figure S3 for a lifitegrast environmental exposure trial design.)

The FDA acknowledged the advancement in trial methodology and encouraged trial designers to consider that even water is known to be an effective component of topically applied treatments for DED. Vehicle responders may be 1 in 5 of the trial population, suggesting this is a significant confounder in a study without a mechanism to screen for vehicle response.[978] In general, comparative clinical trials should use only the investigational drug's vehicle as a control agent. Trials should demonstrate statistical and clinical superiority over a vehicle control or another treatment regimen.[925]

8.3.3.3. Trial population. The FDA defines a DED population as including patients with ocular complaints consistent with dry eye symptoms. Inclusion criteria should include both objective signs and subjective symptoms.[925] Examples of inclusion and exclusion criteria for approved products are included in Table 14. The FDA includes measures of meibomian gland function, meibum quality, and TBUT as both a potential inclusion criterion for a DED population and a potential sign of DED therapeutic effect that can function as a primary sign efficacy end point.[951,954] Studies should include patients from relevant demographic subsets, including both men and women and multiple age, ethnic background, and eye color groups consistent with the US population. Confirmatory efficacy studies do not need to be completed in the United States so long as the study population contains the relevant demographic subsets found within the United States.

DED secondary to cicatrization (following irradiation, alkali burns, Stevens-Johnson syndrome, cicatricial pemphigoid) or the destruction of conjunctival goblet cells (vitamin A deficiency) represent a specific, severely affected

TABLE 14. Mechanism of action and approval pathway

Mechanism of action	Drugs approved for DED and relevant endpoints in their regulatory approvals*					
	Signs of DED			Symptoms of DED		
	Corneal Staining	Conjunctival Hyperemia	Schirmer test score	Eye Dryness VAS Scale	Eye Discomfort VAS Scale	Total OSDI
Calcineurin inhibitor immunosuppressant	IKERVIS		RESTASIS®, CEQUA™, VEVYE			IKERVIS
LFA-1 antagonist	XIIDRA™			XIIDRA™		
Corticosteroid		EYSUVIS™			EYSUVIS™	
Tear Replacement/Stabilizer						
Cholinergic agonist			TYRVAYA™			
Semi-fluorinated alkane	MIEBO™			MIEBO™		
Water retention & binding to fibronectin possibly promoting adhesion & elongation of corneal epithelial cells	HYALEIN® 0.1% & 0.3% HYALEIN® Mini 0.1% & 0.3%					
$P2Y_2$ purinergic receptor agonist	DIQUAS®, DIQUAS® LX					
Quinolinone derivative with mucin secretagogue activity	MUCOSTA®					

*There have been no drugs approved with a mechanism of action specifically for MGD or evaporative DED, based on an improvement of eyelid signs (meibomian gland obstruction, meibum quality or TBUT). Drug approval is irrespective of DED subtype.
DED = dry eye disease, LFA -1 = lymphocyte function-associated antigen-1, OSDI = Ocular Surface Disease Index, VAS = visual analog scale.

patient population. In general, these are considered separate indications, and patients with these conditions should be studied separately from routine DED.

Severe blepharitis or obvious inflammation of the eyelid margin can interfere with the interpretation of trial results. In general, patients with these conditions should be studied separately from routine DED.[925]

8.3.3.4. Demonstration of efficacy. In general, safety and efficacy should be demonstrated in at least 2 adequate and well-controlled, multicenter (at least 2 sites), independent trials. Primary efficacy end points include one of the following 3 options[925,926]:

1. Signs and symptoms: a statistically significant difference between the investigational treatment and vehicle from baseline for at least 1 objective prespecified sign of DED (mean group score of test vs vehicle) AND at least 1 subjective prespecified symptom of DED (mean group score);
2. Corneal staining: a statistically significant difference between the percentage of patients achieving a complete resolution of corneal staining; or
3. Tear production: a statistically significant difference between the percentage of patients achieving a ≥10-mm increase in Schirmer score.

Achieving any one of these 3 end points can support approval of a product for the treatment of the signs and symptoms of DED. Several marketed products have sought approval based on sign and symptom primary end point(s) and have been granted US approval using prespecified secondary end points of a known surrogate end point, a ≥10-mm increase in Schirmer score, when the prespecified signs and symptom end points failed in confirmatory efficacy studies.[927,932,933,939,947,948]

The "≥10-mm increase in Schirmer score" was validated as part of the new drug application for 0.05% cyclosporine and a description of the methods used to validate the end point are described in the "Validation of the Clinical Relevance of the Clinical Sign" section authored by one of the medical reviewers for NDA 21-023.

8.3.3.5. Safety database. The FDA specifies the size of a drug exposure database to support a new drug application based upon Adverse Event detection rates. The clinical program should include enough patients to identify adverse drug events that occur at a rate of 1% or greater. To accomplish this, FDA recommends that approximately 400 or more patients using the investigational drug complete treatment with a concentration of the investigational drug at least as high as proposed for marketing and with a frequency at least as frequent as proposed for marketing. Before submission of a marketing application, the sponsor should ensure at least 300 patients have completed a minimum of 6 weeks of follow-up after the initiation of treatment and at

TABLE 15. Approved drugs and criteria for defining DED

Approved Drug	Studies	Criterion for Defining DED	
		Signs	Symptoms
		Number of Functional Meibomian Glands; Meibum Quality; TBUT; Corneal Staining; Conjunctival Staining; Schirmer Test Score; Eye Redness	OSDI; VAS; SPEED; Ocular Dryness; NEI-VFQ-25; SANDE; Facial Expression Scale
RESTASIS®	192731 -002, -003, and -501	Staining: Sum of corneal & conjunctival staining ≥ +5 in the same eye with corneal staining ≥ +2 (Oxford scheme; 0-15) Schirmer test score (without anaesthesia): ≤ 5 mm/5min, if 0 mm/5min then nasal stimulation ≥ 3 mm/5 min	OSDI: At least 9 responses other than N/A and a minimum score at screening and baseline Facial Expression Score ≥ 3
XIIDRA ™	Phase 2 and Opus-1	Staining: Corneal fluorescein staining score ≥ 2 in at least one region of either eye (0-4 scale) at Screening and Baseline; Change in Inferior Corneal Staining (pre to post CAE ≥ +1) Schirmer test score (without anesthesia): ≥ 1 and ≤ 10 mm/5min Conjunctival redness score: ≥ 1 (0-4 scale) in at least one eye (any eye for OPUS-1)	OSDI ≥ +3 at 2 consecutive time points intra-CAE
	Opus-2 and Opus-3	Active eyelid margin disease not included Staining: Corneal fluorescein staining score ≥ 2 in at least one region of either eye (0-4 scale) at Screening and Baseline; Inferior Corneal Staining ≥ 0.5 at screening and baseline) Schirmer test score (without anesthesia): ≥ 1 and ≤ 10 mm/5min Conjunctival redness score: ≥ 1 (0-4 scale) in at least one eye	Eye Dryness Score ≥ 40 at Screening and Baseline
CEQUA™		Significant eyelid irregularity not included Staining: Lissamine green conjunctival staining sum score of 3 or more to 9 or less of a total possible score of 12 in the same eye	Global symptom score (based on symptoms of dryness, irritation, or both) rated by the patient of 40 or more (range, 0-100)
EYSUVIS™		MGD not included Staining: Corneal fluorescein staining score at Screening and Day 1 of ≥ 6 NEI (National Eye Institute) Schirmer test score (without anesthesia): at Screening of ≤ 10 mm Bulbar conjunctival hyperemia at Screening and Day 1 of ≥ 2 assessed using the CCLRU scale	A score of > 50 mm on Modified SANDE Severity at Screening; > 40 mm on Modified SANDE Severity on the day prior to Day 1

(*continued on next page*)

TABLE 15. (*continued*)

Approved Drug	Studies	Criterion for Defining DED														
		Signs							Symptoms							
		Number of Functional Meibomian Glands	Meibum Quality	TBUT	Corneal Staining	Conjunctival Staining	Schirmer Test Score	Eye Redness	OSDI	VAS	SPEED	Ocular Dryness	NEI-VFQ-25	SANDE	Facial Expression Scale
TYRVAYA™	Onset-1 (OPP-002)	Blepharitis not requiring treatment and mild meibomian gland disease that are typically associated with DED were allowed. Corneal fluorescein staining score of ≥2 in at least one corneal region OR have a sum of ≥4 for all corneal regions Baseline Schirmer test score (with topical anesthesia): ≤10 mm/5 minutes; with a cotton swab nasal stimulation, score at least 7 mm greater in the same eye; <20 mm difference from the study eye score and the fellow eye score														
	Onset-2 (OPP-101)	Blepharitis not requiring treatment and mild Meibomian gland disease that are typically associated with DED were allowed. Corneal fluorescein staining score of ≥2 in at least one corneal region OR a sum of ≥4 for all corneal regions Schirmer test score (with topical anesthesia): ≤10 mm/5 minutes; with a cotton swab nasal stimulation, score at least 7 mm greater in the same eye; <20 mm difference from the study eye score and the fellow eye score							OSDI score ≥23 with ≤3 responses of "Not Applicable") at the Screening Visit							
MIEBO™	GOBI MOJAVE	MGD defined as total MGD score ≥3 at Screening and Baseline (secretion of 5 central glands on the lower eyelid was evaluated, and each was scored from 0-3: 0=normal; 1=thick/yellow, whitish, particulate; 2=paste; 3=none/occluded). Total score ranged from 0-15 TBUT ≤5 seconds at Screening and Baseline Staining: Total corneal fluorescein staining score between 4 and 11 (i.e., sum of inferior, superior, central, nasal, and temporal) according to the NEI scale at Screening and Baseline Schirmer test score (without anesthesia): ≥5 mm at Screening and Baseline							OSDI score ≥25 at Screening and Baseline.							

(*continued on next page*)

TABLE 15. (*continued*)

Approved Drug	Studies	Criterion for Defining DED														
		Signs							Symptoms							
		Number of Functional Meibomian Glands	Meibum Quality	TBUT	Corneal Staining	Conjunctival Staining	Schirmer Test Score	Eye Redness	OSDI	VAS	SPEED	Ocular Dryness	NEI-VFQ-25	SANDE	Facial Expression Scale
VEVYE	CYS-002	Meibomian gland dysfunction (MGD) not included Staining: Total corneal fluorescein staining score of ≥ 6 (e.g. sum of inferior, superior, central, nasal, and temporal) according to the NEI grading at Screening and Baseline Total lissamine green conjunctival score (sum of temporal and nasal) of ≥ 2, based on the Oxford grading at Screening and Baseline Schirmer test score (without anesthesia): Between ≥ 2 mm and ≤ 8 mm at Screening and Baseline							Score of ≥ 40 on the dryness VAS at Screening and Baseline							
	ESSENCE -1 (CYS-003)	Staining: Total corneal fluorescein staining score of ≥ 10 (e.g. sum of inferior, superior, central, nasal, and temporal) according to the NEI grading at Screening and Baseline Total lissamine green conjunctival score (sum of temporal and nasal) of ≥ 2, based on the Oxford grading at Screening and Baseline Schirmer test score (without anesthesia): Between ≥ 1 mm and ≤ 10 mm at Screening and Baseline							Subjective complaints of poor near vision that impact activities of daily living, as defined by at least a moderate impact (score ≥ 3) on at least 1 question on the NEI VFQ-25 Questions 5 to 7 in the main questionnaire or Near Vision Subscale, Questions A3 to A5 in the Appendix of Optional Additional Questions, at the screening visit						
	ESSENCE 2 (CYS-004)	Staining: Total corneal fluorescein staining score of ≥ 10 (e.g. sum of inferior, superior, central, nasal, and temporal) according to the NEI grading at Screening and Baseline; Total lissamine green conjunctival score (sum of temporal and nasal) of ≥ 2, based on the Oxford grading at Screening and Baseline Schirmer test score (without anesthesia): Between ≥ 1 mm and ≤ 10 mm at Screening and Baseline							VAS: ≥ 50 on the dryness at Screening and Baseline							
IKERVIS	Siccanove (NVG06C103)	TBUT: ≤8 seconds Staining: Corneal fluorescein staining ≥2 and ≤4 (modified Oxford scale, scale 0-5); Lissamine green staining >4 (Van Bijsterveld scale, scale 0-9) Schirmer score (unanesthetized): ≥2 mm/5 min and <10 mm/5 min							OSDI score ≥23 VAS: At least one moderate to severe symptom of dry eye with a score ≥2 (severity graded on a 4-point scale) i.e., burning/stinging, foreign body sensation, itching, eye dryness, pain, blurred vision or sticky feeling and photophobia						

(*continued on next page*)

TABLE 15. (*continued*)

Approved Drug	Studies	Criterion for Defining DED													
		Signs							Symptoms						
		Number of Functional Meibomian Glands	Meibum Quality	TBUT	Corneal Staining	Conjunctival Staining	Schirmer Test Score	Eye Redness	OSDI	VAS	SPEED	Ocular Dryness	NEI-VFQ-25	SANDE	Facial Expression Scale
	Sansika (NVG10E117)	Staining: Corneal fluorescein staining score of 4 on the modified Oxford scale Schirmer test score (without anesthesia): ≥2 mm/5 min and <10 mm/5 min							OSDI: ≥23						
HYALEIN® ophthalmic solution 0.3%		Staining: Corneal fluorescein staining score ≥2+													
HYALEIN® Mini ophthalmic solution 0.1%		not listed													
HYALEIN® Mini ophthalmic solution 0.3%		not listed													
DIQUAS®		TBUT: ≤5 seconds Staining: Corneal fluorescein staining score: ≥3 (a maximum score of 9); Rose Bengal staining score: ≥3 (a maximum score of 9); Lissamine green staining score: ≥3 (a maximum score of 9) [Staining of the temporal conjunctiva, cornea, and nasal conjunctiva, with each graded on a 0-3 scale. The scores of the three regions are summed.] Schirmer test score (without anesthesia): ≤5 mm							Subjective symptoms (including visual disturbance)						
MUCOSTA®		Staining: A corneal fluorescein staining score of 4 or more and a LGCS score of 5 or more Schirmer test score (without anesthesia): ≤5 mm							Score of 2 or more for 1 or more dry eye–related ocular symptom(s)						
DIQUAS® LX		TBUT: ≤5 seconds Staining: Corneal fluorescein staining score ≥ 1 Schirmer test score (without anesthesia): ≤5 mm							Dryness score ≥ 1 using the DEQS questionnaire						

CAE = controlled adverse environment, CCLRU = Cornea & Contact Lens Research Unit, DEQS = Dry Eye-related Quality-of-life Score, MGD = meibomian gland dysfunction, NEI-VFQ-25 = National Eye Institute Visual Function Questionnaire-25, OSDI = Ocular Surface Disease Index, ODS = ocular discomfort score, SANDE = Symptom Assessment iN Dry Eye questionnaire, TBUT = tear break-up time; VAS = visual analogue scale

least 100 patients have completed 12 months of follow-up after the initiation of treatment to support long-term use. Acute use, that is, 6 weeks or less of administration each year, does not require long-term follow-up of 12 months.

For reformulations of drug substances that are already approved in the same dosage form, same route of administration, and the same or lower concentration, the FDA recommends the sponsor ensure that a marketing application has safety information from at least 100 patients treated for at least 6 months.

FDA recommends that the following evaluations be performed in each eye and reported separately for each eye (regardless of which eye or eyes are treated):

- Best corrected, distance visual acuity (≥4 m in distance) at every visit.
- A patient comfort examination before and after drug administration at every visit.
- A slitlamp examination of the anterior segment that includes the cornea, conjunctiva, anterior chamber, iris, lids, and lashes. At a minimum, examinations should be performed at baseline, midway through the trial, the end of treatment, and 2 weeks after treatment discontinuation.
- Endothelial cell count, systemic clinical and laboratory evaluations, and dilated fundus examinations at baseline and at the end of trial or at month 3 (whichever is later) in at least 1 trial.

DED also occurs in pediatric patients.[979] Sponsors may consider a pediatric assessment waiver request when submitting their required pediatric study plans under the Pediatric Research Equity Act.[925]

8.3.4. EMA guidance for industry and flexibility for DED approvals

EMA guidelines recommend demonstration of efficacy for 6 months because DED is often chronic, and comparison against artificial tears, which are the standard of care, is recommended.[980] EMA application submissions for lifitegrast and cyclosporine were both withdrawn because of data demonstrating insufficient efficacy.[929,981] The only EMA-approved treatment is cyclosporine 0.1%; however, the indication is not for DED but rather "severe keratitis in patients with DED, not improved despite treatment with tear substitutes."[982]

Regarding the selection of sign end points, the EMA considers both the target population and the mechanism of action of the compound: corneal staining, Schirmer score, and TBUT are established clinical end points. A composite measure is recommended using a validated questionnaire for a symptom end point (eg, OSDI), and use of single worst symptom questions is not recommended. Sponsors are encouraged to consider a relevant effect size rather than only statistical significance. For antiinflammatory products, because inflammation is considered a secondary manifestation, biomarkers may be required to quantify treatment benefit.

The EMA will generally accept development programs completed in the United States assuming the studies comply with the guidelines outlined above. The EMA only requires demonstration of safety and efficacy in one large adequately designed phase 3 trial. The primary end point for a cyclosporine 0.1% phase 3 trial was a sign and symptom composite responder rate. Specifically, the primary end point was the corneal fluorescein staining–OSDI composite responder rate at month 6 (ie, end of Part 1). A corneal fluorescein staining–OSDI responder was defined as a patient simultaneously satisfying the following conditions[982]:

- improvement of 2 points or more from baseline in corneal fluorescein staining based on the modified Oxford scale (ie, change in corneal fluorescein staining ≤–2), and
- improvement by 30% or more from baseline in OSDI (ie, % change ≤–30%).

This is not an end point that has been acceptable to the FDA at the time of writing. The completed confirmatory efficacy trials (SICCANOVE and SANSIKA) failed to demonstrate consistent superiority on the primary end point and the product was subsequently approved on a secondary sign end point by showing a statistically significant reduction in corneal fluorescein staining vs vehicle.[927,983,984] No significant improvement in symptoms was demonstrated along with the observed significant and clinically relevant improvement in corneal staining and the indication was restricted to "severe keratitis treatment" instead of a broader DED population.

8.3.5. PMDA guidance for industry and flexibility for approvals

Historically, there has been a considerable time delay between development and approval in the United States and an ultimate launch in Japan.[985] The PMDA requirements for drug approval in Japan have been harmonized across regions with toxicology and manufacturing generally not requiring additional work or rework.[985]

Working with the PMDA involves a multistep process. This process is initiated with the jizen mendan ("preliminary meeting") which has no fee associated with it, is shorter in length, and requires less of a briefing package. The intention of this meeting is to confirm the materials that are to be submitted and the sponsor's questions that will be discussed at the subsequent *taimen jogen* ("full consultation meeting"). At the full consultation meeting, a more detailed briefing document is submitted in advance and written comments are provided by the PMDA following the meeting.

The PMDA views ethnic differences as critically important. Phase 1 pharmacokinetics studies can be completed in the United States, if done in Japanese American patients defined as first- or second-generation Japanese Americans

of pure Japanese descent. PMDA generally requires a phase 1 pharmacokinetics study even in cases where a global development program may have proceeded into phase 2 or 3 trials in other regions, but with a non-Japanese population. Recently the PMDA issued a notice regarding the basic approach to conducting phase 1 studies in Japanese patients before global clinical trials for drugs whose clinical development has already started overseas,[986,987] stating the following: "In general, it is not mandatory to conduct a Phase I study in each race/ethnicity or country/region before initiating multi-region clinical trials. In principle, an additional Phase I study in Japanese is not needed unless it is deemed necessary after assessing whether the safety/tolerability of the dosage to be evaluated in the multi-region clinical trials in Japanese participants can be explained and the safety is clinically acceptable/manageable based on the data available prior to Japan's participation." This concept seems to have achieved some progress in the development of pharmaceuticals in Japan. The PMDA typically wants to see dose ranging established in the Japanese population specifically because of potential population differences in drug activity and practice patterns. Confirmatory phase 3 study is required to be completed in Japan in part or whole. The PMDA generally requires a single, properly designed phase 3 clinical trial to demonstrate safety and efficacy, similar to the EMA, and discourages post hoc analysis for approval.

Phase 3 trials for the approval of diquafosol sodium ophthalmic solution[928,988-990] and rebamipide ophthalmic suspension[928,991] both required an active comparator (0.1% hyaluronic acid ophthalmic solution was used in both), which necessitated a dosing regimen of 6 times daily. This may have hampered interest in developing the product in Japan because of the risk posed by even vehicle effects at such a high dosing frequency. The introduction of long-acting diquafasol sodium ophthalmic solution in 2022, which is only dosed 3 times daily, may have changed this risk profile. Long-acting diquafasol also employed a vehicle placebo in its phase 3 trial.[992]

All 3 products were tested with primary end points at 4 weeks and all measured primary treatment benefit using staining end points:

1. Diquafosol sodium ophthalmic solution: A noninferiority margin of 0.34 for the study (between-treatment difference in the mean change in the fluorescein and rose bengal staining score [diquafosol – HA])
2. Rebamipide ophthalmic suspension: Change from baseline in corneal fluorescein staining score and the lissamine green conjunctival staining score
3. Long-acting diquafosol sodium ophthalmic solution: Change in corneal fluorescein staining score from baseline to week 4

Similar to those products that were approved in the United States targeting a subpopulation of DED patients with aqueous-deficient DED and inflammation, the phase 3 trials for these products targeted patients with evidence of ocular surface staining, decreased tear production, and dry eye symptom(s).[928,934,988-993] Diquafosol sodium ophthalmic solution and long-acting diquafosol sodium ophthalmic solution also required a TBUT ≤5 seconds, which was also required as part of the development program for perfluorohexyloctane ophthalmic solution.[950,952,953] In these trials, diquafosol sodium ophthalmic solution and long-acting diquafosol sodium ophthalmic solution were reported to promote the secretion of tear fluid with aqueous and mucin components and to increase lipid layer thickness in normal human eyes.[994] Thus, the phase 3 trials were enriched based on the known mechanism of action of a P2Y2 purinergic receptor agonist. It should be noted that a P2Y2 agonist and rebamipide failed in multiple clinical trials in the United States to assess their efficacy in treating DED.[928,934,988-994]

The primary end point for a hyaluronic acid phase 3 study was a sign and symptom composite responder rate.[956] This is not an end point that has been acceptable to the FDA as at the time of writing. The diquafosol ophthalmic solution phase 3 also defined a noninferiority margin between-treatment difference in the mean change in the fluorescein and rose bengal staining score (diquafosol compared with hyaluronic acid).[988,990] The FDA has not accepted a noninferiority margin to support approval of a drug for DED. Thus, the PMDA has allowed flexibility in defining the type of comparison that will support approval: superiority or noninferiority to an active comparator.

The PMDA does not have the same guidance for industry on developing drugs for DED as the FDA. In addition, the diagnostic criteria for DED were revised in 2016[995] and no longer include tear production or keratoconjunctival disorders in the diagnostic criteria. Two of the 3 approved drugs for the treatment of dry eye, diquafosol ophthalmic solution[934,988-990] and rebamipide ophthalmic suspension,[928,991] were approved before the revision, and the third drug, long-acting diquafosol sodium ophthalmic solution,[992,993] had an additional additive that reduced the frequency of application but the efficacy itself did not change, so the primary end point in the trial was the fluorescein corneal staining score. With the revision of diagnostic criteria (ie, tear production and keratoconjunctival disorders are no longer included), it may be necessary to have a robust discussion with regulatory authorities regarding the setting of the primary end point based on the mechanism of action of a new drug.

8.4. DEVICES:

8.4.1. Regulatory pathway (FDA)

The regulatory process for devices is very different from the process for pharmaceuticals.[929,996] This section briefly describes the process used by the FDA, but there are likely differences in other regions of the world.[929] Similar to its role in the approval of drugs, the FDA is responsible for pro-

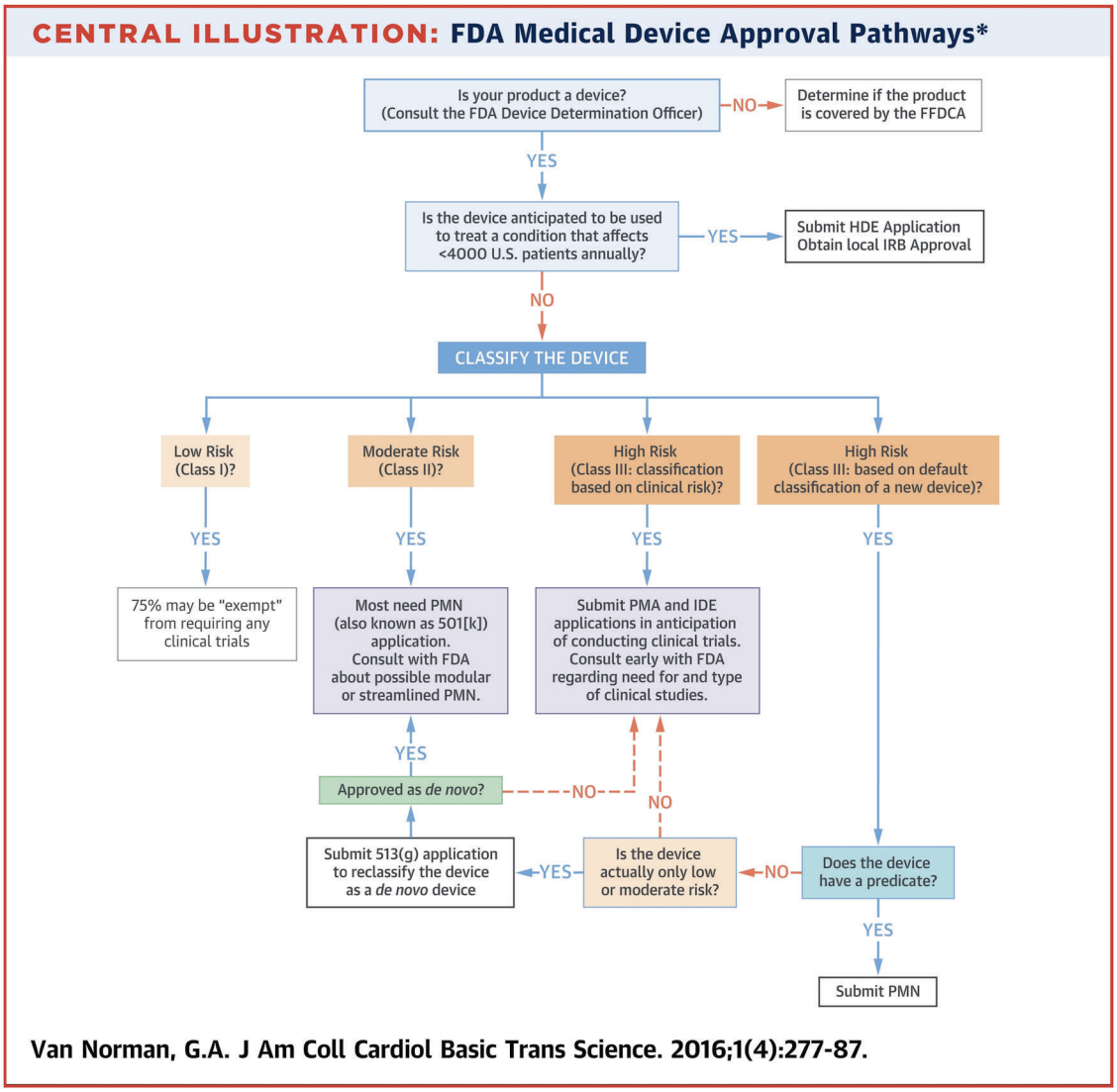

FIGURE 5. FDA Medical Device Approval Pathways. Permission for use of this figure is granted through the creative commons arrangement https://creativecommons.org/licenses/by-nc-nd/4.0/. (from Van Norman 2016).[997]

tecting the public by assuring the safety and efficacy of medical devices. Although both drug and device approval have increased over the last decade, there is an increasing gap favoring new devices in the United States.[996-998] In general, devices have a shorter time for bringing to market and significantly less cost but may have lower profitability.[997] The key steps to FDA device approval start with determining if a product is a device and identify its purpose, and then to determine its risk classification: low, moderate, or high risk (Figure 5).

ProvRisk is assessed to determine the device's chances of presenting harm to patients, including from malfunction or improper use. The risk classification generally indicates the pathway and type of submission required by the FDA. The type of premarket submission depends on the risk assessment:

1. Investigational device exemption: devices used to collect safety and effectiveness data
2. Premarket notification (510k): the device is safe and effective and substantially equivalent to a legally marketed device
3. Premarket approval application: for class III device (high risk) that needs a clinical trial to demonstrate safety and effectiveness
4. *De novo*: for devices that have no existing classification regulation
5. Humanitarian device exemption: for devices for a disease that affects less than 4000 US patients yearly and has demonstrated reasonable assurance of safety and probable benefit
6. Low-risk (class I, eg, the Meibomian Gland Evaluator, marketed by Johnson & Johnson Surgical Vision, Inc)

and moderate-risk (class II, eg, iLux System by Tear Film Innovations, Inc, and the LipiFlow Thermal Pulsation System manufactured by TearScience, Inc) devices can be "exempt" or require a 510(k), whereas the highest-risk (class III) device requires a premarket approval. Class III devices will ultimately require a clinical trial and an Investigational Device Exemption so that an unapproved device can be used in clinical trials.

Trial design for class III devices: Given that it is often difficult or unethical to have a sham control, there is more flexibility on what type of trial is needed and discussion with the FDA will determine what is acceptable.[997] For the control arm of a device trial, one may be able to use an active comparator (ie, compare with an FDA-approved device), use metrics from pre- and post-treatment with a given device, or compare with registry data. Typically, a double-blind randomized clinical trial cannot be done with devices as is done with pharmaceuticals.[998] Mixed devices, pharmaceutical and device combined, are often reviewed by both divisions of the FDA, with one taking the lead, where communications with the FDA determine the regulatory pathway.[929]

8.4.2. Regulatory pathway (Japan, PMDA)

In Japan, as in the United States, the development and approval processes for medical devices differ depending on the type of device. Medical devices are classified into 4 classes according to the degree of risk to the human body. Medical devices in class I require only notification, those (except new devices[1]) in class II require certification by the certification body, and those in class III and IV require approval by government authority.[929,999]

Medical devices classified as class III and IV are considered to have a high risk to the human body and many of them require clinical trials. On the other hand, medical devices used outside the body that are not implanted in the body and do not have a high risk to the human body are often classified as class II. With class II, if the probability of benefit is high when considering risk and benefit, it may be judged that evaluation is possible without the need for clinical trials, as compared with class III and IV. Class I medical devices are those that are considered to have an extremely low risk to the human body.

For medical devices that have already been approved overseas, it is possible to extrapolate test results from overseas clinical trials, although ethnic differences must be taken into consideration. Therefore, unlike drugs, some medical devices are approved without domestic clinical trials by extrapolation to overseas clinical trials.

8.4.3. Artificial tears (US, FDA)

Artificial tears contain FDA-approved demulcents as active ingredient(s). Demulcents are primarily water-soluble, topically applied polymers, which may protect and lubricate mucous membrane surfaces relieving ocular dryness and irritation.[929,997] A list of approved demulcents and their concentration range has been provided by the FDA[1000]:

1. Cellulose derivatives:
   a. Carboxymethyl cellulose (CMC)
   b. Hydroxypropyl methylcellulose (hypromellose)
   c. Hydroxyethylcellulose
   d. Methylcellulose
2. Dextran 70
3. Gelatin
4. Polyols:
   a. Glycerin
   b. Polyethylene glycol (300, 400)
   c. Polysorbate 80
5. Polymers:
   a. Polyvinyl alcohol
   b. Polyvinyl pyrrolidone (povidone)

Federal law does not require premarket approval for nonprescription eye drops, including artificial tears, but does require eye drops to be sterile for safe use. The existence of the FDA's preapproved monograph that covers artificial tears means that if a developer lists an active ingredient that is not on the monograph, they will need to provide clinical data supporting the safety of its use. Manufacturers can list potentially "active ingredients" under "inactive ingredients" to avoid the requirement for clinical trials.

Although clinical data demonstrate that artificial tears show benefit for the management of DED and their control is critical during drug and device trials to remove a confounding source of variance, there are no regulatory standards for study design, clinical end points, patient inclusion and exclusion criteria, or accepted methods to control for potential sponsor bias when evaluating comparative efficacy claims.[1000]

8.4.4. Artificial tears (Japan, PMDA)

Most artificial tears in Japan are classified as OTC drugs that do not require a prescription. Nonprescription drugs require clinical trial results if they contain ingredients that are not included as active ingredients in any of the drugs specified in the Japanese Pharmacopoeia, but do not require clinical trials if they do.[929,997]

8.4.5. Devices approved for DED/MGD

Since the first TFOS DEWS report, there have been several devices FDA-approved targeting meibomian gland secretion or mechanical tear stimulation.[969] The first thermal pulsation device[966,967] was approved by the FDA for the application of localized heat and pressure therapy in adults with chronic cystic conditions of the eyelids, including MGD. It was approved after it met the primary study effectiveness end point of improvement at 2 weeks from baseline in the average number of meibomian glands yielding clear liquid secretion as compared with a warm compress

[1] New medical devices in class II require approval by government authority (PMDA and MHLW: Ministry of Health, Labour and Welfare).

control. Sixty-nine subjects (138 eyes) were randomized to the thermal pulsation treatment and 70 subjects (140 eyes) were randomized to the warm compress control group. The same system is also a medical device approved without clinical trials in Japan.

Subsequent thermal pulsation devices could be approved through a single noninferiority clinical trial that compared the device to the approved system as the predicate device. One example demonstrated noninferiority to the original system using the primary effectiveness end points defined as the change from baseline to 1 month for TBUT and total Meibomian Gland Secretion Score.[969] There are numerous examples of other devices, including those using intense pulsed light, that have been evaluated as treatments of evaporative DED or MGD.[963-965]

The Warming Moist Chamber Goggle (Dr.eye), an NMPA-approved therapeutic device, is indicated for the management of DED and MGD. It facilitates localized thermal and hygrometric conditions, which may enhance tear film stability. Empirical evidence[1001] has demonstrated that this device alleviates ocular discomfort, and improves tear film parameters, beyond topical sodium hyaluronate.

A neurostimulation device received FDA approval as an electromechanical tear stimulator that is a nonimplantable device intended to increase tear production via mechanical stimulation. The device underwent a prospective, open-label, single-arm, multicenter study in 108 subjects and a multicenter, nonsignificant-risk, prospective, double-masked, randomized, sham-controlled, single-visit clinical trial that enrolled 60 subjects. For the latter study, the primary end point achieved was an increase in the mean within-subject Schirmer test score post- vs prestimulation compared with a sham treatment group in subjects with a baseline Schirmer test score <10 mm.

8.5. DESIGN FEATURES TO ENHANCE CLINICAL TRIAL DATA QUALITY AND DECISIONS: This section covers ways to enhance clinical trial data quality and data-supported decisions on whether to proceed with an intervention under development for a particular indication.[2]

8.5.1. Biomarkers, proof of mechanism, and early signal of efficacy

One of the most challenging aspects of randomized controlled trials for DED is the lack of objective, minimally invasive metrics for diagnosing DED for patient selection and for use as efficacy end points. The typical use of signs and symptoms of DED nearly always comes with the caveat that DED affects "a heterogenous group of subjects." Outcome measures in randomized controlled trials although called "objective," such as corneal staining, TBUT, and Schirmer test value, are all subject to bias from the observer and/or biologic and environmental variables such as reflex tearing, time of day, and humidity. Ophthalmology does not have a lot of reliable, validated markers that correlate with clinically relevant findings in DED.[929,1002]

Recent examples of FDA-cleared biomarkers include a point of care immunoassay test for the *in vitro* detection of elevated levels of the MMP-9 protein in human tears, from patients suspected of having DED.[1003] Osmolarity testing is another FDA-approved biomarker.

Biomarkers were used in support of the approval of 0.1% cyclosporine and are referenced in the EU Assessment report (level of HLA-DR expression).[1004] More recently, a genetic marker (TNFR1 marker) was used in evaluating anti-TNF alpha, licaminlimab, and demonstrated improved outcome measures in a genetically defined subgroup analysis.[1005] At the time of writing, no biomarker/surrogate endpoint other than 10-mm increase or more in Schirmer test scores has supported regulatory approval for a DED treatment with the US FDA where they could theoretically serve as a primary sign end point if validated as clinically meaningful with an associated improvement in a primary symptom end point.

8.5.2. Missing data considerations

See TFOS DEWS II Clinical Trials Design report.[929]

8.5.3. Global trial designs

Diagnostic tools and outcome measures have often been highly variable, resulting in inconsistent observations across clinical trials and programs. This has complicated clinical development in that multiple trials may be required to achieve a successful drug approval (eg, lifitegrast ophthalmic solution 5.0%),[938,940-942] but it has also affected harmonization of regulatory requirements across regions. Regulatory requirements can vary considerably across the major DED markets, which include the United States, the European Union, and Japan.

For the clinical development of drug therapies, the commonly selected sign and symptom end points used in the trials have been similar across the major markets: corneal or conjunctival staining (although using different scales), Schirmer test score, and symptom questionnaires. For symptom questionnaires, there seems to be a trend favoring overall symptom questionnaires (eg, OSDI) and visual analog scales (ie, ocular discomfort or dryness).[927,928,931-933,938-942,948,950,953,955,988]

Across the United States, European Union, and Japan, compounds targeted at a similar subpopulation of patients who present predominately with signs of inflammation or its impact (eg, hyperemia, corneal staining, and conjunctival staining) and aqueous-deficient DED (eg, decreased tear production) have been approved.[927,928,931-933,938-942,948,950,953,955,988] The major difference between the regions is the duration of follow-up for the primary end points. The United States allows multiday, natural exposure trials of 2-week duration or longer;

TABLE 16. Digest Sections and Drivers of DED

	Drivers of dry eye disease								
	Tear Film Deficiencies			Eyelid Anomalies		Ocular Surface Abnormalities			
	Lipid	Aqueous	Mucin/glycocalyx	Blink/lid closure	Lid margin	Anatomical misalignment	Neural dysfunction	Ocular surface cell damage/disruption	Primary inflammation /oxidative stress
Sex, gender, and hormones	✓	✓				✓	✓	✓	✓
Epidemiology	✓	✓	✓		✓		✓	✓	✓
Pathophysiology	✓	✓	✓	✓	✓	✓	✓	✓	✓
Tear film	✓	✓	✓	✓	✓	✓			
Pain and sensation							✓	✓	✓
Iatrogeny	✓	✓	✓	✓	✓	✓	✓	✓	✓
Clinical trials design	✓	✓	✓		✓		✓	✓	✓

DED = dry eye disease.

the EMA allows natural exposure trials of 6-month duration or longer; and Japan allows natural exposure trials of 4-week duration or longer.[925,980,985,999]

Taken together, the commonalities between the regions could allow for a single trial with a common disease population and end point to support approval in more than 1 region leveraging multiple statistical analysis plans specifying primarily end points at different time points.[951,954] This allows a single trial to potentially support approval in both the United States and the European Union. To include Japan, a subpopulation of Japanese participants would need to be included. Although this could work for new compounds targeting the patient populations and end points that have already been accepted in the major markets, it is unclear how alternative end points (eg, meibomian gland morphology-related end points) will be accepted globally.

8.5.4. Compliance monitoring

Adherence to prescribed therapy is essential for quality clinical trials data and reliable comparison of treatment modalities. Strategies for assessing adherence to treatment vary in their applicability to ophthalmic trials but may include assessment of the amount of returned investigational product; patient report by regular electronic or other means; direct observation of drug or device use; systemic or ocular monitoring of drug or metabolite levels; or more technical solutions such as electronic monitoring, for example, app control of reusable devices that can map how long and how often they are used, or sensors in topical medication containers.

8.6. CONCLUSIONS: Newer market entrants have used clinical trial designs with enriched populations of patients who take into consideration the mechanism of the drug under study, particularly as it pertains to defining sign end points.[949,950,952,953] This has increased their likelihood of technical success and is aligned with the US FDA Draft Guidance. This guidance states that a statistically significant difference between the investigational treatment and vehicle for at least 1 objective prespecified sign of DED (mean group score for test versus vehicle) AND at least 1 subjective prespecified symptom of DED (mean group score) can be used to support approval. A sign end point consistent with a drug's mechanism of action can be selected to demonstrate that the drug under study has its intended effect. The significance of the symptom end point then establishes the clinical relevance of the observed effect the drug under study has upon how a patient feels, making it clinically relevant.[925] Thus, the recommendations from the TFOS DEWS II, the industry report to identify 3 fundamental elements needed to achieve a significant improvement in trial success rates (Pillars 1-3), and the US FDA Draft Guidance for Industry on DED all coalesce on the same

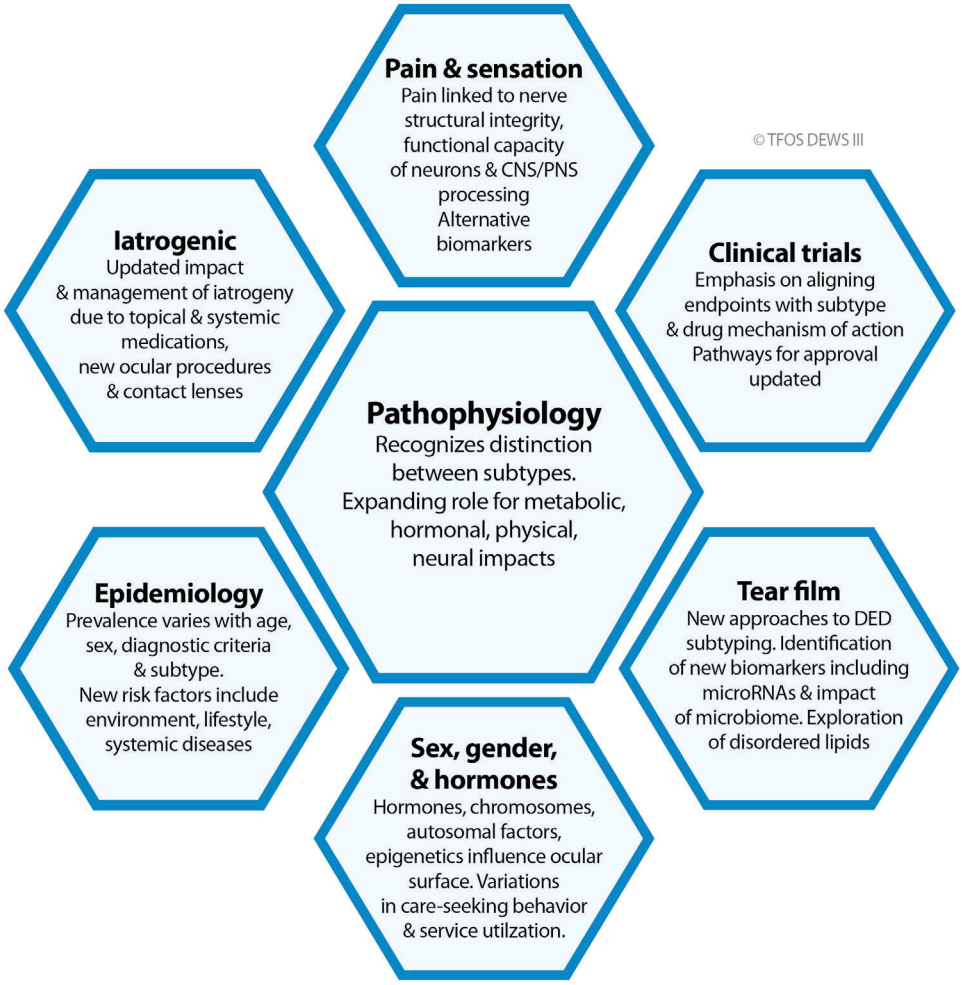

FIGURE 6. Key findings from interdisciplinary reports.

conclusion for achieving regulatory and technical success for getting a new drug approved: match the sign end point to the intended mechanism of the drug under study.

In conjunction with better selection of patients, trial designs have significantly evolved over the 20 years of research. They have gone from little control over confounding variables like artificial tear use[939] to careful control over comparator arms and run-in periods within confirmatory efficacy studies.[930,931,942,949,950,952,953,955] Global regulatory agencies have recognized this evolution with the FDA, even encouraging trial designers to consider that even water is known to be an effective component of topically applied treatments for DED. Thus, comparative clinical trials should use only the vehicle from the investigational drug as a control agent.

The agencies have also recognized that trial end points could change in sensitivity along with disease severity and as a result have also evolved the requirements for replication in confirmatory efficacy studies. The US FDA Draft Guidance recommends the sponsor of a new DED treatment demonstrate efficacy and safety in at least 2 adequate and well-controlled, multicenter independent studies. This is because the agency recognized that different study designs, populations, and time points may be needed to support sign and symptom end points in different studies.[925] Global regulatory agencies also allow prespecified and alpha-corrected secondary end points to support approval even if the primary end points miss statistical significance.[927,932,933,947,948,983,984] Although major differences still remain for trial requirements across the major markets, advances in trial design, patient identification, and statistical methods will undoubtedly continue to advance, allowing for more global confirmatory efficacy studies that can support approvals in more than 1 region.

9. SUMMARY

This report has explored key research published since the 2017 TFOS DEWS II Workshop reports to underpin the

evidence described in the TFOS DEWS III Diagnostic Methodology[1] and Management and Therapy[2] reports and to present how new findings from each of the topic areas considered have informed the drivers of disease. Each topic area has also identified key research needs the short and medium time frame.

Table 16 maps how each of the sections of the Digest inform evidence for the drivers of disease. Figure 6 illustrates the key findings from these interdisciplinary areas, all of which have informed diagnosis and methodology and pathways toward new treatment modalities. The importance of disease subtyping and the relevance of new biomarkers has been consistently recognized.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Fiona Stapleton: Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Visualization, Writing – original draft, Writing – review & editing. **Pablo Argüeso:** Data curation, Writing – original draft, Writing – review & editing. **Penny Asbell:** Data curation, Writing – original draft, Writing – review & editing. **Dimitri Azar:** Data curation, Writing – original draft, Writing – review & editing. **Charles Bosworth:** Data curation, Writing – original draft, Writing – review & editing. **Wei Chen:** Conceptualization, Writing – review & editing, Funding acquisition, Project administration. **Joseph B. Ciolino:** Data curation, Writing – original draft, Writing – review & editing. **Jennifer P. Craig:** Conceptualization, Writing – review & editing, Funding acquisition, Project administration. **Juana Gallar:** Data curation, Writing – original draft, Writing – review & editing. **Anat Galor:** Data curation, Writing – original draft, Writing – review & editing. **José A.P. Gomes:** Data curation, Writing – original draft, Writing – review & editing. **Isabelle Jalbert:** Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Ying Jie:** Data curation, Writing – original draft, Writing – review & editing. **Lyndon Jones:** Conceptualization, Funding acquisition, Project administration, Writing – review & editing. **Kenji Konomi:** Data curation, Writing – original draft, Writing – review & editing. **Yang Liu:** Data curation, Writing – original draft, Writing – review & editing. **Jesus Merayo-Lloves:** Data curation, Writing – original draft, Writing – review & editing. **Fabiola R. Oliveira:** Data curation, Writing – original draft, Writing – review & editing. **Victor L. Perez:** Conceptualization, Writing – original draft, Writing – review & editing, Funding acquisition, Project administration, Visualization, Supervision. **Eduardo M. Rocha:** Data curation, Writing – original draft, Writing – review & editing. **Benjamin D. Sullivan:** Data curation, Writing – original draft, Writing – review & editing. **David A. Sullivan:** Conceptualization, Funding acquisition, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing. **Jelle Vehof:** Data curation, Writing – original draft, Writing – review & editing. **Susan Vitale:** Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Mark Willcox:** Data curation, Writing – original draft, Writing – review & editing. **James S. Wolffsohn:** Conceptualization, Writing – review & editing, Funding acquisition, Project administration. **Murat Dogru:** Conceptualization, Funding acquisition, Project administration, Supervision, Visualization, Writing – original draft, Writing – review & editing.

Support/Funding: The TFOS DEWS III effort was supported by unrestricted donations from Alcon, Bausch + Lomb, Azura, AbbVie, CooperVision, Dompé, Espansione Group, Harrow, Laboratoire Théa, SIFI, SINQI, Tarsus, Topcon and Trukera. Financial Disclosures: F.S.: Grants or contracts (funds paid to institution): Alcon, Roche, Exonate, IOLYX, Nthalmics; Consulting fees (funds paid to institution): CooperVision, Alcon, Mentholatum, CSL Seqirus, Azura Ophthalmics; Payment or honoraria for lectures, presentations, speaker bureaus, manuscript writing or education events: CSL Seqirus, Alcon, InMode, Menicon (all, funds paid to institution), Johnson & Johnson, Santen (both, funds paid to author); Participation on a Data Safety Monitoring Board or Advisory Board (institution): Azura Ophthalmics, CSL Seqirus; Leadership or fiduciary role in other board, society or committee or advocacy group, paid or unpaid: Scientia Clinical Research (unpaid board chair), Vision Research Foundation (paid board member), Brien Holden Foundation (unpaid board member), P.A.: Grants or contracts: Regeneron, National Eye Institute, Research to Prevent Blindness; Consulting fees (funds paid to author): Iolyx, Regeneron, Bausch + Lomb, Centricity, Horizon, Azura, Trefoil, Glia, Harrow, Premark, LinkBiologic, Abbvie, Santen, CSP, Dompe, Senju; Payment or honoraria for lectures, presentations, speaker bureaus, manuscript writing or education events: Vindico, C. B.: Support for attending meetings and/or travel: Azura Ophthalmics; Patents planned, issued or pending: Azura Ophthalmics; Stock or stock options: Azura Ophthalmics, Foray Therapeutics, W. C.: Leadership or fiduciary role in other board, society or committee or advocacy group, paid or unpaid: Tear Film and Ocular Surface Society China (chair), Asia-Pacific Journal of Ophthalmology (editorial board member), J. B. C.: Grants or contracts: Department of Defence, National Institute of Health; Consulting fees (funds paid to author): ORA, W. L. Gore & Associations Inc.; Patents planned, issued or pending: US Publication numbers 20230226006, 11554104, 10918719, 20100239637; Participation on a Data Safety Monitoring Board or Advisory Board: Glaukos Inc.; Receipt of equipment, materials, drugs, medical writing, gifts or other services: Blavatnik Family Foundation; Other financial or non-financial interests: Fontana Bio, J. P. C.: Grants or contracts (funds paid to institution): Alcon, Azura, Laboratoire Théa, Resono Ophthalmic, Topcon, TRG Natural Pharmaceuticals; Consulting fees: Alcon, Bausch + Lomb; Payment or honoraria for lectures, presentations, speaker bureaus, manuscript writing or education events (funds paid to author): Alcon, Bausch + Lomb, Johnson & Johnson Vision, Laboratoire Théa, Resono Ophthalmic, Topcon; Payment for expert testimony (funds paid to author): Optometrists and Dispensing Opticians Board of New Zealand; Support for attending meetings and/or travel: Alcon, Bausch + Lomb, Laboratoire Théa; Participation on a Data Safety Monitoring Board or Advisory Board: Alcon, Bausch + Lomb; Leadership or fiduciary role in other board, society or committee or advocacy group, paid or unpaid: Tear Film and Ocular Surface Society (board of directors), Optometry Council of Australia and New Zealand director), University of Auckland Board of Research (chair), British Contact Lens Association (global ambassador); Receipt of equipment, materials, drugs, medical writing, gifts or other services: E-Swin, Espansione Group, Medmont International, Resono Ophthalmic, Titan Optical, Topcon, TRG Natural Pharmaceuticals, J. G.: Grants or contracts: Ministerio de Ciencia Innovación y Universidades, Generalitat Valenciana; Patents planned, issued or pending: Pharmaceutical composition for the treatment of dry eye, TRPM8 receptor agonist compounds and uses thereof, A. G.: Grants or contracts: Department of Veterans Affairs, Department of Defense Gulf War Illness Research Program, National Eye Institute, National Institute of Health; Consulting fees: Dompe, Alcon, Tarsus, Oyster Point, Bausch + Lomb, EyeCool, AstraZentica, BRIM; Participation on a Data Safety Monitoring Board or Advisory Board: DECLARE DSMB, J. A. P. G.: Grants or contracts: Alcon, Cristalia/Latinofarma, Cnpq, Fapesp; Consulting fees: Alcon, Ofta Vision Health, Cristalia/Latinofarma, Bausch + Lomb, Adapt, Mediphacos; Payment or honoraria for lectures, presentations, speaker bureaus, manuscript writing or education events (funds paid to author): Alcon, Genon, Cristalia/Latinofarma, Abbvie, Bausch + Lomb, Mundipharma; Participation on a Data Safety Monitoring Board or Advisory Board: Alcon, Bausch + Lomb, Cristalia/Latinofarma, Ofta Vision Health, I. J.: Grants or contracts (funds paid to institution): Natural Hazards Research Australia; Payment or honoraria for lectures, presentations, speaker bureaus, manuscript writing or education events: InMode (funds paid to author and institution), Seqirus, Alcon, Melcare (all, funds paid to institution); Receipt of equipment, materials, drugs, medical writing, gifts or other services: AFT Pharmaceuticals, L. J.: Grants or contracts (funds paid to institution): Avizor, Essilor, Euclid, Hoya, iMed Pharma, Integral Biosystems, Johnson & Johnson. All authors attest that they meet the current ICMJE criteria for authorship.

Acknowledgments: The authors thank Rajendra Gyawali (r.gyawali@uq.edu.au), Judy Nam (g.nam@unsw.edu.au), John Everett Serralta (jes528@med.miami.edu), and Pragnya Rao (prd63@miami.edu).

REFERENCES

1. Wolffsohn JS, Benítez-Del-Castillo J, Loya-Garcia D, Inomata T, Iyar G, Liang L, Pult H, Sabater AL, Starr CE, Vehof J, Wang MT, Chen W, Craig JP, Dogru M, Perez VL, Stapleton F, Sullivan DA, Jones L. TFOS collaborator group. TFOS DEWS III Diagnostic Methodology. *Am J Ophthalmol*. 2025 S0002-9394(25)00275-2 Epub ahead of print. doi:10.1016/j.ajo.2025.05.033.
2. Jones L, Craig JP, Markoulli M, Karpecki P, Akpek EK, Basu S, Bitton E, Chen W, Dhaliwal DK, Dogru M, Gomes JAP, Koehler M, Mehta JS, Perez VL, Stapleton F, Sullivan DA, Tauber J, Tong L, Travé-Huarte S, Wolffsohn JS, Alves M, Baudouin C, Downie L, Giannaccare G, Horwath-Winter J, Liu Z, Koh S, Elisabeth M, Otero E, Villani E, Watson S, Yoon KC. TFOS collaborator group. TFOS DEWS III Management and Therapy Report. *Am J Ophthalmol*. 2025 S0002-9394(25)00274-0 Epub ahead of print. doi:10.1016/j.ajo.2025.05.039.
3. Sullivan DA, Rocha EM, Aragona P, et al. TFOS DEWS II Sex, Gender, and Hormones Report. *Ocul Surf*. 2017;15(3):284–333. doi:10.1016/j.jtos.2017.04.001.
4. Galor A, Britten-Jones AC, Feng Y, et al. TFOS lifestyle: impact of lifestyle challenges on the ocular surface. *Ocul Surf*. 2023;28:262–303. doi:10.1016/j.jtos.2023.04.008.
5. Tellefsen S, Morthen MK, Richards SM, et al. Sex effects on gene expression in lacrimal glands of mouse models of Sjögren Syndrome. *Investig Opthalmol Vis Sci*. 2018;59(13):5599. doi:10.1167/iovs.18-25772.
6. Bron AJ, de Paiva CS, Chauhan SK, et al. TFOS DEWS II pathophysiology report. *Ocul Surf*. 2017;15(3):438–510. doi:10.1016/j.jtos.2017.05.011.
7. Toda I, Sullivan BD, Rocha EM, Da Silveira LA, Wickham LA, Sullivan DA. Impact of gender on exocrine gland inflammation in mouse models of Sjögren's syndrome. *Exp Eye Res*. 1999;69(4):355–366. doi:10.1006/exer.1999.0715.
8. Rattner A, Heng JS, Winer BL, Goff LA, Nathans J. Normal and Sjogren's syndrome models of the murine lacrimal gland studied at single-cell resolution. *Proc Natl Acad Sci*. 2023;120(42):e2311983120. doi:10.1073/pnas.2311983120.
9. Hat K, Kaštelan S, Planinić A, Muller D, Ježek D. Pathohistological features of the aging human lacrimal gland. *Croat Med J*. 2023;64(5):307–319. doi:10.3325/cmj.2023.64.307.
10. Chen X, Sullivan BD, Darabad RR, Liu S, Kam WR, Sullivan DA. Are BALB/c mice relevant models for understanding sex-related differences in gene expression in the human meibomian gland? *Cornea*. 2019;38(12):1554–1562. doi:10.1097/ico.0000000000002017.
11. Huang B, Fei F, Wen H, et al. Impacts of gender and age

on meibomian gland in aged people using artificial intelligence. *Front Cell Dev Biol*. 2023;11:1199440. doi:10.3389/fcell.2023.1199440.
12. Lee PK, Chung JL, Kim DR, et al. Categorization of meibomian gland dysfunction using lipid layer thickness and meibomian gland dropout in dry eye patients: a retrospective study. *Korean J Ophthalmol*. 2024;38(1):64–70. doi:10.3341/kjo.2023.0126.
13. Li Y, Lu J, Zhou Q, et al. Analysis of clinical and regional distribution characteristics of obstructive meibomian gland dysfunction in China: a multicenter study. *Curr Eye Res*. 2020;45(11):1373–1379. doi:10.1080/02713683.2020.1752387.
14. Lin X, Wu Y, Chen Y, et al. Characterization of meibomian gland atrophy and the potential risk factors for middle aged to elderly patients with cataracts. *Transl Vis Sci Technol*. 2020;9(7):48. doi:10.1167/tvst.9.7.48.
15. Stapleton F, Alves M, Bunya VY, et al. TFOS DEWS II epidemiology report. *Ocul Surf*. 2017;15(3):334–365. doi:10.1016/j.jtos.2017.05.003.
16. García-Marqués JV, Talens-Estarelles C, García-Lázaro S, Wolffsohn JS, Cerviño A. Systemic, environmental and lifestyle risk factors for dry eye disease in a Mediterranean Caucasian population. *Cont Lens Anterior Eye*. 2022;45(5):101539. doi:10.1016/j.clae.2021.101539.
17. Zhao M, Yu Y, Roy NS, Ying G-s, Asbell P, Bunya VY. Sex-related differences and hormonal effects in the Dry Eye Assessment and Management (DREAM) study. *Br J Ophthalmol*. 2024;108(1):23–29. doi:10.1136/bjo-2022-322238.
18. Borrelli M, Frings A, Geerling G, Finis D. Gender-specific differences in signs and symptoms of dry eye disease. *Curr Eye Res*. 2020;46(3):294–301. doi:10.1080/02713683.2020.1801758.
19. Wolffsohn JS, Wang MTM, Vidal-Rohr M, et al. Demographic and lifestyle risk factors of dry eye disease subtypes: a cross-sectional study. *Ocul Surf*. 2021;21:58–63. doi:10.1016/j.jtos.2021.05.001.
20. Li G, Garzon C, Klawe J, Akpek EK, Ahmad S. Demographic and psychiatric associations with dry eye in a Medicare population. *Cornea*. 2024;43(12):1499–1505. doi:10.1097/ico.0000000000003516.
21. Lu P-T, Lee C-Y, Sun C-C. Sex differences and discordance between symptoms and signs of dry eye disease. *Am J Ophthalmol*. 2024;260:14–20. doi:10.1016/j.ajo.2023.10.008.
22. Wang MTM, Muntz A, Mamidi B, Wolffsohn JS, Craig JP. Modifiable lifestyle risk factors for dry eye disease. *Cont Lens Anterior Eye*. 2021;44(6):101409. doi:10.1016/j.clae.2021.01.004.
23. Craig JP, Wang MTM, Ambler A, Cheyne K, Wilson GA. Characterising the ocular surface and tear film in a population-based birth cohort of 45-year old New Zealand men and women. *Ocul Surf*. 2020;18(4):808–813. doi:10.1016/j.jtos.2020.08.005.
24. Vehof J, Sillevis Smitt-Kamminga N, Nibourg SA, Hammond CJ. Sex differences in clinical characteristics of dry eye disease. *Ocul Surf*. 2018;16(2):242–248. doi:10.1016/j.jtos.2018.01.001.
25. Knop E, Knop N, Millar T, Obata H, Sullivan DA. The International Workshop on Meibomian Gland Dysfunction: report of the Subcommittee on Anatomy, Physiology, and Pathophysiology of the Meibomian Gland. *Investig Opthalmol Vis Sci*. 2011;52(4):1938. doi:10.1167/iovs.10-6997c.
26. Stapleton F, Abad JC, Barabino S, et al. TFOS lifestyle: impact of societal challenges on the ocular surface. *Ocul Surf*. 2023;28:165–199. doi:10.1016/j.jtos.2023.04.006.
27. Abusamak M. Corneal epithelial mapping characteristics in normal eyes using anterior segment spectral domain optical coherence tomography. *Transl Vis Sci Technol*. 2022;11(3):6. doi:10.1167/tvst.11.3.6.
28. Abu-Romman A, Scholand KK, Govindarajan G, et al. Age-related differences in the mouse corneal epithelial transcriptome and their impact on corneal wound healing. *Invest Ophthalmol Vis Sci*. 2024;65(5):21. doi:10.1167/iovs.65.5.21.
29. Pham TL, Kakazu A, He J, Bazan HEP. Mouse strains and sexual divergence in corneal innervation and nerve regeneration. *FASEB J*. 2018;33(3):4598–4609. doi:10.1096/fj.201801957R.
30. Abu-Romman A, Scholand KK, Pal-Ghosh S, et al. Conditional deletion of CD25 in the corneal epithelium reveals sex differences in barrier disruption. *Ocul Surf*. 2023;30:57–72. doi:10.1016/j.jtos.2023.07.008.
31. Mecum NE, Demers D, Sullivan CE, Denis TE, Kalliel JR, Meng ID. Lacrimal gland excision in male and female mice causes ocular pain and anxiety-like behaviors. *Sci Rep*. 2020;10(1):17225. doi:10.1038/s41598-020-73945-w.
32. Culoso A, Lowe C, Evinger C. Sex, blinking, and dry eye. *J Neurophysiol*. 2020;123(2):831–842. doi:10.1152/jn.00635.2019.
33. Sakiani S, Olsen NJ, Kovacs WJ. Gonadal steroids and humoral immunity. *Nat Rev Endocrinol*. 2012;9(1):56–62. doi:10.1038/nrendo.2012.206.
34. Zhao R, Chen X, Ma W, et al. A GPR174–CCL21 module imparts sexual dimorphism to humoral immunity. *Nature*. 2019;577(7790):416–420. doi:10.1038/s41586-019-1873-0.
35. Schmiedel BJ, Singh D, Madrigal A, et al. Impact of genetic polymorphisms on human immune cell gene expression. *Cell*. 2018;175(6):1701–1715 .e16. doi:10.1016/j.cell.2018.10.022.
36. McCoy SS, Sampene E, Baer AN. Association of Sjögren's syndrome with reduced lifetime sex hormone exposure: a case–control study. *Arthritis Care Res (Hoboken)*. 2020;72(9):1315–1322. doi:10.1002/acr.24014.
37. Lopes-Ramos CM, Chen C-Y, Kuijjer ML, et al. Sex differences in gene expression and regulatory networks across 29 human tissues. *Cell Rep*. 2020;31(12):107795. doi:10.1016/j.celrep.2020.107795.
38. Anger JT, Case LK, Baranowski AP, et al. Pain mechanisms in the transgender individual: a review. *Front Pain Res (Lausanne)*. 2024;5:1241015. doi:10.3389/fpain.2024.1241015.
39. Mogil JS. Qualitative sex differences in pain processing: emerging evidence of a biased literature. *Nat Rev Neurosci*. 2020;21(7):353–365. doi:10.1038/s41583-020-0310-6.
40. Belmonte C, Nichols JJ, Cox SM, et al. TFOS DEWS II pain and sensation report. *Ocul Surf*. 2017;15(3):404–437. doi:10.1016/j.jtos.2017.05.002.
41. Li W, MC Lin. Sex disparity in how pain sensitivity influences dry eye symptoms. *Cornea*. 2019;38(10):1291–1298. doi:10.1097/ico.0000000000002050.

42. Li K, Zhang C, Yang Z, Wang Y, Si H. Evaluation of a novel dry eye model induced by oral administration of finasteride. *Mol Med Rep*. 2017;16(6):8763–8770. doi:10.3892/mmr.2017.7754.
43. Morthen MK, Tellefsen S, Richards SM, et al. Testosterone influence on gene expression in lacrimal glands of mouse models of Sjögren syndrome. *Investig Opthalmol Vis Sci*. 2019;60(6):2181. doi:10.1167/iovs.19-26815.
44. Sahin A, Liu Y, Kam WR, Darabad RR, Sullivan DA. Dihydrotestosterone suppression of proinflammatory gene expression in human meibomian gland epithelial cells. *Ocul Surf*. 2020;18(2):199–205. doi:10.1016/j.jtos.2020.02.006.
45. Zibandeh N, Yıldız E, Özer B, Taş AY, Sahin A. Androgen suppresses hyperosmolarity-induced inflammatory mediators in human corneal epithelial cells. *Cornea*. 2020;39(7):886–891. doi:10.1097/ico.0000000000002291.
46. Aydin Kurna S, Oflaz Hacisalihoglu A, Altun A, et al. Effects of systemic anti-androgen drugs on the ocular surface. *J Fr Ophtalmol*. 2022;45(6):619–627. doi:10.1016/j.jfo.2021.06.007.
47. Shimizu S, Arita R, Kawashima M, Inoue S, Itoh K, Tsubota K. Morphological changes of meibomian glands in men with benign prostate hyperplasia. *Cornea*. 2021;40(4):445–448. doi:10.1097/ICO.0000000000002454.
48. Supalaset S, Tananuvat N, Pongsatha S, Chaidaroon W, Ausayakhun S. A randomized controlled double-masked study of transdermal androgen in dry eye patients associated with androgen deficiency. *Am J Ophthalmol*. 2019;197:136–144. doi:10.1016/j.ajo.2018.09.021.
49. Lixiang Wang, Huang L, Qiu Shuang, Deng Y. Effect of androgen replacement therapy on mixed dry eye in a rabbit model. *Zhonghua Yi Xue Za Zhi*. 2021;101(32):2525–2530. doi:10.3760/cma.j.cn112137-20210322-00713.
50. Kiyat P, Palamar M, Gerceker Turk B, Yagci A. Evaluation of dry eye and meibomian gland dysfunction in female androgenetic alopecia patients. *Int Ophthalmol*. 2021;41(11):3749–3757. doi:10.1007/s10792-021-01946-3.
51. Carmina E, Azziz R, Bergfeld W, et al. Female pattern hair loss and androgen excess: a report from the multidisciplinary androgen excess and PCOS committee. *J Clin Endocrinol Metab*. 2019;104(7):2875–2891. doi:10.1210/jc.2018-02548.
52. Qi X, Yang Y, Xiong D, Wu S, Cui G, Zhang Q. ER-1 deficiency induces inflammation and lipid deposition in meibomian gland and lacrimal gland. *Biochem Biophys Res Commun*. 2024;696:149526. doi:10.1016/j.bbrc.2024.149526.
53. Nalvarte I, Antonson P. Estrogen receptor knockout mice and their effects on fertility. *Receptors*. 2023;2(1):116–126. doi:10.3390/receptors2010007.
54. Hewitt SC, Couse JF, Korach KS. Estrogen receptor transcription and transactivation Estrogen receptor knockout mice: what their phenotypes reveal about mechanisms of estrogen action. *Breast Cancer Res*. 2000;2(5):345–352. doi:10.1186/bcr79.
55. Winn NC, Jurrissen TJ, Grunewald ZI, et al. Estrogen receptor-α signaling maintains immunometabolic function in males and is obligatory for exercise-induced amelioration of nonalcoholic fatty liver. *Am J Physiol Endocrinol Metab*. 2019;316(2):E156–E167. doi:10.1152/ajpendo.00259.2018.
56. Li C, Zhang X, Wang Y, Li M. Development and long-term evaluation of ocular surface conditions using ovariectomized cynomolgus monkey. *Eye Contact Lens*. 2019;45(4):246–253. doi:10.1097/icl.0000000000000563.
57. Kumar V, Sur VP, Guha R, Konar A, Hazra S. Estrogen modulates corneal nociception and maintains corneal homeostasis in rat eye. *Cornea*. 2017;37(4):508–514. doi:10.1097/ico.0000000000001437.
58. Rodríguez-Landa JF. Considerations of timing postovariectomy in mice and rats in studying anxiety- and depression-like behaviors associated with surgical menopause in women. *Front Behav Neurosci*. 2022;16:829274. doi:10.3389/fnbeh.2022.829274.
59. Bereiter DA, Rahman M, Ahmed F, Thompson R, Luong N, Olson JK. Title: P2x7 receptor activation and estrogen status drive neuroinflammatory mechanisms in a rat model for dry eye. *Front Pharmacol*. 2022;13:827244. doi:10.3389/fphar.2022.827244.
60. Rahimi Darabad R, Suzuki T, Richards SM, et al. Does estrogen deficiency cause lacrimal gland inflammation and aqueous-deficient dry eye in mice? *Exp Eye Res*. 2014;127:153–160. doi:10.1016/j.exer.2014.07.017.
61. Rahimi Darabad R, Suzuki T, Richards SM, et al. Influence of aromatase absence on the gene expression and histology of the mouse meibomian gland. *Investig Opthalmol Vis Sci*. 2013;54(2):987. doi:10.1167/iovs.12-10992.
62. Machairoudia G, Kazantzis D, Chatziralli I, et al. Prevalence and risk factors of dry eye disease in patients treated with aromatase inhibitors for breast cancer. *Clin Exp Optom*. 2023;107(4):415–419. doi:10.1080/08164622.2023.2239247.
63. Ağın A, Kocabeyoglu S, Yucel Gencoglu A, Aksoy S, Karakaya J, Irkec M. The effects of systemic aromatase inhibitors on meibomian glands and corneal structure. *Eye*. 2021;36(6):1185–1193. doi:10.1038/s41433-021-01612-z.
64. Khoo P, Groeneveld T, Boyle F, O'Neill S, Forster B, Watson SL. Dry eye signs and symptoms in patients on aromatase inhibitor therapy. *Eye*. 2021;36(4):766–772. doi:10.1038/s41433-021-01538-6.
65. Bicer T, Imamoglu GI, Dogan AS, et al. The effects of adjuvant hormonotherapy on tear functions in patients with breast cancer. *Int Ophthalmol*. 2020;40(8):2077–2083. doi:10.1007/s10792-020-01384-7.
66. Kharb R, Haider K, Neha K, Yar MS. Aromatase inhibitors: role in postmenopausal breast cancer. *Arch Pharm (Weinheim)*. 2020;353(8):e2000081. doi:10.1002/ardp.202000081.
67. García-Sánchez J, Mafla-España MA, Torregrosa MD, Cauli O. Adjuvant aromatase inhibitor treatment worsens depressive symptoms and sleep quality in postmenopausal women with localized breast cancer: a one-year follow-up study. *Breast*. 2022;66:310–316. doi:10.1016/j.breast.2022.11.007.
68. Zhu Y, Huang X, Lin L, et al. Sleep quality is associated with severe meibomian gland disruption in dry eye. *Front Med*. 2022;9:812705. doi:10.3389/fmed.2022.812705.
69. Boga A, Stapleton F, Chapman M, Golebiowski B. Effects of elevated serum estrogen on dry eye in women undergoing

in vitro fertilisation. *Ocul Surf*. 2023;29:511–520. doi:10.1016/j.jtos.2023.06.015.
70. Çolak E, Ulusoy MO, Ceran MU, Taşdemir Ü, Kal A, Özçimen EE. Assessment of tear parameters in patients undergoing ovulation induction for a short period using optical coherence tomography of the anterior segment. *Arq Bras Oftalmol*. 2020;83(6):511–516. doi:10.5935/0004-2749.20200095.
71. Huang LH, Kuo CP, Lu YC, Lee MS, Lee SH. Association of emotional distress and quality of sleep among women receiving in-vitro fertilization treatment. *Taiwanese J Obstet Gynecol*. 2019;58(1):168–172. doi:10.1016/j.tjog.2018.11.031.
72. Schmidl D, Szalai L, Kiss OG, Schmetterer L, Garhöfer G. A Phase II, multicenter, randomized, placebo-controlled, double-masked trial of a topical estradiol ophthalmic formulation in postmenopausal women with moderate-to-severe dry eye disease. *Adv Ther*. 2021;38(4):1975–1986. doi:10.1007/s12325-021-01680-3.
73. Yang M, Fjærvoll HK, Fjærvoll KA, et al. Sex-based differences in conjunctival goblet cell responses to pro-inflammatory and pro-resolving mediators. *Sci Rep*. 2022;12(1):16305. doi:10.1038/s41598-022-20177-9.
74. Straub RH. The complex role of estrogens in inflammation. *Endocr Rev*. 2007;28(5):521–574. doi:10.1210/er.2007-0001.
75. Meng ID, Barton ST, Goodney I, Russell R, Mecum NE. Progesterone application to the rat forehead produces corneal antinociception. *Investig Opthalmol Vis Sci*. 2019;60(5):1706. doi:10.1167/iovs.18-26049.
76. Luo ZK, Domenech-Estarellas EA, Han A, et al. Efficacy and safety of 1% progesterone gel to the forehead for ocular chronic graft-versus-host disease. *Transplant Cell Ther*. 2021;27(5):433.e1–433.e8. doi:10.1016/j.jtct.2021.02.008.
77. Piette PCM. The pharmacodynamics and safety of progesterone. *Best Pract Res Clin Obstet Gynaecol*. 2020;69:13–29. doi:10.1016/j.bpobgyn.2020.06.002.
78. Kontula K, Paavonen T, Luukkainen T, Andersson LC. Binding of progestins to the glucocorticoid receptor. *Biochem Pharmacol*. 1983;32(9):1511–1518. doi:10.1016/0006-2952(83)90474-4.
79. Robciuc A, Savolainen-Peltonen H, Haanpää M, Moilanen JAO, Mikkola TS. Sex steroid hormone analysis in human tear fluid using a liquid chromatography—mass spectrometry method. *Int J Mol Sci*. 2022;23(23):14864. doi:10.3390/ijms232314864.
80. Phan MAT, Gibson E, Golebiowski B, Stapleton F, Jenner AM, Bucknall MP. Analysis of sex steroids in human tears using LC-MS and GC-MS: considerations and developments to improve method sensitivity and accuracy. *Exp Eye Res*. 2022;225:109283. doi:10.1016/j.exer.2022.109283.
81. Gibson EJ, Bucknall MP, Golebiowski B, Stapleton F. Comparative limitations and benefits of liquid chromatography – mass spectrometry techniques for analysis of sex steroids in tears. *Exp Eye Res*. 2019;179:168–178. doi:10.1016/j.exer.2018.11.015.
82. Methodologies to diagnose and monitor dry eye disease: report of the diagnostic methodology subcommittee of the international dry eye workshop (2007). *Ocul Surf*. 2007;5(2):108–152. doi:10.1016/s1542-0124(12)70083-6.
83. Patel R, Zhu M, Robertson DM. Shifting the IGF-axis: an age-related decline in human tear IGF-1 correlates with clinical signs of dry eye. *Growth Horm IGF Res*. 2018;40:69–73. doi:10.1016/j.ghir.2018.02.001.
84. Stuard Sambhariya W, Trautmann IJ, Robertson DM. Insulin-like growth factor binding protein-3 mediates hyperosmolar stress–induced mitophagy through the mechanistic target of rapamycin. *J Biol Chem*. 2023;299(11):105239. doi:10.1016/j.jbc.2023.105239.
85. Bisetty Khan E. Prevalence of dry eye syndrome in a South African diabetic paediatric population. *Cont Lens Anterior Eye*. 2024;47(5):102175. doi:10.1016/j.clae.2024.102175.
86. Bayat AH, Aydemir E, Aydemir GA, Gencer H. Assessment of tear film anomalies in childhood obesity. *Klin Monbl Augenheilkd*. 2021;239(03):331–337. doi:10.1055/a-1668-0276.
87. Kasturi N, Manchikanti V, Rajappa M, Gochhait D. Ocular surface disorder among adult patients with type II diabetes mellitus and its correlation with tear film markers: a pilot study. *Taiwan J Ophthalmol*. 2021;11(2):156. doi:10.4103/tjo.tjo_56_20.
88. Zagon IS, Sassani JW, Purushothaman I, McLaughlin PJ. Dysregulation of the OGF–OGFr pathway correlates with elevated serum OGF and ocular surface complications in the diabetic rat. *Exp Biol Med*. 2020;245(15):1414–1421. doi:10.1177/1535370220940273.
89. Burgos-Blasco B, Diaz-Valle D, Rego-Lorca D, et al. Topical insulin, a novel corneal epithelial regeneration agent in dry eye disease. *Eur J Ophthalmol*. 2023;34(3):719–725. doi:10.1177/11206721231206790.
90. Tahmaz V, Menghesha L, Stern ME, Holtick U, Scheid C, Steven P. Insulin eye drops for severe refractory chronic ocular graft-versus-host disease. *Bone Marrow Transplant*. 2024;59(7):1031–1033. doi:10.1038/s41409-024-02272-9.
91. Aniah Azmi N, Bastion MLC. Short-term results of trial of topical insulin for treatment of dry eyes in diabetics. *Eye Contact Lens*. 2020;46(1):S25–S32. doi:10.1097/icl.0000000000000623.
92. Cid-Bertomeu P, Vilaltella M, Martínez M, Mir M, Huerva V. Topical insulin for ocular surface disease. *J Ocul Pharmacol Ther*. 2024;40(4):204–214. doi:10.1089/jop.2024.0016.
93. Cruz-Cazarim ELC, Cazarim MS, Ogunjimi AT, Petrilli R, Rocha EM, Lopez RFV. Prospective insulin-based ophthalmic delivery systems for the treatment of dry eye syndrome and corneal injuries. *Eur J Pharm Biopharm*. 2019;140:1–10. doi:10.1016/j.ejpb.2019.04.014.
94. Xia Y, Zhang Y, Du Y, Wang Z, Cheng L, Du Z. Comprehensive dry eye therapy: overcoming ocular surface barrier and combating inflammation, oxidation, and mitochondrial damage. *J Nanobiotechnology*. 2024;22(1):233. doi:10.1186/s12951-024-02503-7.
95. Dasari S, Naha K, Hande M, Vivek G. Hot and cold: coexistent Graves' disease and Hashimoto's thyroiditis in a patient with Schmidt's syndrome. *BMJ Case Rep*. 2014 bcr2013010432. doi:10.1136/bcr-2013-010432.
96. Yu K, Bunya V, Maguire M, et al. Systemic conditions associated with severity of dry eye signs and symptoms in the Dry Eye Assessment and Management Study. *Ophthalmology*. 2021;128(10):1384–1392. doi:10.1016/j.ophtha.2021.03.030.

97. Bikbov MM, Gilmanshin TR, Zainullin RM, et al. Prevalence and associations of dry eye disease and meibomian gland dysfunction in the Ural Eye and Medical Study. *Sci Rep*. 2022;12(1):18849. doi:10.1038/s41598-022-22580-8.
98. Garcia-Queiruga J, Pena-Verdeal H, Sabucedo-Villamarin B, Giraldez MJ, Garcia-Resua C, Yebra-Pimentel E. A cross-sectional study of non-modifiable and modifiable risk factors of dry eye disease states. *Cont Lens Anterior Eye*. 2023;46(3):101800. doi:10.1016/j.clae.2022.101800.
99. Dossari SK, Alkhars AZ, Albaqshi AA, et al. Prevalence of dry eye disease and its risk factors among the general population of Saudi Arabia: a cross-sectional survey. *Cureus*. 2022;14(12):E32552. doi:10.7759/cureus.32552.
100. Lai EW, Tai YH, Wu HL, et al. The association between autoimmune thyroid disease and ocular surface damage: a retrospective population-based cohort study. *J Clin Med*. 2023;12(9):3203. doi:10.3390/jcm12093203.
101. Rana HS, Akella SS, Clabeaux CE, Skurski ZP, Aakalu VK. Ocular surface disease in thyroid eye disease: a narrative review. *Ocul Surf*. 2022;24:67–73. doi:10.1016/j.jtos.2022.02.001.
102. Faustino-Barros JF, Saranzo Sant'Ana AM, Dias LC, et al. Distinct inflammatory and oxidative effects of diabetes mellitus and hypothyroidism in the lacrimal functional unit. *Int J Mol Sci*. 2023;24(8):6974. doi:10.3390/ijms24086974.
103. Liao X, Aljufairi FMAA, Sebastian JU, et al. Investigation of lacrimal gland and extraocular muscle in thyroid eye disease patients with severe subjective dry eye disease: a multiparametric magnetic resonance imaging study. *Graefes Arch Clin Exp Ophthalmol*. 2024;262(8):2651–2659. doi:10.1007/s00417-024-06424-x.
104. Zhang K, Yu X, Zhang Y, et al. Identification of key genes in salivary gland in Sjögren's syndrome complicated with Hashimoto thyroiditis: common pathogenesis and potential diagnostic markers. *Medicine*. 2023;102(39):e35188. doi:10.1097/md.0000000000035188.
105. Allam IY, Lazreg S, Shafik Shaheen M, Doheim MF, Mohammed MA. Ocular surface changes in patients with thyroid eye disease: an observational clinical study. *Clin Ophthalmol*. 2021;15:2481–2488. doi:10.2147/opth.s317708.
106. Park J, Baek S. Dry eye syndrome in thyroid eye disease patients: the role of increased incomplete blinking and meibomian gland loss. *Acta Ophthalmol*. 2018;97(5):e800–e806. doi:10.1111/aos.14000.
107. Alanazi SA, Alomran AA, Abusharha A, et al. An assessment of the ocular tear film in patients with thyroid disorders. *Clin Ophthalmol*. 2019;13:1019–1026. doi:10.2147/opth.s210044.
108. Liao X, Lai KKH, Aljufairi FMAA, et al. Ocular surface changes in treatment-naive thyroid eye disease. *J Clin Med*. 2023;12(9):3066. doi:10.3390/jcm12093066.
109. Lai KKH, Liao X, Aljufairi FMAA, et al. Ocular surface and meibomian gland evaluation in euthyroid Graves' ophthalmopathy. *Int Ophthalmol*. 2024;44(1):124. doi:10.1007/s10792-024-02919-y.
110. Riguetto C, Barbosa EB, Atihe CC, Reis F, Alves M, Zantut-Wittmann D. Ocular surface disease related to the inflammatory and non-inflammatory phases of thyroid eye disease. *Clin Ophthalmol*. 2023;17:3465–3475. doi:10.2147/opth.s430861.
111. Takahashi Y, Vaidya A, Kakizaki H. Risk factors for development of superior limbic keratoconjunctivitis in thyroid eye disease in Japanese. *Graefes Arch Clin Exp Ophthalmol*. 2022;261(3):833–839. doi:10.1007/s00417-022-05827-y.
112. Kiziltoprak H, Koc M, Tekin K, et al. Local ocular surface alterations in children with Hashimoto's thyroiditis. *Ocul Immunol Inflamm*. 2019;28(5):791–797. doi:10.1080/09273948.2019.1642495.
113. Kashkouli MB, Alemzadeh SA, Aghaei H, et al. Subjective versus objective dry eye disease in patients with moderate-severe thyroid eye disease. *Ocul Surf*. 2018;16(4):458–462. doi:10.1016/j.jtos.2018.07.003.
114. Starčević A, Radojičić Z, Djurić Stefanović A, et al. Morphometric and volumetric analysis of lacrimal glands in patients with thyroid eye disease. *Sci Rep*. 2023;13(1):16345. doi:10.1038/s41598-023-43083-0.
115. Wong NTY, Yuen KFK, Aljufairi FMAA, et al. Magnetic resonance imaging parameters on lacrimal gland in thyroid eye disease: a systematic review and meta-analysis. *BMC Ophthalmol*. 2023;23(1):347. doi:10.1186/s12886-023-03008-x.
116. Park J, Kim J, Lee H, Park M, Baek S. Functional and structural evaluation of the meibomian gland using a LipiView interferometer in thyroid eye disease. *Can J Ophthalmol*. 2018;53(4):373–379. doi:10.1016/j.jcjo.2017.11.006.
117. Winter S, Diamond M, Green J, et al. Transgender people: health at the margins of society. *Lancet*. 2016;388(10042):390–400. doi:10.1016/S0140-6736(16)00683-8.
118. Nieves-Ríos C, Pulido JS, Thornton S, et al. Instances of ocular findings in transgender patients undergoing hormonal therapy. *Am J Ophthalmol Case Rep*. 2023;32:101965. doi:10.1016/j.ajoc.2023.101965.
119. Berrian K, Exsted MD, Lampe NM, Pease SL, Akré ERL. Barriers to quality healthcare among transgender and gender nonconforming adults. *Health Serv Res*. 2025;60(1):e14362. doi:10.1111/1475-6773.14362.
120. Maoto M, Davis B. Breaking barriers: how transwomen meet their healthcare needs. *Afr J Prim Health Care Fam Med*. 2024;16(1):e1–e6. doi:10.4102/phcfm.v16i1.4598.
121. Zhang X, Neuville P, Skokan AJ. Sexual health in transgender and gender diverse people. *Curr Opin Urol*. 2024;34(5):330–335. doi:10.1097/mou.0000000000001205.
122. Coelho R, Gonçalves R, Mendes F, Macedo G. Gastroenterology healthcare in LGBTQ+ individuals. *Eur J Gastroenterol Hepatol*. 2024;36(9):1059–1067. doi:10.1097/meg.0000000000002808.
123. Ceolin C, Papa MV, Scala A, Sergi G, Garolla A. Getting old in the desired gender: a systematic review on aging diseases in transgender people. *J Endocrinol Investig*. 2024;47(8):1851–1862. doi:10.1007/s40618-024-02353-y.
124. Betsi G, Goulia P, Sandhu S, Xekouki P. Puberty suppression in adolescents with gender dysphoria: an emerging issue with multiple implications. *Front Endocrinol*. 2024;15:1309904. doi:10.3389/fendo.2024.1309904.
125. Heng YJ, Baker GM, VJ Fein-Zachary, et al. Effect of testosterone therapy on breast tissue composition and mammographic breast density in trans masculine individuals. *Breast Cancer Res*. 2024;26(1):109. doi:10.1186/s13058-024-01867-w.

126. Hashemi L, Marijic Buljubasic A, Budoff MJ, et al. Gender-affirming hormone treatment and metabolic syndrome among transgender veterans. *JAMA Network Open*. 2024;7(7):e2419696. doi:10.1001/jamanetworkopen.2024.19696.
127. de Silva NL, Dimakopoulou A, Quinton O, Jayasena CN. Metabolic and cardiovascular risks of hormone treatment for transgender individuals. *Best Pract Res Clin Endocrinol Metab*. 2024;38(5):101907. doi:10.1016/j.beem.2024.101907.
128. Tienforti D, Savignano G, Spagnolo L, Di Giulio F, Baroni MG, Barbonetti A. Biochemical liver damage during gender affirming therapy in trans adults assigned female at birth: a meta-analysis. *J Endocrinol Investig*. 2025;48(1):161–171. doi:10.1007/s40618-024-02418-y.
129. Jelicic Kadic A, Vucic K, Dosenovic S, Sapunar D, Puljak L. Extracting data from figures with software was faster, with higher interrater reliability than manual extraction. *J Clin Epidemiol*. 2016;74:119–123. doi:10.1016/j.jclinepi.2016.01.002.
130. Wolffsohn JS, Arita R, Chalmers R, et al. TFOS DEWS II diagnostic methodology report. *Ocul Surf*. 2017;15(3):539–574. doi:10.1016/j.jtos.2017.05.001.
131. Arita R, Mizoguchi T, Kawashima M, et al. Meibomian gland dysfunction and dry eye are similar but different based on a population-based study: the Hirado-Takushima Study in Japan. *Am J Ophthalmol*. 2019;207:410–418. doi:10.1016/j.ajo.2019.02.024.
132. Ma J, Zhu H, Guo W, et al. Association of different digital media experiences with paediatric dry eye in China: a population-based study. *BMJ Open*. 2022;12(11):e062850. doi:10.1136/bmjopen-2022-062850.
133. Stang A, Schmidt B, Schramm S, et al. Synergism between coexisting eye diseases and sex in increasing the prevalence of the dry eye syndrome. *Sci Rep*. 2024;14(1):314. doi:10.1038/s41598-023-50871-1.
134. Uchino M, Nishiwaki Y, Michikawa T, et al. Prevalence and risk factors of dry eye disease in Japan: Koumi study. *Ophthalmology*. 2011;118(12):2361–2367. doi:10.1016/j.ophtha.2011.05.029.
135. Vehof J, Wang B, Kozareva D, Hysi PG, Snieder H, Hammond CJ. The heritability of dry eye disease in a female twin cohort. *Invest Ophthalmol Vis Sci*. 2014;55(11):7278–7283. doi:10.1167/iovs.14-15200.
136. Schaumberg DA, Dana R, Buring JE, Sullivan DA. Prevalence of dry eye disease among US men: estimates from the Physicians' Health Studies. *Arch Ophthalmol*. 2009;127(6):763–768. doi:10.1001/archophthalmol.2009.103.
137. Schaumberg DA, Sullivan DA, Buring JE, Dana MR. Prevalence of dry eye syndrome among US women. *Am J Ophthalmol*. 2003;136(2):318–326. doi:10.1016/s0002-9394(03)00218-6.
138. Zhang Y, Chen H, Wu X. Prevalence and risk factors associated with dry eye syndrome among senior high school students in a county of Shandong Province, China. *Ophthal Epidemiol*. 2012;19(4):226–230. doi:10.3109/09286586.2012.670742.
139. Ahn JH, Choi YH, Paik HJ, Kim MK, Wee WR, Kim DH. Sex differences in the effect of aging on dry eye disease. *Clin Interv Aging*. 2017;12:1331–1338. doi:10.2147/CIA.S140912.
140. Magno MS, Utheim TP, Snieder H, Hammond CJ, Vehof J. The relationship between dry eye and sleep quality. *Ocul Surf*. 2021;20:13–19. doi:10.1016/j.jtos.2020.12.009.
141. Malet F, Le Goff M, Colin J, et al. Dry eye disease in French elderly subjects: the Alienor Study. *Acta Ophthalmol*. 2014;92(6):e429–e436. doi:10.1111/aos.12174.
142. Paulsen AJ, Cruickshanks KJ, Fischer ME, et al. Dry eye in the Beaver Dam Offspring Study: prevalence, risk factors, and health-related quality of life. *Am J Ophthalmol*. 2014;157(4):799–806. doi:10.1016/j.ajo.2013.12.023.
143. Munch K, Nohre M, Westenberger A, et al. Prevalence and correlates of dry eye in a German population sample. *Cornea*. 2024;43(6):685–692. doi:10.1097/ICO.0000000000003374.
144. Kai JY, Wu YB, Shi B, et al. Dry eye symptoms and health-related quality of life among Chinese individuals: a national-based study. *Br J Ophthalmol*. 2024;108(11):1500–1507. doi:10.1136/bjo-2023-324677.
145. Li X, Wang Z, Mu J, et al. Prevalence and associated risk factors of dry eye disease in Hotan, Xinjiang: a cross-sectional study. *BMC Ophthalmol*. 2023;23(1):214. doi:10.1186/s12886-023-02955-9.
146. Kim JM, Choi YJ. Impact of dietary nutrients on the prevalence of dry eye syndrome among Korean women aged 40 and above: evidence from the Korea National Health and Nutrition Examination Survey. *Nutrients*. 2024;16(3):372. doi:10.3390/nu16030372.
147. Viet Vu CH, Uchino M, Kawashima M, et al. Lack of social support and social trust as potential risk factors for dry eye disease: JPHC-NEXT study. *Ocul Surf*. 2019;17(2):278–284. doi:10.1016/j.jtos.2019.01.005.
148. Uchino M, Schaumberg DA, Dogru M, et al. Prevalence of dry eye disease among Japanese visual display terminal users. *Ophthalmology*. 2008;115(11):1982–1988. doi:10.1016/j.ophtha.2008.06.022.
149. McCarty CA, Bansal AK, Livingston PM, Stanislavsky YL, Taylor HR. The epidemiology of dry eye in Melbourne, Australia. *Ophthalmology*. 1998;105(6):1114–1119. doi:10.1016/S0161-6420(98)96016-X.
150. Ahn JM, Lee SH, Rim TH, et al. Prevalence of and risk factors associated with dry eye: the Korea National Health and Nutrition Examination Survey 2010-2011. *Am J Ophthalmol*. 2014;158(6):1205–1214 .e7. doi:10.1016/j.ajo.2014.08.021.
151. Hashemi H, Khabazkhoob M, Kheirkhah A, et al. Prevalence of dry eye syndrome in an adult population. *Clin Exp Ophthalmol*. 2014;42(3):242–248. doi:10.1111/ceo.12183.
152. Lu P, Chen X, Liu X, et al. Dry eye syndrome in elderly Tibetans at high altitude: a population-based study in China. *Cornea*. 2008;27(5):545–551. doi:10.1097/ICO.0b013e318165b1b7.
153. Tong L, Saw SM, Lamoureux EL, et al. A questionnaire-based assessment of symptoms associated with tear film dysfunction and lid margin disease in an Asian population. *Ophthal Epidemiol*. 2009;16(1):31–37. doi:10.1080/09286580802521317.
154. Um SB, Kim NH, Lee HK, Song JS, Kim HC. Spatial epidemiology of dry eye disease: findings from South Korea.

Int J Health Geogr. 2014;13:31. doi:10.1186/1476-072X-13-31.
155. Guo B, Lu P, Chen X, Zhang W, Chen R. Prevalence of dry eye disease in Mongolians at high altitude in China: the Henan Eye Study. *Ophthal Epidemiol*. 2010;17(4):234–241. doi:10.3109/09286586.2010.498659.
156. Tan LL, Morgan P, Cai ZQ, Straughan RA. Prevalence of and risk factors for symptomatic dry eye disease in Singapore. *Clin Exp Optom*. 2015;98(1):45–53. doi:10.1111/cxo.12210.
157. Schein OD, Tielsch JM, Munoz B, Bandeen-Roche K, West S. Relation between signs and symptoms of dry eye in the elderly. A population-based perspective. *Ophthalmology*. 1997;104(9):1395–1401.
158. Lin PY, Tsai SY, Cheng CY, Liu JH, Chou P, Hsu WM. Prevalence of dry eye among an elderly Chinese population in Taiwan: the Shihpai Eye Study. *Ophthalmology*. 2003;110(6):1096–1101. doi:10.1016/S0161-6420(03)00262-8.
159. Lee AJ, Lee J, Saw SM, et al. Prevalence and risk factors associated with dry eye symptoms: a population based study in Indonesia. *Br J Ophthalmol*. 2002;86(12):1347–1351. doi:10.1136/bjo.86.12.1347.
160. Bakkar MM, Shihadeh WA, Haddad MF, Khader YS. Epidemiology of symptoms of dry eye disease (DED) in Jordan: a cross-sectional non-clinical population-based study. *Cont Lens Anterior Eye*. 2016;39(3):197–202. doi:10.1016/j.clae.2016.01.003.
161. Ferrero A, Alassane S, Binquet C, et al. Dry eye disease in the elderly in a French population-based study (the Montrachet study: maculopathy, optic nerve, nuTRition, neurovAsCular and HEarT diseases): prevalence and associated factors. *Ocul Surf*. 2018;16(1):112–119. doi:10.1016/j.jtos.2017.09.008.
162. Shanti Y, Shehada R, Bakkar MM, Qaddumi J. Prevalence and associated risk factors of dry eye disease in 16 northern West Bank towns in Palestine: a cross-sectional study. *BMC Ophthalmol*. 2020;20(1):26. doi:10.1186/s12886-019-1290-z.
163. Yasir ZH, Chauhan D, Khandekar R, Souru C, Varghese S. Prevalence and determinants of dry eye disease among 40 years and older population of Riyadh (except capital), Saudi Arabia. *Middle East Afr J Ophthalmol*. 2019;26(1):27–32. doi:10.4103/meajo.MEAJO_194_18.
164. Bikbov MM, Kazakbaeva GM, Rakhimova EM, et al. The prevalence of dry eye in a very old population. *Acta Ophthalmol*. 2022;100(3):262–268. doi:10.1111/aos.14937.
165. Kobia-Acquah E, Ankamah-Lomotey S, Owusu E, et al. Prevalence and associated risk factors of symptomatic dry eye in Ghana: a cross-sectional population-based study. *Cont Lens Anterior Eye*. 2021;44(6):101404. doi:10.1016/j.clae.2020.12.067.
166. Choi HR, Kim NH, Lee JM, et al. Risk factors influencing the occurrence and severity of symptomatic dry eye syndrome: a cross-sectional study. *Ophthal Epidemiol*. 2021;28(6):488–494. doi:10.1080/09286586.2021.1879172.
167. Kim SW, Brown DJ, Jester JV. Transcriptome analysis after PPARγ activation in human meibomian gland epithelial cells (hMGEC). *Ocul Surf*. 2019;17(4):809–816. doi:10.1016/j.jtos.2019.02.003.
168. Olaniyan SI, Fasina O, Bekibele CO, Ogundipe AO. Dry eye disease in an adult population in South-West Nigeria. *Cont Lens Anterior Eye*. 2016;39(5):359–364. doi:10.1016/j.clae.2016.06.008.
169. Tandon R, Vashist P, Gupta N, et al. Association of dry eye disease and sun exposure in geographically diverse adult (>/=40 years) populations of India: the SEED (Sun Exposure, Environment and Dry Eye Disease) study - Second report of the ICMR-EYE SEE study group. *Ocul Surf*. 2020;18(4):718–730. doi:10.1016/j.jtos.2020.07.016.
170. Dana R, Bradley JL, Guerin A, et al. Estimated prevalence and incidence of dry eye disease based on coding analysis of a large, all-age United States Health Care System. *Am J Ophthalmol*. 2019;202:47–54. doi:10.1016/j.ajo.2019.01.026.
171. Roh HC, Lee JK, Kim M, et al. Systemic comorbidities of dry eye syndrome: the Korean National Health and Nutrition Examination Survey V, 2010 to 2012. *Cornea*. 2016;35(2):187–192. doi:10.1097/ICO.0000000000000657.
172. Lim MN, Lee JY, Hyon JY, Han SB. Association of self-reported psychiatric and systemic risk factors in dry eye disease in adult Korean population. *Eye (Lond)*. 2024;38(5):917–922. doi:10.1038/s41433-023-02803-6.
173. Siak JJK, Tong L, Wong WL, et al. Prevalence and risk factors of meibomian gland dysfunction: the Singapore Malay Eye Study. *Cornea*. 2012;31(11):1223–1228. doi:10.1097/ICO.0b013e31823f0977.
174. Viso E, Rodriguez-Ares MT, Abelenda D, Oubina B, Gude F. Prevalence of asymptomatic and symptomatic meibomian gland dysfunction in the general population of Spain. *Invest Ophthalmol Vis Sci*. 2012;53(6):2601–2606. doi:10.1167/iovs.11-9228.
175. Lin JB, Shen X, Pfeifer CW, et al. Dry eye disease in mice activates adaptive corneal epithelial regeneration distinct from constitutive renewal in homeostasis. *Proc Natl Acad Sci U S A*. 2023;120(2):e2204134120. doi:10.1073/pnas.2204134120.
176. Hashemi H, Rastad H, Emamian MH, Fotouhi A. Meibomian gland dysfunction and its determinants in Iranian adults: a population-based study. *Cont Lens Anterior Eye*. 2017;40(4):213–216. doi:10.1016/j.clae.2017.05.003.
177. Aapola U, Nattinen J, Suurkuukka I, et al. Ocular surface health of the Finnish elderly population. *Acta Ophthalmol*. 2022;100(8):894–902. doi:10.1111/aos.15130.
178. Aljarousha M, Badarudin NE, Che Azemin MZ, Aljeesh Y, Amer A, Abdul Rahim MAS. Prevalence and risk factors of dry eye disease in the South of Palestine. *Malays J Med Sci*. 2024;31(2):72–97. doi:10.21315/mjms2024.31.2.8.
179. Alshamrani AA, Almousa AS, Almulhim AA, et al. Prevalence and risk factors of dry eye symptoms in a Saudi Arabian population. *Middle East Afr J Ophthalmol*. 2017;24(2):67–73. doi:10.4103/meajo.MEAJO_281_16.
180. Betiku AO, Oduyoye OO, Jagun OO, Olajide OS, Adebusoye SO, Aham-Onyebuchi UO. Prevalence and risk factors associated with dry eye disease among adults in a population-based setting in South-West Nigeria. *Niger J Clin Pract*. 2022;25(3):354–360. doi:10.4103/njcp.njcp_1598_21.

181. Caffery B, Srinivasan S, Reaume CJ, et al. Prevalence of dry eye disease in Ontario, Canada: a population-based survey. *Ocul Surf*. 2019;17(3):526–531. doi:10.1016/j.jtos.2019.02.011.
182. Castro JS, Selegatto IB, Castro RS, et al. Prevalence and risk factors of self-reported dry eye in Brazil using a short symptom questionnaire. *Sci Rep*. 2018;8(1):2076. doi:10.1038/s41598-018-20273-9.
183. Chatterjee S, Agrawal D, Sanowar G, Kandoi R. Prevalence of symptoms of dry eye disease in an urban Indian population. *Indian J Ophthalmol*. 2021;69(5):1061–1066. doi:10.4103/ijo.IJO_1796_20.
184. Farrand KF, Fridman M, Stillman IO, Schaumberg DA. Prevalence of diagnosed dry eye disease in the United States among adults aged 18 years and older. *Am J Ophthalmol*. 2017;182:90–98. doi:10.1016/j.ajo.2017.06.033.
185. Gong YY, Zhang F, Zhou J, et al. Prevalence of dry eye in Uyghur and Han ethnic groups in Western China. *Ophthal Epidemiol*. 2017;24(3):181–187. doi:10.1080/09286586.2016.1263996.
186. Graue-Hernandez EO, Serna-Ojeda JC, Estrada-Reyes C, Navas A, Arrieta-Camacho J, Jimenez-Corona A. Dry eye symptoms and associated risk factors among adults aged 50 or more years in Central Mexico. *Salud Publica Mex*. 2018;60(5):520–527. doi:10.21149/9024.
187. Hashemi H, Asharlous A, Aghamirsalim M, et al. Meibomian gland dysfunction in geriatric population: Tehran geriatric eye study. *Int Ophthalmol*. 2021;41(7):2539–2546. doi:10.1007/s10792-021-01812-2.
188. Kim KI, Park YS, Kim RH, Kim JH. Factors associated with dry eye symptoms in elderly Koreans: the Fifth Korea National Health and Nutrition Examination Survey 2010-2012. *Korean J Fam Med*. 2019;40(1):22–30. doi:10.4082/kjfm.17.0058.
189. Lim EWL, Chong CCY, Nusinovici S, et al. Relationship between dry eye symptoms and quality of life: associations and mediation analysis. *Br J Ophthalmol*. Nov 2023;107(11):1606–1612. doi:10.1136/bjo-2022-321246.
190. Lin F, Cai Y, Fei X, Wang Y, Zhou M, Liu Y. Prevalence of dry eye disease among Chinese high school students during the COVID-19 outbreak. *BMC Ophthalmol*. 2022;22(1):190. doi:10.1186/s12886-022-02408-9.
191. Mai ELC, Lin CC, Lian I, Liao R, Chen M, Chang C. Population-based study on the epidemiology of dry eye disease and its association with presbyopia and other risk factors. *Int Ophthalmol*. 2019;39(12):2731–2739. doi:10.1007/s10792-019-01117-5.
192. Marculino LGC, Hazarbassanov RM, Hazarbassanov N, et al. Prevalence and risk factors for dry eye disease: the Sao Paulo Dry Eye Study. *Arq Bras Oftalmol*. 2022;85(6):549–557. doi:10.5935/0004-2749.202200100.
193. Ooi KG, Lee MH, Burlutsky G, Gopinath B, Mitchell P, Watson S. Association of dyslipidaemia and oral statin use, and dry eye disease symptoms in the Blue Mountains Eye Study. *Clin Exp Ophthalmol*. 2019;47(2):187–192. doi:10.1111/ceo.13388.
194. Park HW, Park JW. The association between symptoms of dry eye syndrome and metabolic outcome in a general population in Korea. *J Korean Med Sci*. 2016;31(7):1121–1126. doi:10.3346/jkms.2016.31.7.1121.
195. Reitmeir P, Linkohr B, Heier M, et al. Common eye diseases in older adults of southern Germany: results from the KORA-Age study. *Age Ageing*. 2017;46(3):481–486. doi:10.1093/ageing/afw234.
196. Tonkerdmongkol D, Poyomtip T, Poolsanam C, Watcharapalakorn A, Tawonkasiwattanakun P. Prevalence and associated factors for self-reported symptoms of dry eye among Thai school children during the COVID-19 outbreak. *PLoS One*. 2023;18(4):e0284928. doi:10.1371/journal.pone.0284928.
197. Wu SS, Fernando K, Allerton C, Jansen KU, Vincent MS, Dolsten M. Reviving an R&D pipeline: a step change in the Phase II success rate. *Drug Discov Today*. 2021;26(2):308–314. doi:10.1016/j.drudis.2020.10.019.
198. Donthineni PR, Kammari P, Shanbhag SS, Singh V, Das AV, Basu S. Incidence, demographics, types and risk factors of dry eye disease in India: electronic medical records driven big data analytics report I. *Ocul Surf*. 2019;17(2):250–256. doi:10.1016/j.jtos.2019.02.007.
199. Lin IC, Kuo YK, Liu HY, Chien LN. Trends in diagnosed dry eye disease incidence, 2001 to 2015: a nationwide population-based study in Taiwan. *Cornea*. 2022;41(11):1372–1377. doi:10.1097/ICO.0000000000002987.
200. Christen WG, Cook NR, Manson JE, et al. Efficacy of marine omega-3 fatty acid supplementation vs placebo in reducing incidence of dry eye disease in healthy US adults: a randomized clinical trial. *JAMA Ophthalmol*. 2022;140(7):707–714. doi:10.1001/jamaophthalmol.2022.1818.
201. Millan A, Viso E, Gude F, Parafita-Fernandez A, Morana N, Rodriguez-Ares MT. Incidence and risk factors of dry eye in a Spanish adult population: 11-year follow-up from the Salnes Eye Study. *Cornea*. 2018;37(12):1527–1534. doi:10.1097/ICO.0000000000001713.
202. Man REK, Veerappan AR, Tan SP, et al. Incidence and risk factors of symptomatic dry eye disease in Asian Malays from the Singapore Malay Eye Study. *Ocul Surf*. 2017;15(4):742–748. doi:10.1016/j.jtos.2017.04.004.
203. Lienert JP, Tarko L, Uchino M, Christen WG, Schaumberg DA. Long-term natural history of dry eye disease from the patient's perspective. *Ophthalmology*. 2016;123(2):425–433. doi:10.1016/j.ophtha.2015.10.011.
204. Jones L, Downie LE, Korb D, et al. TFOS DEWS II management and therapy report. *Ocul Surf*. 2017;15(3):575–628. doi:10.1016/j.jtos.2017.05.006.
205. Cui D, Mathews P, Li G, VanCourt S, Akpek E. Outcomes of Sjögren's versus non-Sjögren's related dry eye in a longitudinal, tertiary clinic-based sample. *PLoS One*. 2021;16(12):e0261241. doi:10.1371/journal.pone.0261241.
206. Garcia-Queiruga J, Pena-Verdeal H, Sabucedo-Villamarin B, Garcia-Resua C, Giraldez MJ, Yebra-Pimentel E. Temporal progression of entry factors into the vicious circle of dry eye in untreated sufferers. *Life (Basel)*. 2024;14(7):806. doi:10.3390/life14070806.
207. Wang MTM, Muntz A, Lim J, et al. Ageing and the natural history of dry eye disease: a prospective registry-based cross-sectional study. *Ocul Surf*. 2020;18(4):736–741. doi:10.1016/j.jtos.2020.07.003.

208. Wang MTM, Craig JP. Natural history of dry eye disease: perspectives from inter-ethnic comparison studies. *Ocul Surf*. 2019;17(3):424–433. doi:10.1016/j.jtos.2019.03.004.
209. Wan X, Wu Y, Zhai Z, et al. Factors affecting long-term changes of meibomian gland in MGD patients. *Graefes Arch Clin Exp Ophthalmol*. 2024;262(2):527–535. doi:10.1007/s00417-023-06210-1.
210. Turk MA, Hayworth JL, Nevskaya T, Pope JE. Ocular manifestations in rheumatoid arthritis, connective tissue disease, and vasculitis: a systematic review and metaanalysis. *J Rheumatol*. 2021;48(1):25–34. doi:10.3899/jrheum.190768.
211. Yang W, Yang K, Pan Y, et al. A literature-derived dataset on risk factors for dry eye disease. *Sci Data*. 2023;10(1):21. doi:10.1038/s41597-023-01931-8.
212. Wang L, Xie Y, Deng Y. Prevalence of dry eye in patients with systemic lupus erythematosus: a meta-analysis. *BMJ Open*. 2021;11(9):e047081. doi:10.1136/bmjopen-2020-047081.
213. Qian L, Wei W. Identified risk factors for dry eye syndrome: a systematic review and meta-analysis. *PLoS One*. 2022;17(8):e0271267. doi:10.1371/journal.pone.0271267.
214. Gorimanipalli B, Khamar P, Sethu S, Shetty R. Hormones and dry eye disease. *Indian J Ophthalmol*. 2023;71(4):1276–1284. doi:10.4103/IJO.IJO_2887_22.
215. Vehof J, Snieder H, Jansonius N, Hammond CJ. Prevalence and risk factors of dry eye in 79,866 participants of the population-based Lifelines cohort study in the Netherlands. *Ocul Surf*. 2021;19:83–93. doi:10.1016/j.jtos.2020.04.005.
216. Ko YT, Wu YM, Wu HL, et al. Inflammatory bowel disease and the associated risk of dry eye and ocular surface injury: a nationwide matched cohort study. *BMC Ophthalmol*. 2023;23(1):415. doi:10.1186/s12886-023-03165-z.
217. Wu PC, Ma SH, Huang YY, Chang JY, Chang YT, Dai YX. Psoriasis and dry eye disease: a systematic review and meta-analysis. *Dermatology*. 2022;238(5):876–885. doi:10.1159/000522167.
218. Yoo TK, Oh E. Diabetes mellitus is associated with dry eye syndrome: a meta-analysis. *Int Ophthalmol*. 2019;39(11):2611–2620. doi:10.1007/s10792-019-01110-y.
219. Sullivan DA, Sullivan BD, Evans JE, et al. Androgen deficiency, meibomian gland dysfunction, and evaporative dry eye. *Ann N Y Acad Sci*. 2002;966:211–222. doi:10.1111/j.1749-6632.2002.tb04217.x.
220. Vehof J, Hysi PG, Hammond CJ. A metabolome-wide study of dry eye disease reveals serum androgens as biomarkers. *Ophthalmology*. 2017;124(4):505–511. doi:10.1016/j.ophtha.2016.12.011.
221. Bonini S, Mantelli F, Moretti C, Lambiase A, Bonini S, Micera A. Itchy-dry eye associated with polycystic ovary syndrome. *Am J Ophthalmol*. 2007;143(5):763–771. doi:10.1016/j.ajo.2007.01.030.
222. Sobolewska B, Schaller M, Zierhut M. Rosacea and dry eye disease. *Ocul Immunol Inflamm*. 2022;30(3):570–579. doi:10.1080/09273948.2021.2025251.
223. Tavassoli S, Wong N, Chan E. Ocular manifestations of rosacea: a clinical review. *Clin Exp Ophthalmol*. 2021;49(2):104–117. doi:10.1111/ceo.13900.
224. Koca S, Oral AY. Assessments of the ocular surface and meibomian gland morphology in patients with treatment-naive acne vulgaris. *Arq Bras Oftalmol*. 2023;86(2):145–150. doi:10.5935/0004-2749.20230025.
225. Huang Q, Zheng Y, Zhang C, et al. Association between asthma and dry eye disease: a meta-analysis based on observational studies. *BMJ Open*. 2021;11(12):e045275. doi:10.1136/bmjopen-2020-045275.
226. Akasaki Y, Inomata T, Sung J, et al. Prevalence of comorbidity between dry eye and allergic conjunctivitis: a systematic review and meta-analysis. *J Clin Med*. 2022;11(13):3643. doi:10.3390/jcm11133643.
227. Lee CJ, Levitt RC, Felix ER, Sarantopoulos CD, Galor A. Evidence that dry eye is a comorbid pain condition in a U.S. veteran population. *Pain Rep*. 2017;2(6):e629. doi:10.1097/PR9.0000000000000629.
228. Vehof J, Kozareva D, Hysi PG, Hammond CJ. Prevalence and risk factors of dry eye disease in a British female cohort. *Br J Ophthalmol*. 2014;98(12):1712–1717. doi:10.1136/bjophthalmol-2014-305201.
229. Liu S, Dong H, Fang S, Zhang L. Risk of dry eye in headache patients: a systematic review and meta-analysis. *Ann Med*. 2022;54(1):2876–2885. doi:10.1080/07853890.2022.2133165.
230. Basilious A, Xu CY, MS Malvankar-Mehta. Dry eye disease and psychiatric disorders: a systematic review and meta-analysis. *Eur J Ophthalmol*. 2022;32(4):1872–1889. doi:10.1177/11206721211060963.
231. Wan KH, Chen LJ, Young AL. Depression and anxiety in dry eye disease: a systematic review and meta-analysis. *Eye (Lond)*. 2016;30(12):1558–1567. doi:10.1038/eye.2016.186.
232. Tsai CY, Jiesisibieke ZL, Tung TH. Association between dry eye disease and depression: an umbrella review. *Front Public Health*. 2022;10:910608. doi:10.3389/fpubh.2022.910608.
233. Lin CW, Su YC, Liu JD, et al. Impact of obstructive sleep apnea and continuous positive airway pressure treatment on dry eye disease: a systematic review and meta-analysis. *Nat Sci Sleep*. 2024;16:1921–1935. doi:10.2147/NSS.S495883.
234. Ayaki M, Tsubota K, Kawashima M, Kishimoto T, Mimura M, Negishi K. Sleep disorders are a prevalent and serious comorbidity in dry eye. *Invest Ophthalmol Vis Sci*. 2018;59(14):DES143–DES150. doi:10.1167/iovs.17-23467.
235. Nagino K, Sung J, Oyama G, et al. Prevalence and characteristics of dry eye disease in Parkinson's disease: a systematic review and meta-analysis. *Sci Rep*. 2022;12(1):18348. doi:10.1038/s41598-022-22037-y.
236. Nichols KK, Foulks GN, Bron AJ, et al. The International Workshop on Meibomian Gland Dysfunction: executive summary. *Invest Ophthalmol Vis Sci*. 2011;52(4):1922–1929. doi:10.1167/iovs.10-6997a.
237. Schaumberg DA, Nichols JJ, Papas EB, Tong L, Uchino M, Nichols KK. The International Workshop on Meibomian Gland Dysfunction: report of the subcommittee on the epidemiology of, and associated risk factors for, MGD. *Invest Ophthalmol Vis Sci*. 2011;52(4):1994–2005. doi:10.1167/iovs.10-6997e.
238. Lin A, Ahmad S, Amescua G, et al. Blepharitis preferred practice pattern(R). *Ophthalmology*. 2024;131(4):P50–P86. doi:10.1016/j.ophtha.2023.12.036.
239. Zhang AC, Muntz A, Wang MTM, Craig JP, Downie LE. Ocular demodex: a systematic review of the clinical liter-

ature. *Ophthalmic Physiol Opt*. 2020;40(4):389–432. doi:10.1111/opo.12691.
240. Ekin MA, Ugurlu SK, Imre SS, Kahraman HG. The role of meibomian gland dysfunction on the development of dry eye disease in patients with facial nerve palsy. *Arq Bras Oftalmol*. 2021;85(2):128–135. doi:10.5935/0004-2749.20220021.
241. Mistry RK, Hohman MH, Al-Sayed AA. Facial nerve trauma. *StatPearls*. 2024.
242. Kucuk E, Yilmaz U, Zor KR. Tear film functions and dry eye symptoms in young patients with pterygium. *Beyoglu Eye J*. 2020;5(1):26–31. doi:10.14744/bej.2019.18853.
243. Napoli PE, Sanna R, Iovino C, Fossarello M. Resolution of pinguecula-related dry eye disease after argon laser photocoagulation. *Int Med Case Rep J*. 2017;10:247–250. doi:10.2147/IMCRJ.S138786.
244. Dong N, Li W, Lin H, et al. Abnormal epithelial differentiation and tear film alteration in pinguecula. *Invest Ophthalmol Vis Sci*. 2009;50(6):2710–2715. doi:10.1167/iovs.08-2905.
245. Jeong J, Rand GM, Kwon T, Kwon JW. The improvement of dry eye symptoms after pinguecula excision and conjunctival autograft with fibrin glue. *J Ophthalmol*. 2019;2019:6438157. doi:10.1155/2019/6438157.
246. Balci O. Clinical characteristics of patients with conjunctivochalasis. *Clin Ophthalmol*. 2014;8:1655–1660. doi:10.2147/OPTH.S61851.
247. Chhadva P, Alexander A, McClellan AL, McManus KT, Seiden B, Galor A. The impact of conjunctivochalasis on dry eye symptoms and signs. *Invest Ophthalmol Vis Sci*. 2015;56(5):2867–2871. doi:10.1167/iovs.14-16337.
248. Di Pascuale MA, Espana EM, Kawakita T, Tseng SC. Clinical characteristics of conjunctivochalasis with or without aqueous tear deficiency. *Br J Ophthalmol*. 2004;88(3):388–392.
249. Bruscolini A, Abbouda A, Locuratolo N, Restivo L, Trimboli P, Romanelli F. Dry eye syndrome in non-exophthalmic Graves' disease. *Semin Ophthalmol*. 2015;30(5-6):372–376. doi:10.3109/08820538.2013.874491.
250. Gurdal C, Sarac O, Genc I, Kirimlioglu H, Takmaz T, Can I. Ocular surface and dry eye in Graves' disease. *Curr Eye Res*. 2011;36(1):8–13. doi:10.3109/02713683.2010.526285.
251. Miura M, Inomata T, Nakamura M, et al. Prevalence and characteristics of dry eye disease after cataract surgery: a systematic review and meta-analysis. *Ophthalmol Ther*. 2022;11(4):1309–1332. doi:10.1007/s40123-022-00513-y.
252. Sambhi RS, Sambhi GDS, Mather R, MS Malvankar-Mehta. Dry eye after refractive surgery: a meta-analysis. *Can J Ophthalmol*. 2020;55(2):99–106. doi:10.1016/j.jcjo.2019.07.005.
253. Shen Z, Zhu Y, Song X, Yan J, Yao K. Dry eye after Small Incision Lenticule Extraction (SMILE) versus Femtosecond Laser-Assisted in Situ Keratomileusis (FS-LASIK) for myopia: a meta-analysis. *PLoS One*. 2016;11(12):e0168081. doi:10.1371/journal.pone.0168081.
254. Kobashi H, Kamiya K, Shimizu K. Dry eye after small incision lenticule extraction and femtosecond laser-assisted LASIK: meta-analysis. *Cornea*. 2017;36(1):85–91. doi:10.1097/ICO.0000000000000999.
255. Lu Q, Lu Y, Zhu X. Dry eye and phacoemulsification cataract surgery: a systematic review and meta-analysis. *Front Med (Lausanne)*. 2021;8:649030. doi:10.3389/fmed.2021.649030.
256. Gao M, Xia F, Wang P, Feng Z, Wang X. Influence of serial intravitreal injections on measures of dry eye: a systemic review and meta-analysis. *Cont Lens Anterior Eye*. 2024;47(2):102127. doi:10.1016/j.clae.2024.102127.
257. Gomes JAP, Azar DT, Baudouin C, et al. TFOS lifestyle: impact of elective medications and procedures on the ocular surface. *Ocul Surf*. 2023;29:331–385. doi:10.1016/j.jtos.2023.04.011.
258. Gomes JAP, Azar DT, Baudouin C, et al. TFOS DEWS II iatrogenic report. *Ocul Surf*. 2017;15(3):511–538. doi:10.1016/j.jtos.2017.05.004.
259. Sullivan DA, da Costa AX, Del Duca E, et al. TFOS lifestyle: impact of cosmetics on the ocular surface. *Ocul Surf*. 2023;29:77–130. doi:10.1016/j.jtos.2023.04.005.
260. Ogawa Y, Kawakami Y, Tsubota K. Cascade of inflammatory, fibrotic processes, and stress-induced senescence in chronic GVHD-related dry eye disease. *Int J Mol Sci*. 2021;22(11):6114. doi:10.3390/ijms22116114.
261. Soleimani M, Mahdavi Sharif P, Cheraqpour K, et al. Ocular graft-versus-host disease (oGVHD): from A to Z. *Surv Ophthalmol*. 2023;68(4):697–712. doi:10.1016/j.survophthal.2023.02.006.
262. Wolpert LE, Snieder H, Jansonius NM, Utheim TP, Hammond CJ, Vehof J. Medication use and dry eye symptoms: a large, hypothesis-free, population-based study in the Netherlands. *Ocul Surf*. Oct 2021;22:1–12. doi:10.1016/j.jtos.2021.06.009.
263. Hampel U, Schuster AK, Nickels S, et al. Schirmer test results: are they associated with topical or systemic medication? *Ocul Surf*. 2020;18(1):141–147. doi:10.1016/j.jtos.2019.11.003.
264. Katipoglu Z, Abay RN. The relationship between dry eye disease and anticholinergic burden. *Eye (Lond)*. 2023;37(14):2921–2925. doi:10.1038/s41433-023-02442-x.
265. Monge-Carmona R, Caro-Magdaleno M, MC Sánchez-González. Association between the use of prostaglandin analogues and ocular surface disease: a systematic review. *Eye (Lond)*. 2025;39(1):28–39. doi:10.1038/s41433-024-03372-y.
266. Stoicescu EA, Iancu RC, Popa Cherecheanu A, Iancu G. Ocular adverse effects of anti-cancer chemotherapy. *J Med Life*. 2023;16(6):818–821. doi:10.25122/jml-2023-0041.
267. Kam KW, Di Zazzo A, De Gregorio C, Narang P, Jhanji V, Basu S. A review on drug-induced dry eye disease. *Indian J Ophthalmol*. 2023;71(4):1263–1269. doi:10.4103/IJO.IJO_2782_22.
268. Boucher R, Haigh O, Barreau E, et al. Ocular surface toxicities associated with modern anticancer therapies. *Surv Ophthalmol*. 2024;69(2):198–210. doi:10.1016/j.survophthal.2023.10.002.
269. Inglis H, Boyle FM, Friedlander ML, Watson SL. Dry eyes and AIs: if you don't ask you won't find out. *Breast*. 2015;24(6):694–698. doi:10.1016/j.breast.2015.08.008.
270. Elubous KA, Toubasi AA, Elubous A, Alryalat SA, Abous H. Ocular manifestations of systemic isotretinoin in patients with acne: a systemic review and meta-analysis. *Cutan Ocul Toxicol*. 2022;41(2):113–122. doi:10.1080/15569527.2022.2050747.

271. Caffery BE, Josephson JE. Ocular side effects of isotretinoin therapy. *J Am Optom Assoc*. 1988;59(3):221–224.
272. Mathers WD, Shields WJ, Sachdev MS, Petroll WM, Jester JV. Meibomian gland morphology and tear osmolarity: changes with Accutane therapy. *Cornea*. 1991;10(4):286–290.
273. Serino F, Dattilo V, Cennamo M, et al. Dupilumab-associated blepharoconjunctivitis: clinical and morphological aspects. *Biomedicines*. 2023;11(12):3104. doi:10.3390/biomedicines11123104.
274. Wu D, Daniel BS, Lai AJX, et al. Dupilumab-associated ocular manifestations: a review of clinical presentations and management. *Surv Ophthalmol*. 2022;67(5):1419–1442. doi:10.1016/j.survophthal.2022.02.002.
275. Marolo P, Ribero S, Caselgrandi P, et al. Ocular surface disease in patients with atopic dermatitis treated with dupilumab: a prospective case-control study. *Cornea*. 2024;43(2):221–227. doi:10.1097/ICO.0000000000003341.
276. Chien HW, Lin CW, Lee CY, Huang JY, Yang SF, Wang K. The use of androgen deprivation therapy for prostate cancer and its effect on the subsequent dry eye disease: a population-based cohort study. *Int J Med Sci*. 2022;19(7):1103–1109. doi:10.7150/ijms.73417.
277. Alves M, Asbell P, Dogru M, et al. TFOS Lifestyle Report: Impact of environmental conditions on the ocular surface. *Ocul Surf*. 2023;29:1–52. doi:10.1016/j.jtos.2023.04.007.
278. Nichols JJ, Willcox MD, Bron AJ, et al. The TFOS International Workshop on Contact Lens Discomfort: executive summary. *Invest Ophthalmol Vis Sci*. 2013;54(11):TFOS7–TFOS13. doi:10.1167/iovs.13-13212.
279. Fjaervoll H, Fjaervoll K, Magno M, et al. The association between visual display terminal use and dry eye: a review. *Acta Ophthalmol*. 2022;100(4):357–375. doi:10.1111/aos.15049.
280. Wolffsohn JS, Lingham G, Downie LE, et al. TFOS lifestyle: impact of the digital environment on the ocular surface. *Ocul Surf*. 2023;28:213–252. doi:10.1016/j.jtos.2023.04.004.
281. Al-Mohtaseb Z, Schachter S, Shen Lee B, Garlich J, Trattler W. The relationship between dry eye disease and digital screen use. *Clin Ophthalmol*. 2021;15:3811–3820. doi:10.2147/OPTH.S321591.
282. Cai Y, Wei J, Zhou J, Zou W. Prevalence and incidence of dry eye disease in Asia: a systematic review and meta-analysis. *Ophthalmic Res*. 2022;65(6):647–658. doi:10.1159/000525696.
283. Markoulli M, Ahmad S, Arcot J, et al. TFOS lifestyle: impact of nutrition on the ocular surface. *Ocul Surf*. 2023;29:226–271. doi:10.1016/j.jtos.2023.04.003.
284. Liu J, Dong Y, Wang Y. Vitamin D deficiency is associated with dry eye syndrome: a systematic review and meta-analysis. *Acta Ophthalmol*. 2020;98(8):749–754. doi:10.1111/aos.14470.
285. Askari G, Rafie N, Miraghajani M, Heidari Z, Arab A. Association between vitamin D and dry eye disease: a systematic review and meta-analysis of observational studies. *Cont Lens Anterior Eye*. 2020;43(5):418–425. doi:10.1016/j.clae.2020.03.001.
286. Labetoulle M, Baudouin C, Benitez Del Castillo JM, et al. How gut microbiota may impact ocular surface homeostasis and related disorders. *Prog Retin Eye Res*. 2024;100:101250. doi:10.1016/j.preteyeres.2024.101250.
287. Moon J, Yoon CH, Choi SH, Kim MK. Can gut microbiota affect dry eye syndrome? *Int J Mol Sci*. 2020;21(22):8443. doi:10.3390/ijms21228443.
288. Watane A, Raolji S, Cavuoto K, Galor A. Microbiome and immune-mediated dry eye: a review. *BMJ Open Ophthalmol*. 2022;7(1):e000956. doi:10.1136/bmjophth-2021-000956.
289. Hsu CC, Chuang HK, Hsiao YJ, et al. Predicting risks of dry eye disease development using a genome-wide polygenic risk score model. *Transl Vis Sci Technol*. 2024;13(5):13. doi:10.1167/tvst.13.5.13.
290. Vehof J, Zavos HMS, Lachance G, Hammond CJ, Williams FMK. Shared genetic factors underlie chronic pain syndromes. *Pain*. 2014;155(8):1562–1568. doi:10.1016/j.pain.2014.05.002.
291. Bjordal O, Norheim KB, Rodahl E, Jonsson R, Omdal R. Primary Sjogren's syndrome and the eye. *Surv Ophthalmol*. 2020;65(2):119–132. doi:10.1016/j.survophthal.2019.10.004.
292. Kiliccioglu A, Oncel D, Celebi ARC. Autoimmune disease-related dry eye diseases and their placement under the revised classification systems: an update. *Cureus*. 2023;15(12):e50276. doi:10.7759/cureus.50276.
293. Aoki T, Yokoi N, Nagata K, Deguchi H, Sekiyama Y, Sotozono C. Investigation of the relationship between ocular sarcoidosis and dry eye. *Sci Rep*. 2022;12(1):3469. doi:10.1038/s41598-022-07435-6.
294. Bustamante-Arias A, Ruiz Lozano RE, Rodriguez-Garcia A. Dry eye disease, a prominent manifestation of systemic autoimmune disorders. *Eur J Ophthalmol*. 2022;32(6):3142–3162. doi:10.1177/11206721221088259.
295. Ruiz-Lozano RE, Hernandez-Camarena JC, Valdez-Garcia JE, et al. Ocular involvement and complications of lichen planus, lichen planus pigmentosus, and lichen planopilaris: a comprehensive review. *Dermatol Ther*. 2021;34(6):e15137. doi:10.1111/dth.15137.
296. Mohsenin A, Huang JJ. Ocular manifestations of systemic inflammatory diseases. *Conn Med*. 2012;76(9):533–544.
297. Selter JH, Gire AI, Sikder S. The relationship between Graves' ophthalmopathy and dry eye syndrome. *Clin Ophthalmol*. 2015;9:57–62. doi:10.2147/OPTH.S76583.
298. Achtsidis V, Eleftheriadou I, Kozanidou E, et al. Dry eye syndrome in subjects with diabetes and association with neuropathy. *Diabetes Care*. 2014;37(10):e210–e211. doi:10.2337/dc14-0860.
299. Sriprasert I, Warren DW, Mircheff AK, Stanczyk FZ. Dry eye in postmenopausal women: a hormonal disorder. *Menopause*. 2016;23(3):343–351. doi:10.1097/GME.0000000000000530.
300. Chen X, Rao J, Zheng Z, et al. Integrated tear proteome and metabolome reveal panels of inflammatory-related molecules via key regulatory pathways in dry eye syndrome. *J Proteome Res*. 2019;18(5):2321–2330. doi:10.1021/acs.jproteome.9b00149.
301. Leonardi A, Modugno RL, Salami E. Allergy and dry eye disease. *Ocul Immunol Inflamm*. 2021;29(6):1168–1176. doi:10.1080/09273948.2020.1841804.
302. Aumond S, Bitton E. Palpebral and facial skin infestation by Demodex folliculorum. *Cont Lens Anterior Eye*. 2020;43(2):115–122. doi:10.1016/j.clae.2019.09.001.

303. Galor A, Covington D, Levitt AE, et al. Neuropathic ocular pain due to dry eye is associated with multiple comorbid chronic pain syndromes. *J Pain*. 2016;17(3):310–318. doi:10.1016/j.jpain.2015.10.019.
304. Salinas R, Puig M, Fry CL, Johnson DA, Kheirkhah A. Floppy eyelid syndrome: a comprehensive review. *Ocul Surf*. 2020;18(1):31–39. doi:10.1016/j.jtos.2019.10.002.
305. Acar M, Firat H, Yuceege M, Ardic S. Long-term effects of PAP on ocular surface in obstructive sleep apnea syndrome. *Can J Ophthalmol*. 2014;49(2):217–221. doi:10.1016/j.jcjo.2013.11.010.
306. Hayirci E, Yagci A, Palamar M, Basoglu OK, Veral A. The effect of continuous positive airway pressure treatment for obstructive sleep apnea syndrome on the ocular surface. *Cornea*. 2012;31(6):604–608. doi:10.1097/ICO.0b013e31824a2040.
307. Kheirandish-Gozal L, Gozal D. Obstructive sleep apnea and inflammation: proof of concept based on two illustrative cytokines. *Int J Mol Sci*. 2019;20(3):459. doi:10.3390/ijms20030459.
308. Jeng YT, Lin SY, Hu HY, Lee OK, Kuo LL. Osteoporosis and dry eye syndrome: a previously unappreciated association that may alert active prevention of fall. *PLoS One*. 2018;13(11):e0207008. doi:10.1371/journal.pone.0207008.
309. Ungureanu L, Chaudhuri KR, Diaconu S. Falup-Pecurariu C. Dry eye in Parkinson's disease: a narrative review. *Front Neurol*. 2023;14:1236366. doi:10.3389/fneur.2023.1236366.
310. Hassanzadeh S, Varmaghani M, Zarei-Ghanavati S, Heravian Shandiz J, Azimi Khorasani A. Global prevalence of meibomian gland dysfunction: a systematic review and meta-analysis. *Ocul Immunol Inflamm*. 2021;29(1):66–75. doi:10.1080/09273948.2020.1755441.
311. Nelson JD, Shimazaki J, Benitez-del-Castillo JM, et al. The international workshop on meibomian gland dysfunction: report of the definition and classification subcommittee. *Invest Ophthalmol Vis Sci*. 2011;52(4):1930–1937. doi:10.1167/iovs.10-6997b.
312. Devebacak A, Teker ME, Palamar M. The influence of pterygium on meibomian glands and dry eye parameters. *Optom Vis Sci*. 2023;100(3):207–210. doi:10.1097/OPX.0000000000001996.
313. Fineide F, Lagali N, Adil MY, et al. Topical glaucoma medications—clinical implications for the ocular surface. *Ocul Surf*. 2022;26:19–49. doi:10.1016/j.jtos.2022.07.007.
314. Fineide F, Magno M, Dahlo K, et al. Topical glaucoma medications—possible implications on the meibomian glands. *Acta Ophthalmol*. 2024;102(7):735–748. doi:10.1111/aos.16728.
315. Wang MTM, Tien L, Han A, et al. Impact of blinking on ocular surface and tear film parameters. *Ocul Surf*. 2018;16(4):424–429. doi:10.1016/j.jtos.2018.06.001.
316. Nair S, Kaur M, Sharma N, Titiyal JS. Refractive surgery and dry eye—an update. *Indian J Ophthalmol*. 2023;71(4):1105–1114. doi:10.4103/IJO.IJO_3406_22.
317. Naderi K, Gormley J, O'Brart D. Cataract surgery and dry eye disease: a review. *Eur J Ophthalmol*. 2020;30(5):840–855. doi:10.1177/1120672120929958.
318. Ipek T, Hanga MP, Hartwig A, Wolffsohn JS, O'Donnell C. Dry eye following cataract surgery: the effect of light exposure using an in-vitro model. *Cont Lens Anterior Eye*. 2018;41(1):128–131. doi:10.1016/j.clae.2017.11.003.
319. Matulonis UA, Birrer MJ, O'Malley DM, et al. Evaluation of prophylactic corticosteroid eye drop use in the management of corneal abnormalities induced by the antibody-drug conjugate mirvetuximab soravtansine. *Clin Cancer Res*. 2019;25(6):1727–1736. doi:10.1158/1078-0432.Ccr-18-2474.
320. Ge H, Di G, Song P, Han W, Chen P, Wang Y. Role of vitamin A on the ocular surface. *Exp Eye Res*. 2025;250:110179. doi:10.1016/j.exer.2024.110179.
321. Samarawickrama C, Chew S, Watson S. Retinoic acid and the ocular surface. *Surv Ophthalmol*. 2015;60(3):183–195. doi:10.1016/j.survophthal.2014.10.001.
322. Schiffman RM, Christianson MD, Jacobsen G, Hirsch JD, Reis BL. Reliability and validity of the Ocular Surface Disease Index. *Arch Ophthalmol*. 2000;118(5):615–621.
323. Mehra D, Galor A. Digital screen use and dry eye: a review. *Asia Pac J Ophthalmol (Phila)*. 2020;9(6):491–497. doi:10.1097/APO.0000000000000328.
324. Fjaervoll K, Fjaervoll H, Magno M, et al. Review on the possible pathophysiological mechanisms underlying visual display terminal-associated dry eye disease. *Acta Ophthalmol*. 2022;100(8):861–877. doi:10.1111/aos.15150.
325. Pontelli RCN, Rocha BA, Garcia DM, et al. Endocrine disrupting chemicals associated with dry eye syndrome. *Ocul Surf*. 2020;18(3):487–493. doi:10.1016/j.jtos.2020.01.001.
326. Tariq MA, Amin H, Ahmed B, Ali U, Mohiuddin A. Association of dry eye disease with smoking: a systematic review and meta-analysis. *Indian J Ophthalmol*. 2022;70(6):1892–1904. doi:10.4103/ijo.IJO_2193_21.
327. Bandara NA, Burgos-Blasco B, Zhou XR, et al. The impact of vaping on the ocular surface: a systematic review of the literature. *J Clin Med*. 2024;13(9):2619. doi:10.3390/jcm13092619.
328. Makrynioti D, Zagoriti Z, Koutsojannis C, Morgan PB, Lagoumintzis G. Ocular conditions and dry eye due to traditional and new forms of smoking: a review. *Cont Lens Anterior Eye*. 2020;43(3):277–284. doi:10.1016/j.clae.2020.02.009.
329. Md Isa NA, Koh PY, Doraj P. The tear function in electronic cigarette smokers. *Optom Vis Sci*. 2019;96(9):678–685. doi:10.1097/opx.0000000000001422.
330. Courtin R, Pereira B, Naughton G, et al. Prevalence of dry eye disease in visual display terminal workers: a systematic review and meta-analysis. *BMJ Open*. 2016;6(1):e009675. doi:10.1136/bmjopen-2015-009675.
331. Song P, Xia W, Wang M, et al. Variations of dry eye disease prevalence by age, sex and geographic characteristics in China: a systematic review and meta-analysis. *J Glob Health*. 2018;8(2):020503. doi:10.7189/jogh.08.020503.
332. Uchino M. What we know about the epidemiology of dry eye disease in Japan. *Invest Ophthalmol Vis Sci*. 2018;59(14):DES1–DES6. doi:10.1167/iovs.17-23491.
333. McCann P, Abraham AG, Mukhopadhyay A, et al. Prevalence and incidence of dry eye and meibomian gland dysfunction in the United States: a systematic review and meta-analysis. *JAMA Ophthalmol*. 2022;140(12):1181–1192. doi:10.1001/jamaophthalmol.2022.4394.
334. Goodman CF, Doan T, Mehra D, et al. Case-control study examining the composition of the gut microbiome

in individuals with and without immune-mediated dry eye. *Cornea*. 2023;42(11):1340–1348. doi:10.1097/ICO.0000000000003195.
335. Napolitano P, Filippelli M, Davinelli S, Bartollino S, dell'Omo R, Costagliola C. Influence of gut microbiota on eye diseases: an overview. *Ann Med*. 2021;53(1):750–761. doi:10.1080/07853890.2021.1925150.
336. Pilkington M, Lloyd D, Guo B, Watson SL, Ooi KGJ. Effects of dietary imbalances of micro- and macronutrients on the ocular microbiome and its implications in dry eye disease. *Exploration of Medicine*. 2024;5(1):127–147. doi:10.37349/emed.2024.00211.
337. Wu Y, Xie Y, Yuan Y, et al. The Mediterranean diet and age-related eye diseases: a systematic review. *Nutrients*. 2023;15(9):2043. doi:10.3390/nu15092043.
338. Contreras-Ruiz L, Ryan DS, Sia RK, Bower KS, Dartt DA, Masli S. Polymorphism in *THBS1* gene is associated with post-refractive surgery chronic ocular surface inflammation. *Ophthalmology*. 2014;121(7):1389–1397. doi:10.1016/j.ophtha.2014.01.033.
339. He Y, Li X, Bao Y, Sun J, Liu J. The correlation of polymorphism of estrogen receptor gene to dry eye syndrome in postmenopausal women. Article in Chinese. *Yan Ke Xue Bao*. 2006;22(4):233–236.
340. Imbert Y, Darling DS, Jumblatt MM, et al. MUC1 splice variants in human ocular surface tissues: possible differences between dry eye patients and normal controls. *Exp Eye Res*. 2006;83(3):493–501. doi:10.1016/j.exer.2006.01.031.
341. Imbert Y, Foulks GN, Brennan MD, et al. MUC1 and estrogen receptor alpha gene polymorphisms in dry eye patients. *Exp Eye Res*. 2009;88(3):334–338. doi:10.1016/j.exer.2008.05.019.
342. Na KS, Mok JW, Kim JY, Joo CK. Proinflammatory gene polymorphisms are potentially associated with Korean non-Sjogren dry eye patients. *Mol Vis*. 2011;17:2818–2823.
343. Ben-Eli H, Gomel N, Aframian DJ, et al. SNP variations in IL10, TNFalpha and TNFAIP3 genes in patients with dry eye syndrome and Sjogren's syndrome. *J Inflamm (Lond)*. 2019;16:6. doi:10.1186/s12950-019-0209-z.
344. Polanska V, Sery O, Fojtik Z, Hlinomazova Z. The presence of dry eye syndrome and corneal complications in patients with rheumatoid arthritis and its association with -174 gene polymorphism for interleukin 6. Vyskyt syndromu sucheho oka a rohovkovych komplikaci u pacientu s revmatoidni artritidou a jeho souvislost s polymorfismem -174 genu pro interleukin 6. Article in Czech. *Cesk Slov Oftalmol*. 2008;64(2):77–80.
345. Rabensteiner DF, Aminfar H, Boldin I, Schwantzer G, Horwath-Winter J. The prevalence of meibomian gland dysfunction, tear film and ocular surface parameters in an Austrian dry eye clinic population. *Acta Ophthalmol*. 2018;96(6):e707–e711. doi:10.1111/aos.13732.
346. Sullivan BD, Smith GT, Gupta A, Harman F, Ansari E. Impact of clinician subjectivity on the assessment of dry eye disease prevalence in a UK public health care patient population. *Clin Ophthalmol*. 2024;18:743–753. doi:10.2147/OPTH.S452149.
347. Arita R, Itoh K, Inoue K, Amano S. Noncontact infrared meibography to document age-related changes of the meibomian glands in a normal population. *Ophthalmology*. 2008;115(5):911–915. doi:10.1016/j.ophtha.2007.06.031.
348. Morthen MK, Magno MS, Utheim TP, Snieder H, Hammond CJ, Vehof J. The physical and mental burden of dry eye disease: a large population-based study investigating the relationship with health-related quality of life and its determinants. *Ocul Surf*. 2021;21:107–117. doi:10.1016/j.jtos.2021.05.006.
349. McDonald M, Patel DA, Keith MS, Snedecor SJ. Economic and humanistic burden of dry eye disease in Europe, North America, and Asia: a systematic literature review. *Ocul Surf*. 2016;14(2):144–167. doi:10.1016/j.jtos.2015.11.002.
350. Boboridis KG, Messmer EM, Benítez-Del-Castillo J, et al. Patient-reported burden and overall impact of dry eye disease across eight European countries: a cross-sectional web-based survey. *BMJ Open*. 2023;13(3):e067007. doi:10.1136/bmjopen-2022-067007.
351. Hossain P, Siffel C, Joseph C, Meunier J, Markowitz JT, Dana R. Patient-reported burden of dry eye disease in the UK: a cross-sectional web-based survey. *BMJ Open*. 2021;11(3):e039209. doi:10.1136/bmjopen-2020-039209.
352. McDonnell PJ, Pflugfelder SC, Stern ME, et al. Study design and baseline findings from the progression of ocular findings (PROOF) natural history study of dry eye. *BMC Ophthalmol*. 2017;17(1):265. doi:10.1186/s12886-017-0646-5.
353. Tong L, Waduthantri S, Wong TY, et al. Impact of symptomatic dry eye on vision-related daily activities: the Singapore Malay Eye Study. *Eye (Lond)*. 2010;24(9):1486–1491. doi:10.1038/eye.2010.67.
354. Li M, Gong L, Chapin WJ, Zhu M. Assessment of vision-related quality of life in dry eye patients. *Invest Ophthalmol Vis Sci*. 2012;53(9):5722–5727. doi:10.1167/iovs.11-9094.
355. Schiffman RM, Walt JG, Jacobsen G, Doyle JJ, Lebovics G, Sumner W. Utility assessment among patients with dry eye disease. *Ophthalmology*. 2003;110(7):1412–1419. doi:10.1016/s0161-6420(03)00462-7.
356. Wlodarczyk J, Fairchild C. United States cost-effectiveness study of two dry eye ophthalmic lubricants. *Ophthalmic Epidemiol*. 2009;16(1):22–30. doi:10.1080/09286580802521309.
357. Brown MM, Brown GC, Brown HC, Peet J, Roth Z. Value-based medicine, comparative effectiveness, and cost-effectiveness analysis of topical cyclosporine for the treatment of dry eye syndrome. *Arch Ophthalmol*. 2009;127(2):146–152. doi:10.1001/archophthalmol.2008.608.
358. Perumal N, Funke S, Pfeiffer N, Grus FH. Proteomics analysis of human tears from aqueous-deficient and evaporative dry eye patients. *Sci Rep*. 2016;6:29629. doi:10.1038/srep29629.
359. Augustin AJ, Spitznas M, Kaviani N, et al. Oxidative reactions in the tear fluid of patients suffering from dry eyes. *Graefes Arch Clin Exp Ophthalmol*. 1995;233(11):694–698. doi:10.1007/BF00164671.
360. Boehm N, Funke S, Wiegand M, Wehrwein N, Pfeiffer N, Grus FH. Alterations in the tear proteome of dry eye patients—a matter of the clinical phenotype. *Invest Ophthalmol Vis Sci*. 2013;54(3):2385–2392. doi:10.1167/iovs.11-8751.
361. Enriquez-de-Salamanca A, Castellanos E, Stern ME, et al. Tear cytokine and chemokine analysis and clinical

correlations in evaporative-type dry eye disease. *Mol Vis*. 2010;16:862–873.
362. Hamm-Alvarez SF, Janga SR, Edman MC, et al. Tear cathepsin S as a candidate biomarker for Sjogren's syndrome. *Arthritis Rheumatol*. 2014;66(7):1872–1881. doi:10.1002/art.38633.
363. Wang HH, Chen WY, Huang YH, et al. Interleukin-20 is involved in dry eye disease and is a potential therapeutic target. *J Biomed Sci*. 2022;29(1):36. doi:10.1186/s12929-022-00821-2.
364. Chotikavanich S, de Paiva CS, Li de Q, et al. Production and activity of matrix metalloproteinase-9 on the ocular surface increase in dysfunctional tear syndrome. *Invest Ophthalmol Vis Sci*. 2009;50(7):3203–3209. doi:10.1167/iovs.08-2476.
365. Kantor NB, Tovar A, Wang T, Galor A. How does ocular graft-versus-host disease fit under the dry eye umbrella? A review. *Clin Exp Ophthalmol*. 2024;52(2):167–185. doi:10.1111/ceo.14347.
366. Ogawa Y. Sjogren's syndrome, non-Sjogren's syndrome, and graft-versus-host disease related dry eye. *Invest Ophthalmol Vis Sci*. 2018;59(14):DES71–DES79. doi:10.1167/iovs.17-23750.
367. Gilbard JP, Rossi SR, Heyda KG. Tear film and ocular surface changes after closure of the meibomian gland orifices in the rabbit. *Ophthalmology*. 1989;96(8):1180–1186. doi:10.1016/s0161-6420(89)32753-9.
368. Byun YS, Rho CR, Cho K, Choi JA, Na KS, Joo CK. Cyclosporine 0.05% ophthalmic emulsion for dry eye in Korea: a prospective, multicenter, open-label, surveillance study. *Korean J Ophthalmol*. 2011;25(6):369–374. doi:10.3341/kjo.2011.25.6.369.
369. Iaccheri B, Torroni G, Cagini C, et al. Corneal confocal scanning laser microscopy in patients with dry eye disease treated with topical cyclosporine. *Eye (Lond)*. 2017;31(5):788–794. doi:10.1038/eye.2017.3.
370. Cook N, Mullins A, Gautam R, et al. Evaluating patient experiences in dry eye disease through social media listening research. *Ophthalmol Ther*. 2019:407–420. doi:10.1007/s40123-019-0188-4.
371. Park Y, Song JS, Choi CY, Yoon KC, Lee HK, Kim HS. A randomized multicenter study comparing 0.1%, 0.15%, and 0.3% sodium hyaluronate with 0.05% cyclosporine in the treatment of dry eye. *J Ocul Pharmacol Ther*. 17;33(2):66-72. doi:10.1089/jop.2016.0086
372. Schwartz LM, Woloshin S. A clear-eyed view of restasis and chronic dry eye disease. *JAMA Intern Med*. 2018;178(2):181–182. doi:10.1001/jamainternmed.2017.7904.
373. Williamson JF, Huynh K, Weaver MA, Davis RM. Perceptions of dry eye disease management in current clinical practice. *Eye Contact Lens*. 2014;40(2):111–115. doi:10.1097/ICL.0000000000000020.
374. Traish AM. Health risks associated with long-term finasteride and dutasteride use: it's time to sound the alarm. *World J Mens Health*. 2020;38(3):323–337. doi:10.5534/wjmh.200012.
375. Barrera MJ, Aguilera S, Castro I, et al. Dysfunctional mitochondria as critical players in the inflammation of autoimmune diseases: potential role in Sjogren's syndrome. *Autoimmun Rev*. 2021;20(8):102867. doi:10.1016/j.autrev.2021.102867.
376. Datta S, Baudouin C, Brignole-Baudouin F, Denoyer A, Cortopassi GA. The eye drop preservative benzalkonium chloride potently induces mitochondrial dysfunction and preferentially affects LHON mutant cells. *Invest Ophthalmol Vis Sci*. 2017;58(4):2406–2412. doi:10.1167/iovs.16-20903.
377. Li Y, Liu H, Zeng W, Wei J. Edaravone protects against hyperosmolarity-induced oxidative stress and apoptosis in primary human corneal epithelial cells. *PLoS One*. 2017;12(3):e0174437. doi:10.1371/journal.pone.0174437.
378. Sacca SC, Cutolo CA, Ferrari D, Corazza P, Traverso CE. The eye, oxidative damage and polyunsaturated fatty acids. *Nutrients*. 2018;10(6):668. doi:10.3390/nu10060668.
379. Stuard WL, Guner MK, Robertson DM. IGFBP-3 regulates mitochondrial hyperfusion and metabolic activity in ocular surface epithelia during hyperosmolar stress. *Int J Mol Sci*. 2022;23(7):4066. doi:10.3390/ijms23074066.
380. Wang B, Peng L, Ouyang H, et al. Induction of DDIT4 impairs autophagy through oxidative stress in dry eye. *Invest Ophthalmol Vis Sci*. 2019;60(8):2836–2847. doi:10.1167/iovs.19-27072.
381. Alves M, Calegari VC, Cunha DA, Saad MJ, Velloso LA, Rocha EM. Increased expression of advanced glycation end-products and their receptor, and activation of nuclear factor kappa-B in lacrimal glands of diabetic rats. *Diabetologia*. 2005;48(12):2675–2681. doi:10.1007/s00125-005-0010-9.
382. Alves M, Cunha DA, Calegari VC, et al. Nuclear factor-kappaB and advanced glycation end-products expression in lacrimal glands of aging rats. *J Endocrinol*. 2005;187(1):159–166. doi:10.1677/joe.1.06209.
383. Grasso A, Di Zazzo A, Giannaccare G, et al. Sex hormones related ocular dryness in breast cancer women. *J Clin Med*. 2021;10(12):2620. doi:10.3390/jcm10122620.
384. Sullivan DA. Androgen deficiency & dry eye syndromes. *Arch Soc Esp Oftalmol*. 2004;79(2):49–50.
385. Kamath V, Arvind A, Bhuvana K. Lid wiper epitheliopathy in symptomatic and asymptomatic dry eye subjects. *Indian J Ophthalmol*. 2023;71(4):1499–1502. doi:10.4103/IJO.IJO_2709_22.
386. van Setten GB. Impact of attrition, intercellular shear in dry eye disease: when cells are challenged and neurons are triggered. *Int J Mol Sci*. 2020;21(12):4333. doi:10.3390/ijms21124333.
387. van Setten GB. Expression of GPR-68 in human corneal and conjunctival epithelium. Possible indicator and mediator of attrition associated inflammation at the ocular surface. *J Fr Ophtalmol*. 2023;46(1):19–24. doi:10.1016/j.jfo.2022.07.006.
388. van Setten GB, Mueller-Lierheim W, Baudouin C. Dry eye etiology: focus on friction. *Klin Monbl Augenheilkd*. Oct 2020;237(10):1235–1236 Atiologie des trockenen Auges: Reibung im Fokus. doi:10.1055/a-0898-3857.
389. Vu CHV, Kawashima M, Yamada M, et al. Influence of meibomian gland dysfunction and friction-related disease on the severity of dry eye. *Ophthalmology*. 2018;125(8):1181–1188. doi:10.1016/j.ophtha.2018.01.025.
390. Chen K, Li Y, Zhang X, Ullah R, Tong J, Shen Y. The role of the PI3K/AKT signalling pathway in the corneal

epithelium: recent updates. *Cell Death Dis*. 2022;13(5):513. doi:10.1038/s41419-022-04963-x.
391. Han Y, Zhang Y, Yuan K, Wu Y, Jin X, Huang X. Hyperosmolarity promotes macrophage pyroptosis by driving the glycolytic reprogramming of corneal epithelial cells in dry eye disease. *Front Med*. 2023;17(4):781–795. doi:10.1007/s11684-023-0986-x.
392. Lema C, Reins RY, Redfern RL. High-mobility group box 1 in dry eye inflammation. *Invest Ophthalmol Vis Sci*. 2018;59(5):1741–1750. doi:10.1167/iovs.17-23363.
393. Li H, Li J, Hou C, Li J, Peng H, Wang Q. The effect of astaxanthin on inflammation in hyperosmolarity of experimental dry eye model in vitro and in vivo. *Exp Eye Res*. 2020;197:108113. doi:10.1016/j.exer.2020.108113.
394. Lv Y, Chu C, Liu K, et al. A combination of CMC and alpha-MSH inhibited ROS activated NLRP3 inflammasome in hyperosmolarity stressed HCECs and scopolamine-induced dry eye rats. *Sci Rep*. 2021;11(1):1184. doi:10.1038/s41598-020-80849-2.
395. Magny R, Kessal K, Regazzetti A, et al. Lipidomic analysis of epithelial corneal cells following hyperosmolarity and benzalkonium chloride exposure: new insights in dry eye disease. *Biochim Biophys Acta Mol Cell Biol Lipids*. 2020;1865(9):158728. doi:10.1016/j.bbalip.2020.158728.
396. Ren Y, Feng J, Lin Y, et al. MiR-223 inhibits hyperosmolarity-induced inflammation through downregulating NLRP3 activation in human corneal epithelial cells and dry eye patients. *Exp Eye Res*. 2022;220:109096. doi:10.1016/j.exer.2022.109096.
397. Zhang Y, Li JM, Lu R, et al. Imbalanced IL-37/TNF-alpha/CTSS signaling disrupts corneal epithelial barrier in a dry eye model in vitro. *Ocul Surf*. 2022;26:234–243. doi:10.1016/j.jtos.2022.10.001.
398. Zhang Y, Yang M, Zhao SX, Nie L, Shen LJ, Han W. Hyperosmolarity disrupts tight junction via TNF-alpha/MMP pathway in primary human corneal epithelial cells. *Int J Ophthalmol*. 2022;15(5):683–689. doi:10.18240/ijo.2022.05.01.
399. Ortiz G, Chao C, Jamali A, et al. Effect of dry eye disease on the kinetics of lacrimal gland dendritic cells as visualized by intravital multi-photon microscopy. *Front Immunol*. 2020;11:1713. doi:10.3389/fimmu.2020.01713.
400. de Souza RG, de Paiva CS, Alves MR. Age-related autoimmune changes in lacrimal glands. *Immune Netw*. 2019;19(1):e3. doi:10.4110/in.2019.19.e3.
401. Nair AP, D'Souza S, Shetty R, et al. Altered ocular surface immune cell profile in patients with dry eye disease. *Ocul Surf*. 2021;21:96–106. doi:10.1016/j.jtos.2021.04.002.
402. Pflugfelder SC, de Paiva CS. The pathophysiology of dry eye disease: what we know and future directions for research. *Ophthalmology*. 2017;124(11S):S4–S13. doi:10.1016/j.ophtha.2017.07.010.
403. Stern ME, Theofilopoulos AN, Steven P, et al. Immunologic basis for development of keratoconjunctivitis sicca in systemic autoimmune diseases: role of innate immune sensors. *Ocul Surf*. 2024;32:130–138. doi:10.1016/j.jtos.2024.02.003.
404. Benitez-Del-Castillo J, Cantu-Dibildox J, Sanz-Gonzalez SM, Zanon-Moreno V, Pinazo-Duran MD. Cytokine expression in tears of patients with glaucoma or dry eye disease: a prospective, observational cohort study. *Eur J Ophthalmol*. 2019;29(4):437–443. doi:10.1177/1120672118795399.
405. Henrioux F, Navel V, Belville C, et al. Inflammation of dry eye syndrome: a cellular study of the epithelial and macrophagic involvement of NFAT5 and RAGE. *Int J Mol Sci*. 2023;24(13):11052. doi:10.3390/ijms241311052.
406. Kelagere Y, Scholand KK, DeJong EN, et al. TNF is a critical cytokine in age-related dry eye disease. *Ocul Surf*. 2023;30:119–128. doi:10.1016/j.jtos.2023.08.004.
407. Kumar NR, Praveen M, Narasimhan R, et al. Tear biomarkers in dry eye disease: progress in the last decade. *Indian J Ophthalmol*. 2023;71(4):1190–1202. doi:10.4103/IJO.IJO_2981_22.
408. Perez VL, Stern ME, Pflugfelder SC. Inflammatory basis for dry eye disease flares. *Exp Eye Res*. Dec 2020;201:108294. doi:10.1016/j.exer.2020.108294.
409. Roda M, Corazza I, Bacchi Reggiani ML, et al. Dry eye disease and tear cytokine levels—a meta-analysis. *Int J Mol Sci*. 2020;21(9):3111. doi:10.3390/ijms21093111.
410. Roy NS, Wei Y, Ying GS, et al. Association of tear cytokine concentrations with symptoms and signs of dry eye disease: baseline data from the Dry Eye Assessment and Management (DREAM) study. *Curr Eye Res*. 2023;48(4):339–347. doi:10.1080/02713683.2022.2162088.
411. de Souza GA, Godoy LM, Mann M. Identification of 491 proteins in the tear fluid proteome reveals a large number of proteases and protease inhibitors. *Genome Biol*. 2006;7(8):R72. doi:10.1186/gb-2006-7-8-R72.
412. Edman MC, Janga SR, Meng Z, et al. Increased cathepsin S activity associated with decreased protease inhibitory capacity contributes to altered tear proteins in Sjogren's syndrome patients. *Sci Rep*. 2018;8(1):11044. doi:10.1038/s41598-018-29411-9.
413. Joossen C, Baan A, Moreno-Cinos C, et al. A novel serine protease inhibitor as potential treatment for dry eye syndrome and ocular inflammation. *Sci Rep*. 2020;10(1):17268. doi:10.1038/s41598-020-74159-w.
414. Choi W, Lian C, Ying L, et al. Expression of lipid peroxidation markers in the tear film and ocular surface of patients with non-Sjogren syndrome: potential biomarkers for dry eye disease. *Curr Eye Res*. Sep 2016;41(9):1143–1149. doi:10.3109/02713683.2015.1098707.
415. An S, Raju I, Surenkhuu B, et al. Neutrophil extracellular traps (NETs) contribute to pathological changes of ocular graft-vs.-host disease (oGVHD) dry eye: implications for novel biomarkers and therapeutic strategies. *Ocul Surf*. 2019;17(3):589–614. doi:10.1016/j.jtos.2019.03.010.
416. Mahajan A, Hasikova L, Hampel U, et al. Aggregated neutrophil extracellular traps occlude meibomian glands during ocular surface inflammation. *Ocul Surf*. 2021;20:1–12. doi:10.1016/j.jtos.2020.12.005.
417. Sonawane S, Khanolkar V, Namavari A, et al. Ocular surface extracellular DNA and nuclease activity imbalance: a new paradigm for inflammation in dry eye disease. *Invest Ophthalmol Vis Sci*. 2012;53(13):8253–8263. doi:10.1167/iovs.12-10430.
418. Moon J, Choi SH, Yoon CH, Kim MK. Gut dysbiosis is prevailing in Sjogren's syndrome and is related to dry eye severity. *PLoS One*. 2020;15(2):e0229029. doi:10.1371/journal.pone.0229029.
419. Yoon CH, Ryu JS, Moon J, Kim MK. Association be-

tween aging-dependent gut microbiome dysbiosis and dry eye severity in C57BL/6 male mouse model: a pilot study. *BMC Microbiol*. 2021;21(1):106. doi:10.1186/s12866-021-02173-7.
420. Fakih D, Zhao Z, Nicolle P, et al. Chronic dry eye induced corneal hypersensitivity, neuroinflammatory responses, and synaptic plasticity in the mouse trigeminal brainstem. *J Neuroinflammation*. 2019;16(1):268. doi:10.1186/s12974-019-1656-4.
421. Sonkodi B, Marsovszky L, Csorba A, et al. Neural regeneration in dry eye secondary to systemic lupus erythematosus is also disrupted like in rheumatoid arthritis, but in a progressive fashion. *Int J Mol Sci*. 2023;24(13):10680. doi:10.3390/ijms241310680.
422. Sonkodi B, Resch MD, Hortobagyi T. Is the sex difference a clue to the pathomechanism of dry eye disease? Watch out for the NGF-TrkA-Piezo2 signaling axis and the Piezo2 channelopathy. *J Mol Neurosci*. 2022;72(8):1598–1608. doi:10.1007/s12031-022-02015-9.
423. Wang S, Naderi A, Kahale F, et al. Substance P regulates memory Th17 cell generation and maintenance in chronic dry eye disease. *J Leukoc Biol*. 2024;166(6):1446–1453. doi:10.1093/jleuko/qiae142.
424. Wu M, Downie LE, Grover LM, et al. The neuroregenerative effects of topical decorin on the injured mouse cornea. *J Neuroinflammation*. 2020;17(1):142. doi:10.1186/s12974-020-01812-6.
425. Yu M, Lee SM, Lee H, et al. Neurokinin-1 receptor antagonism ameliorates dry eye disease by inhibiting antigen-presenting cell maturation and T helper 17 cell activation. *Am J Pathol*. 2020;190(1):125–133. doi:10.1016/j.ajpath.2019.09.020.
426. Uchino Y. The ocular surface glycocalyx and its alteration in dry eye disease: a review. *Invest Ophthalmol Vis Sci*. 2018;59(14):DES157–DES162. doi:10.1167/iovs.17-23756.
427. Goto E, Endo K, Suzuki A, Fujikura Y, Matsumoto Y, Tsubota K. Tear evaporation dynamics in normal subjects and subjects with obstructive meibomian gland dysfunction. *Invest Ophthalmol Vis Sci*. 2003;44(2):533–539. doi:10.1167/iovs.02-0170.
428. McCulley JP, Shine WE. Meibomian gland function and the tear lipid layer. *Ocul Surf*. 2003;1(3):97–106. doi:10.1016/s1542-0124(12)70138-6.
429. Seo J, Byun WY, Alisafaei F, et al. Multiscale reverse engineering of the human ocular surface. *Nat Med*. 2019;25(8):1310–1318. doi:10.1038/s41591-019-0531-2.
430. Sullivan DA. Tearful relationships? Sex, hormones, the lacrimal gland, and aqueous-deficient dry eye. *Ocul Surf*. 2004;2(2):92–123. doi:10.1016/s1542-0124(12)70147-7.
431. Baudouin C, Rolando M, Benitez Del Castillo JM, et al. Reconsidering the central role of mucins in dry eye and ocular surface diseases. *Prog Retin Eye Res*. 2019;71:68–87. doi:10.1016/j.preteyeres.2018.11.007.
432. Rolando M, Barabino S, Giannaccare G, Aragona P. Dealing with the persistent pathogenic issues of dry eye disease: the importance of external and internal stimuli and tissue responses. *J Clin Med*. 2023;12(6):2205. doi:10.3390/jcm12062205.
433. Gilbard JP. Tear film osmolarity and keratoconjunctivitis sicca. *CLAO J*. 1985;11(3):243–250.
434. Luo L, Li DQ, Pflugfelder SC. Hyperosmolarity-induced apoptosis in human corneal epithelial cells is mediated by cytochrome c and MAPK pathways. *Cornea*. 2007;26(4):452–460. doi:10.1097/ICO.0b013e318030d259.
435. Chen Z, Tong L, Li Z, et al. Hyperosmolarity-induced cornification of human corneal epithelial cells is regulated by JNK MAPK. *Invest Ophthalmol Vis Sci*. 2008;49(2):539–549. doi:10.1167/iovs.07-0569.
436. Igarashi T, Fujimoto C, Suzuki H, et al. Short-time exposure of hyperosmolarity triggers interleukin-6 expression in corneal epithelial cells. *Cornea*. 2014;33(12):1342–1347. doi:10.1097/ICO.0000000000000256.
437. Luo L, Li DQ, Corrales RM, Pflugfelder SC. Hyperosmolar saline is a proinflammatory stress on the mouse ocular surface. *Eye Contact Lens*. 2005;31(5):186–193. doi:10.1097/01.icl.0000162759.79740.46.
438. Peng F, Jiang D, Xu W, et al. AMPK/MFF activation: role in mitochondrial fission and mitophagy in dry eye. *Invest Ophthalmol Vis Sci*. 2022;63(12):18. doi:10.1167/iovs.63.12.18.
439. Liu H, Begley C, Chen M, et al. A link between tear instability and hyperosmolarity in dry eye. *Invest Ophthalmol Vis Sci*. 2009;50(8):3671–3679. doi:10.1167/iovs.08-2689.
440. Peng CC, Cerretani C, Braun RJ, Radke CJ. Evaporation-driven instability of the precorneal tear film. *Adv Colloid Interface Sci*. 2014;206:250–264. doi:10.1016/j.cis.2013.06.001.
441. Wakamatsu TH, Dogru M, Tsubota K. Tearful relations: oxidative stress, inflammation and eye diseases. *Arq Bras Oftalmol*. 2008;71(6 suppl):72–79. doi:10.1590/s0004-27492008000700015.
442. Shetty R, Subramani M, Murugeswari P, et al. Resveratrol rescues human corneal epithelial cells cultured in hyperosmolar conditions: potential for dry eye disease treatment. *Cornea*. 2020;39(12):1520–1532. doi:10.1097/ICO.0000000000002495.
443. Ayala A, Munoz MF, Arguelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxid Med Cell Longev*. 2014;2014:360438. doi:10.1155/2014/360438.
444. Ouyang W, Wang S, Yan D, et al. The cGAS-STING pathway-dependent sensing of mitochondrial DNA mediates ocular surface inflammation. *Signal Transduct Target Ther*. 2023;8(1):371. doi:10.1038/s41392-023-01624-z.
445. Tan X, Chen Q, Chen Z, Sun Z, Chen W, Wei R. Mitochondrial DNA-activated cGAS-STING signaling in environmental dry eye. *Invest Ophthalmol Vis Sci*. 2024;65(4):33. doi:10.1167/iovs.65.4.33.
446. Jackson DC, Zeng W, Wong CY, et al. Tear interferon-gamma as a biomarker for evaporative dry eye disease. *Invest Ophthalmol Vis Sci*. 2016;57(11):4824–4830. doi:10.1167/iovs.16-19757.
447. Ko JH, Kim S, Ryu JS, Song HJ, Oh JY. Interferon-gamma elicits the ocular surface pathology mimicking dry eye through direct modulation of resident corneal cells. *Cell Death Discov*. 2023;9(1):209. doi:10.1038/s41420-023-01511-0.
448. Wu D, Zhang J, Qian T, et al. IFN-gamma regulates the expression of MICA in human corneal epithelium through

miRNA4448 and NFkappaB. *Front Immunol*. 2018;9:1530. doi:10.3389/fimmu.2018.01530.
449. Zhang X, Chen W, De Paiva CS, et al. Interferon-gamma exacerbates dry eye-induced apoptosis in conjunctiva through dual apoptotic pathways. *Invest Ophthalmol Vis Sci*. 2011;52(9):6279–6285. doi:10.1167/iovs.10-7081.
450. Yang X, Zuo X, Zeng H, et al. IFN-gamma facilitates corneal epithelial cell pyroptosis through the JAK2/STAT1 pathway in dry eye. *Invest Ophthalmol Vis Sci*. 2023;64(3):34. doi:10.1167/iovs.64.3.34.
451. Deng R, Hua X, Li J, et al. Oxidative stress markers induced by hyperosmolarity in primary human corneal epithelial cells. *PLoS One*. 2015;10(5):e0126561. doi:10.1371/journal.pone.0126561.
452. Li J, Ruzhi D, Hua X, et al. Blueberry component pterostilbene protects corneal epithelial cells from inflammation via anti-oxidative pathway. *Sci Rep*. 2016;6:19408. doi:10.1038/srep19408.
453. Marek V, Melik-Parsadaniantz S, Villette T, et al. Blue light phototoxicity toward human corneal and conjunctival epithelial cells in basal and hyperosmolar conditions. *Free Radic Biol Med*. 2018;126:27–40. doi:10.1016/j.freeradbiomed.2018.07.012.
454. Wang B, Zeng H, Zuo X, et al. TLR4-dependent DUOX2 activation triggered oxidative stress and promoted HMGB1 release in dry eye. *Front Med (Lausanne)*. 2021;8:781616. doi:10.3389/fmed.2021.781616.
455. Reins RY, Lema C, Courson J, Kunnen CME, Redfern RL. MyD88 deficiency protects against dry eye-induced damage. *Invest Ophthalmol Vis Sci*. 2018;59(7):2967–2976. doi:10.1167/iovs.17-23397.
456. Alven A, Lema C, Redfern RL. Impact of low humidity on damage-associated molecular patterns at the ocular surface during dry eye disease. *Optom Vis Sci*. 2021;98(11):1231–1238. doi:10.1097/OPX.0000000000001802.
457. Heimann H, Coupland SE, Gochman R, Hellmich M, Foerster MH. Alterations in expression of mucin, tenascin-c and syndecan-1 in the conjunctiva following retinal surgery and plaque radiotherapy. *Graefes Arch Clin Exp Ophthalmol*. 2001;239(7):488–495. doi:10.1007/s004170100301.
458. Lai CT, Yao WC, Lin SY, et al. Changes of ocular surface and the inflammatory response in a rabbit model of short-term exposure keratopathy. *PLoS One*. 2015;10(9):e0137186. doi:10.1371/journal.pone.0137186.
459. Kam W, Sullivan DA, Sullivan BD, Venkiteshwar M. Does hyperosmolarity induce an irreversible process leading to human corneal epithelial cell death? *Invest Ophthalmol Vis Sci*. 2016;57(12) 6181-6181.
460. Martinez-Carrasco R, Fini ME. Dynasore protects corneal epithelial cells subjected to hyperosmolar stress in an in vitro model of dry eye epitheliopathy. *Int J Mol Sci*. 2023;24(5):4754. doi:10.3390/ijms24054754.
461. Moore JE, Vasey GT, Dartt DA, et al. Effect of tear hyperosmolarity and signs of clinical ocular surface pathology upon conjunctival goblet cell function in the human ocular surface. *Invest Ophthalmol Vis Sci*. 2011;52(9):6174–6180. doi:10.1167/iovs.10-7022.
462. Masli S, Akpek EK. Reduced tear thrombospondin-1/matrix metalloproteinase-9 ratio can aid in detecting Sjogren's syndrome etiology in patients with dry eye. *Clin Transl Sci*. 2022;15(8):1999–2009. doi:10.1111/cts.13316.
463. Virtanen T, Konttinen YT, Honkanen N, Harkonen M, Tervo T. Tear fluid plasmin activity of dry eye patients with Sjogren's syndrome. *Acta Ophthalmol Scand*. 1997;75(2):137–141. doi:10.1111/j.1600-0420.1997.tb00109.x.
464. Schargus M, Ivanova S, Kakkassery V, Dick HB, Joachim S. Correlation of tear film osmolarity and 2 different MMP-9 tests with common dry eye tests in a cohort of non-dry eye patients. *Cornea*. 2015;34(7):739–744. doi:10.1097/ICO.0000000000000449.
465. Arafat SN, Robert MC, Abud T, et al. Elevated neutrophil elastase in tears of ocular graft-versus-host disease patients. *Am J Ophthalmol*. 2017;176:46–52. doi:10.1016/j.ajo.2016.12.026.
466. Benitez-Del-Castillo JM, Soria J, Acera A, Munoz AM, Rodriguez S, Suarez T. Quantification of a panel for dry-eye protein biomarkers in tears: a comparative pilot study using standard ELISA and customized microarrays. *Mol Vis*. 2021;27:243–261.
467. Regmi SC, Samsom ML, Heynen ML, et al. Degradation of proteoglycan 4/lubricin by cathepsin S: potential mechanism for diminished ocular surface lubrication in Sjogren's syndrome. *Exp Eye Res*. 2017;161:1–9. doi:10.1016/j.exer.2017.05.006.
468. Berchicci L, Aragona E, Arrigo A, et al. Conjunctival matrix metalloproteinase-9 clinical assessment in early ocular graft versus host disease. *J Ophthalmol*. 2021;2021:9958713. doi:10.1155/2021/9958713.
469. Zhou Q, Yang L, Wang Q, Li Y, Wei C, Xie L. Mechanistic investigations of diabetic ocular surface diseases. *Front Endocrinol (Lausanne)*. 2022;13:1079541. doi:10.3389/fendo.2022.1079541.
470. Schmidt TA, Sullivan DA, Knop E, et al. Transcription, translation, and function of lubricin, a boundary lubricant, at the ocular surface. *JAMA Ophthalmol*. 2013;131(6):766–776. doi:10.1001/jamaophthalmol.2013.2385.
471. Iwata S, Lemp MA, Holly FJ, Dohlman CH. Evaporation rate of water from the precorneal tear film and cornea in the rabbit. *Invest Ophthalmol*. 1969;8(6):613–619.
472. Georgiev GA, Eftimov P, Yokoi N. Contribution of mucins towards the physical properties of the tear film: a modern update. *Int J Mol Sci*. 2019;20(24):6132. doi:10.3390/ijms20246132.
473. Ablamowicz AF, Nichols JJ. Ocular surface membrane-associated mucins. *Ocul Surf*. 2016;14(3):331–341. doi:10.1016/j.jtos.2016.03.003.
474. Dilly PN. On the nature and the role of the subsurface vesicles in the outer epithelial cells of the conjunctiva. *Br J Ophthalmol*. 1985;69(7):477–481. doi:10.1136/bjo.69.7.477.
475. Martinez-Carrasco R, Rachagani S, Batra SK, Argueso P, Fini ME. Roles unveiled for membrane-associated mucins at the ocular surface using a Muc4 knockout mouse model. *Sci Rep*. 2023;13(1):13558. doi:10.1038/s41598-023-40491-0.
476. Weng J, Ross C, Baker J, Alfuraih S, Shamloo K, Sharma A. Diabetes-associated hyperglycemia causes rapid-onset ocular surface damage. *Invest Ophthalmol Vis Sci*. 2023;64(14):11. doi:10.1167/iovs.64.14.11.
477. Lin N, Chen X, Liu H, et al. Ectoine enhances mucin production via restoring IL-13/IFN-gamma balance in

a murine dry eye model. *Invest Ophthalmol Vis Sci*. 2024;65(6):39. doi:10.1167/iovs.65.6.39.
478. Weng J, Trinh S, Lee R, Metwale R, Sharma A. Impact of high glucose on ocular surface glycocalyx components: implications for diabetes-associated ocular surface damage. *Int J Mol Sci*. 2022;23(22):14289. doi:10.3390/ijms232214289.
479. Mani R, Shobha PS, Thilagavathi S, et al. Altered mucins and aquaporins indicate dry eye outcome in patients undergoing vitreo-retinal surgery. *PLoS One*. 2020;15(5):e0233517. doi:10.1371/journal.pone.0233517.
480. Shamloo K, Barbarino A, Alfuraih S, Sharma A. Graft versus host disease-associated dry eye: role of ocular surface mucins and the effect of rebamipide, a mucin secretagogue. *Invest Ophthalmol Vis Sci*. 2019;60(14):4511–4519. doi:10.1167/iovs.19-27843.
481. AbuSamra DB, Martinez-Carrasco R, Argueso P. Galectins differentially regulate the surface glycosylation of human monocytes. *Biomolecules*. 2022;12(9):1168. doi:10.3390/biom12091168.
482. Fini ME, Jeong S, Gong H, et al. Membrane-associated mucins of the ocular surface: new genes, new protein functions and new biological roles in human and mouse. *Prog Retin Eye Res*. 2020;75:100777. doi:10.1016/j.preteyeres.2019.100777.
483. Menon NG, Goyal R, Lema C, et al. Proteoglycan 4 (PRG4) expression and function in dry eye associated inflammation. *Exp Eye Res*. 2021;208:108628. doi:10.1016/j.exer.2021.108628.
484. Nakamura Y, Yokoi N, Tokushige H, Kinoshita S. Sialic acid in human tear fluid decreases in dry eye. *Jpn J Ophthalmol*. 2004;48(6):519–523. doi:10.1007/s10384-004-0111-x.
485. Hata-Mizuno M, Uchino Y, Uchino M, et al. Analysis of the association between galectin-3 concentration in tears and the severity of dry eye disease: a case-control study. *J Clin Med*. 2021;11(1):66. doi:10.3390/jcm11010066.
486. Uchino Y, Mauris J, Woodward AM, et al. Alteration of galectin-3 in tears of patients with dry eye disease. *Am J Ophthalmol*. 2015;159(6):1027–1035 e3. doi:10.1016/j.ajo.2015.02.008.
487. Uchino Y, Woodward AM, Mauris J, et al. Galectin-3 is an amplifier of the interleukin-1beta-mediated inflammatory response in corneal keratinocytes. *Immunology*. 2018;154(3):490–499. doi:10.1111/imm.12899.
488. Hoarau A, Polette M, Coraux C. Lung hyaluronasome: involvement of low molecular weight Ha (Lmw-Ha) in innate immunity. *Biomolecules*. 2022;12(5):658. doi:10.3390/biom12050658.
489. Muller-Lierheim WGK. Why chain length of hyaluronan in eye drops matters. *Diagnostics (Basel)*. 2020;10(8):511. doi:10.3390/diagnostics10080511.
490. Lemp MA, Crews LA, Bron AJ, Foulks GN, Sullivan BD. Distribution of aqueous-deficient and evaporative dry eye in a clinic-based patient cohort: a retrospective study. *Cornea*. 2012;31(5):472–478. doi:10.1097/ICO.0b013e318225415a.
491. Liu W, Lin T, Gong L. Analysis of cytokine levels in meibum and clinical correlations with meibomian gland dysfunction. *Dis Markers*. 2022;2022:4259067. doi:10.1155/2022/4259067.
492. Duong HT, Phan MAT, Madigan MC, et al. Culture of primary human meibomian gland cells from surgically excised eyelid tissue. *Exp Eye Res*. 2023;235:109636. doi:10.1016/j.exer.2023.109636.
493. Peng X, Du YL, Liu ST, et al. Isolation and culture of human meibomian gland ductal cells. *Invest Ophthalmol Vis Sci*. 2023;64(15):29. doi:10.1167/iovs.64.15.29.
494. Nuwormegbe S, Park NY, Park HJ, Jin Y, Kim SW, Jester JV. Induction of meibocyte differentiation by three-dimensional, matrigel culture of immortalized human meibomian gland epithelial cells to form acinar organoids. *Ocul Surf*. 2022;26:271–282. doi:10.1016/j.jtos.2022.10.004.
495. Asano N, Hampel U, Garreis F, et al. Differentiation patterns of immortalized human meibomian gland epithelial cells in three-dimensional culture. *Invest Ophthalmol Vis Sci*. 2018;59(3):1343–1353. doi:10.1167/iovs.17-23266.
496. Xu KK, Huang YK, Liu X, Zhang MC, Xie HT. Organotypic culture of mouse meibomian gland: a novel model to study meibomian gland dysfunction in vitro. *Invest Ophthalmol Vis Sci*. 2020;61(4):30. doi:10.1167/iovs.61.4.30.
497. Wang G, Xu L, Shi R, et al. Organotypic culture model of mouse meibomian gland as a screening platform for risk factors related to meibomian gland dysfunction. *Ocul Surf*. 2023;30:73–84. doi:10.1016/j.jtos.2023.08.007.
498. Zahn I, Garreis F, Schicht M, et al. A new organotypic 3D slice culture of mouse meibomian glands reveals impact of melanocortins. *Int J Mol Sci*. 2022;23(23):14947. doi:10.3390/ijms232314947.
499. Liu R, Tu M, Xue J, Xiao B, Li J, Liang L. Oleic acid induces lipogenesis and NLRP3 inflammasome activation in organotypic mouse meibomian gland and human meibomian gland epithelial cells. *Exp Eye Res*. 2024;241:109851. doi:10.1016/j.exer.2024.109851.
500. Kim SW, Xie Y, Nguyen PQ, et al. PPARγ regulates meibocyte differentiation and lipid synthesis of cultured human meibomian gland epithelial cells (hMGEC). *Ocul Surf*. 2018;16(4):463–469. doi:10.1016/j.jtos.2018.07.004.
501. Kim SW, Rho CR, Kim J, et al. Eicosapentaenoic acid (EPA) activates PPARγ signaling leading to cell cycle exit, lipid accumulation, and autophagy in human meibomian gland epithelial cells (hMGEC). *Ocul Surf*. 2020;18(3):427–437. doi:10.1016/j.jtos.2020.04.012.
502. Phan MAT, Madigan MC, Willcox M, Stapleton F, Golebiowski B. Semi-quantification of lipids in human meibomian gland epithelial cells using dual staining microplate assays. *Exp Eye Res*. 2021;210:108719. doi:10.1016/j.exer.2021.108719.
503. Ziemanski JF, Wilson L, Barnes S, Nichols KK. Saturation of cholesteryl esters produced by human meibomian gland epithelial cells after treatment with rosiglitazone. *Ocul Surf*. 2021;20:39–47. doi:10.1016/j.jtos.2020.11.011.
504. Ziemanski JF, Wilson L, Barnes S, Nichols KK. Triacylglycerol lipidome from human meibomian gland epithelial cells: description, response to culture conditions, and perspective on function. *Exp Eye Res*. 2021;207:108573. doi:10.1016/j.exer.2021.108573.
505. Liu Y, Chen D, Chen X, Kam WR, Hatton MP, Sullivan DA. Hypoxia: a breath of fresh air for the meibomian gland. *Ocul Surf*. 2019;17(2):310–317. doi:10.1016/j.jtos.2018.12.001.
506. Kim H, Cho KJ, Durkin AJ, Tromberg BJ, Park I. Quantitative measurement of optical properties and Hb con-

centration in a rodent model of inflammatory Meibomian gland dysfunction using spatial frequency domain imaging. *Biomed Opt Express*. 2022;13(3):1261–1274. doi:10.1364/boe.449150.
507. Liu Y, Wang J, Chen D, Kam WR, Sullivan DA. The role of hypoxia-inducible factor 1alpha in the regulation of human meibomian gland epithelial cells. *Invest Ophthalmol Vis Sci*. 2020;61(3):1. doi:10.1167/iovs.61.3.1.
508. Miyake H, Oda T, Katsuta O, Seno M, Nakamura M. A novel model of meibomian gland dysfunction induced with complete Freund's adjuvant in rabbits. *Vision (Basel)*. 2017;1(1):10. doi:10.3390/vision1010010.
509. Lu Y, Yin Y, Gong L. Meibomian gland dysfunction model induced with complete Freund's adjuvant in C57BL/6 mice. *Int J Ophthalmol*. 2020;13(11):1705–1712. doi:10.18240/ijo.2020.11.04.
510. Dong ZY, Ying M, Zheng J, Hu LJ, Xie JY, Ma Y. Evaluation of a rat meibomian gland dysfunction model induced by closure of meibomian gland orifices. *Int J Ophthalmol*. 2018;11(7):1077–1083. doi:10.18240/ijo.2018.07.01.
511. Jin K, Kawashima M, Ito M, Arita R, Sano K, Tsubota K. A new modified experimental meibomian gland injury model: partial loss of gland due to orifice cauterization and the alleviating potential of 22-oxacalcitriol. *J Clin Med*. 2020;10(1):6. doi:10.3390/jcm10010006.
512. Bu J, Wu Y, Li K, et al. Transitory alkali exposure on meibomian gland orifices induces meibomian gland dysfunction. *Ocul Surf*. 2023;29:406–415. doi:10.1016/j.jtos.2023.06.007.
513. Butovich IA, Wilkerson A, Yuksel S. Depletion of cholesteryl esters causes meibomian gland dysfunction-like symptoms in a soat1-null mouse model. *Int J Mol Sci*. 2021;22(4):1583. doi:10.3390/ijms22041583.
514. Wilkerson A, Yuksel S, Acharya R, Butovich IA. Physiological effects of soat1 inactivation on homeostasis of the mouse ocular surface. *Invest Ophthalmol Vis Sci*. 2024;65(8):2. doi:10.1167/iovs.65.8.2.
515. Bu J, Wu Y, Cai X, et al. Hyperlipidemia induces meibomian gland dysfunction. *Ocul Surf*. 2019;17(4):777–786. doi:10.1016/j.jtos.2019.06.002.
516. Watanabe K, Yoshida M, Okumura T, Sassa T, Kihara A, Uchiyama A. Improvement of evaporative dry eye with meibomian gland dysfunction in model mice by treatment with ophthalmic solution containing mineral oil. *Transl Vis Sci Technol*. 2021;10(4):21. doi:10.1167/tvst.10.4.21.
517. Reneker LW, Wang L, Irlmeier RT, Huang AJW. Fibroblast growth factor receptor 2 (FGFR2) is required for meibomian gland homeostasis in the adult mouse. *Invest Ophthalmol Vis Sci*. 2017;58(5):2638–2646. doi:10.1167/iovs.16-21204.
518. Yang X, Zhong X, Huang AJ, Reneker LW. Spontaneous acinar and ductal regrowth after meibomian gland atrophy induced by deletion of FGFR2 in a mouse model. *Ocul Surf*. 2022;26:300–309. doi:10.1016/j.jtos.2021.11.005.
519. Chen X, Liu Y, Zheng T, et al. Eyes on the lid: the impact of fibroblast growth factor receptor targeting drug on human meibomian gland. *Eye Contact Lens*. 2024;50(2):102–105. doi:10.1097/icl.0000000000001058.
520. Serrano-Morales JM, Álvarez-Santaliestra N, MC Sánchez-González, Ballesteros-Sánchez A, Sánchez-González JM. Impact of dyslipidemia on tear film and meibomian gland dysfunction: a cross-sectional study of the interplay between serum lipid profile and ocular surface health. *J Ophthalmol*. 2024;2024:7345270. doi:10.1155/2024/7345270.
521. Osae EA, Bullock T, Chintapalati M, et al. Obese mice with dyslipidemia exhibit meibomian gland hypertrophy and alterations in meibum composition and aqueous tear production. *Int J Mol Sci*. 2020;21(22):8772. doi:10.3390/ijms21228772.
522. Bu J, Zhang M, Wu Y, et al. High-fat diet induces inflammation of meibomian gland. *Invest Ophthalmol Vis Sci*. 2021;62(10):13. doi:10.1167/iovs.62.10.13.
523. Zou S, Liu J, Si H, et al. High-fat intake reshapes the circadian transcriptome profile and metabolism in murine meibomian glands. *Front Nutr*. 2023;10:1146916. doi:10.3389/fnut.2023.1146916.
524. Wu KI, Chen CY, Jou TS, Jimmy Juang JM, Lu JY, Wang IJ. Effect of 3-hydroxy-3-methyl-glutaryl-coenzyme a reductase inhibitors on the meibomian gland morphology in patients with dyslipidemia. *Am J Ophthalmol*. 2020;219:240–252. doi:10.1016/j.ajo.2020.06.029.
525. Butovich IA, Wilkerson A, Yuksel S. Differential effects of dietary cholesterol and triglycerides on the lipid homeostasis in meibomian glands. *J Steroid Biochem Mol Biol*. 2021;211:105894. doi:10.1016/j.jsbmb.2021.105894.
526. Guliani BP, Bhalla A, Naik MP. Association of the severity of meibomian gland dysfunction with dyslipidemia in Indian population. *Indian J Ophthalmol*. 2018;66(10):1411–1416. doi:10.4103/ijo.IJO_1256_17.
527. Butovich IA, Suzuki T. Delineating a novel metabolic high triglycerides-low waxes syndrome that affects lipid homeostasis in meibomian and sebaceous glands. *Exp Eye Res*. 2020;199:108189. doi:10.1016/j.exer.2020.108189.
528. Al-Namaeh M. A systematic review of the effect of omega-3 supplements on meibomian gland dysfunction. *Ther Adv Ophthalmol*. 2020;12:2515841420952188. doi:10.1177/2515841420952188.
529. Jeyalatha MV, Qu Y, Liu Z, et al. Function of meibomian gland: contribution of proteins. *Exp Eye Res*. 2017;163:29–36. doi:10.1016/j.exer.2017.06.009.
530. Li S, Zhou J, Bu J, et al. Ectodysplasin A protein promotes corneal epithelial cell proliferation. *J Biol Chem*. 2017;292(32):13391–13401. doi:10.1074/jbc.M117.803809.
531. Alam J, Yaman E, Silva GCV, et al. Single cell analysis of short-term dry eye induced changes in cornea immune cell populations. *Front Med (Lausanne)*. 2024;11:1362336. doi:10.3389/fmed.2024.1362336.
532. Alam J, de Paiva CS, Pflugfelder SC. Desiccation induced conjunctival monocyte recruitment and activation—implications for keratoconjunctivitis. *Front Immunol*. 2021;12:701415. doi:10.3389/fimmu.2021.701415.
533. Liu Z, Xie H, Li L, et al. Single-cell landscape reveals the epithelial cell-centric pro-inflammatory immune microenvironment in dry eye development. *Mucosal Immunol*. 2024;17(3):491–507. doi:10.1016/j.mucimm.2023.11.008.
534. Nair AP, D'Souza S, Khamar P, Nuijts R, Sethu S, Shetty R. Ocular surface immune cell diversity in dry eye disease. *Indian J Ophthalmol*. 2023;71(4):1237–1247. doi:10.4103/IJO.IJO_2986_22.
535. Li L, Li Y, Zhu X, et al. Conjunctiva resident gam-

madelta T cells expressed high level of IL-17A and promoted the severity of dry eye. *Invest Ophthalmol Vis Sci*. 2022;63(12):13. doi:10.1167/iovs.63.12.13.
536. Ji YW, Lee JL, Kang HG, et al. Corneal lymphangiogenesis facilitates ocular surface inflammation and cell trafficking in dry eye disease. *Ocul Surf*. 2018;16(3):306–313. doi:10.1016/j.jtos.2018.03.008.
537. Ortiz G, Blanco T, Singh RB, et al. IL-6 induces Treg dysfunction in desiccating stress-induced dry eye disease. *Exp Eye Res*. 2024;246:110006. doi:10.1016/j.exer.2024.110006.
538. Zhao D, Ji H, Zhao H, Xu Y, He A, He Y. BMSC-derived exosomes regulate the Treg/Th17 balance through the miR-21-5p/TLR4/MyD88/NF-kappaB pathway to alleviate dry eye symptoms in mice. *In Vitro Cell Dev Biol Anim*. 2024;60(6):644–656. doi:10.1007/s11626-024-00910-6.
539. Schaefer L, Trujillo-Vargas CM, Midani FS, Pflugfelder SC, Britton RA, de Paiva CS. Gut Microbiota from Sjogren syndrome patients causes decreased T regulatory cells in the lymphoid organs and desiccation-induced corneal barrier disruption in mice. *Front Med (Lausanne)*. 2022;9:852918. doi:10.3389/fmed.2022.852918.
540. Coursey TG, Bian F, Zaheer M, Pflugfelder SC, Volpe EA, de Paiva CS. Age-related spontaneous lacrimal keratoconjunctivitis is accompanied by dysfunctional T regulatory cells. *Mucosal Immunol*. 2017;10(3):743–756. doi:10.1038/mi.2016.83.
541. Bedggood P, Wu M, Zhang X, et al. Improved tracking of corneal immune cell dynamics using in vivo confocal microscopy. *Biomed Opt Express*. 2024;15(11):6277–6298. doi:10.1364/BOE.536553.
542. Willcox MDP, Argueso P, Georgiev GA, et al. TFOS DEWS II tear film report. *Ocul Surf*. 2017;15(3):366–403. doi:10.1016/j.jtos.2017.03.006.
543. Bandlitz S, Peter B, Pflugi T, et al. Agreement and repeatability of four different devices to measure non-invasive tear breakup time (NIBUT). *Cont Lens Anterior Eye*. 2020;43(5):507–511. doi:10.1016/j.clae.2020.02.018.
544. Lim J, Wang MTM, Craig JP. Evaluating the diagnostic ability of two automated non-invasive tear film stability measurement techniques. *Cont Lens Anterior Eye*. 2021;44(4):101362. doi:10.1016/j.clae.2020.08.006.
545. Singh S, Srivastav S, Modiwala Z, Ali MH, Repeatability Basu S. reproducibility and agreement between three different diagnostic imaging platforms for tear film evaluation of normal and dry eye disease. *Eye (Lond)*. 2023;37(10):2042–2047. doi:10.1038/s41433-022-02281-2.
546. Wang MT, Murphy PJ, Blades KJ, Craig JP. Comparison of non-invasive tear film stability measurement techniques. *Clin Exp Optom*. 2018;101(1):13–17. doi:10.1111/cxo.12546.
547. Wang MTM, Craig JP. Comparative evaluation of clinical methods of tear film stability assessment: a randomized crossover trial. *JAMA Ophthalmol*. 2018;136(3):291–294. doi:10.1001/jamaophthalmol.2017.6489.
548. Szczesna-Iskander DH, Llorens-Quintana C. Agreement between invasive and noninvasive measurement of tear film breakup time. *Sci Rep*. 2024;14(1):3852. doi:10.1038/s41598-024-54219-1.
549. Abusharha A, Yami AA, Alsreea K, et al. Repeatability and reproducibility of tear film evaporation rate measurement using a new closed-chamber evaporimeter. *Open J Ophthalmol*. 2021;15:117–121.
550. Fagehi R, El-Hiti GA, Alsubaie MH, et al. Measurements of tear evaporation rate in subjects with refractive errors using a portable evaporimeter. *Healthcare (Basel)*. 2022;10(2):405. doi:10.3390/healthcare10020405.
551. Alanazi MA, Alanazi SA, Baashen MA, Almutairi MS, El-Hiti GA. Assessment of tear film parameters in females with refractive errors using a single device: an observational study. *J Pak Med Assoc*. 2024;74(3) 599-562. doi:10.47391/JPMA.9994.
552. Ji YW, Lee J, Lee H, Seo KY, Kim EK, Kim TI. Automated measurement of tear film dynamics and lipid layer thickness for assessment of non-Sjogren dry eye syndrome with meibomian gland dysfunction. *Cornea*. 2017;36(2):176–182. doi:10.1097/ICO.0000000000001101.
553. Lee Y, Hyon JY, Jeon HS. Characteristics of dry eye patients with thick tear film lipid layers evaluated by a LipiView II interferometer. *Graefes Arch Clin Exp Ophthalmol*. 2021;259(5):1235–1241. doi:10.1007/s00417-020-05044-5.
554. Qi Y, Zhang C, Zhao S, Huang Y, Yang R. A novel non-invasive ocular surface analyzer for the assessment of dry eye with meibomian gland dysfunction. *Exp Ther Med*. 2017;13(6):2983–2988. doi:10.3892/etm.2017.4364.
555. Shen J, Xu Z, Wang Y, et al. Influential signs of dry eye-related ocular symptoms in participants with unstable tear film. *Cornea*. 2024;43(6):751–756. doi:10.1097/ICO.0000000000003473.
556. Alanazi SA, Aldawood MA, Badawood YS, El-Hiti GA, Masmali AM. A comparative study of the quality of non-stimulated and stimulated tears in normal eye male subjects using the tear ferning test. *Clin Optom (Auckl)*. 2019;11:65–71. doi:10.2147/OPTO.S214597.
557. Szczesna-Iskander DH, Muzyka-Wozniak M, Llorens Quintana C. The efficacy of ocular surface assessment approaches in evaluating dry eye treatment with artificial tears. *Sci Rep*. 2022;12(1):21835. doi:10.1038/s41598-022-26327-3.
558. Bai Y, Ngo W, Khanal S, Nichols KK, Nichols JJ. Human precorneal tear film and lipid layer dynamics in meibomian gland dysfunction. *Ocul Surf*. 2021;21:250–256. doi:10.1016/j.jtos.2021.03.006.
559. Li J, Ma J, Hu M, Yu J, Zhao Y. Assessment of tear film lipid layer thickness in patients with meibomian gland dysfunction at different ages. *BMC Ophthalmol*. 2020;20(1):394. doi:10.1186/s12886-020-01667-8.
560. Yi HC, Lee YP, Shin YJ. Influence of nasal tear osmolarity on ocular symptoms related to dry eye disease. *Am J Ophthalmol*. 2018;189:71–76. doi:10.1016/j.ajo.2018.02.008.
561. Yazdani M, Fiskadal J, Chen X, et al. Tear film break-up time and dry eye disease severity in a large Norwegian cohort. *J Clin Med*. 2021;10(4):884. doi:10.3390/jcm10040884.
562. Tashbayev B, Utheim TP, Utheim OA, et al. Utility of tear osmolarity measurement in diagnosis of dry eye disease. *Sci Rep*. 2020;10(1):5542. doi:10.1038/s41598-020-62583-x.
563. Shimazaki J, Sakata M, Den S, Iwasaki M, Toda I. Tear film osmolarity measurement in Japanese dry eye patients using a handheld osmolarity system. *Diagnostics (Basel)*. 2020;10(10):789. doi:10.3390/diagnostics10100789.
564. Nilsen C, Graae Jensen P, Gundersen M, et al. The sig-

nificance of inter-eye osmolarity difference in dry eye diagnostics. *Clin Ophthalmol*. 2023;17:829–835. doi:10.2147/OPTH.S402556.
565. Mathews PM, Karakus S, Agrawal D, Hindman HB, Ramulu PY, Akpek EK. Tear osmolarity and correlation with ocular surface parameters in patients with dry eye. *Cornea*. 2017;36(11):1352–1357. doi:10.1097/ICO.0000000000001364.
566. Kim M, Kim HS, Na KS. Correlation between tear osmolarity and other ocular surface parameters in primary Sjögren's syndrome. *Korean J Ophthalmol*. 2017;31:25–31.
567. Greiner JV, Ying GS, Pistilli M, et al. Association of tear osmolarity with signs and symptoms of dry eye disease in the Dry Eye Assessment and Management (DREAM) study. *Invest Ophthalmol Vis Sci*. 2023;64(1):5. doi:10.1167/iovs.64.1.5.
568. Titiyal JS, Goswami A, Kaur M, et al. Impact of topical cyclosporine-A or topical chloroquine on post-LASIK ocular surface stability - a randomized controlled trial. *Curr Eye Res*. 2023;48(6):557–563. doi:10.1080/02713683.2023.2182747.
569. Rajpoot M, Singh D, Pandey K, Bhargava R. Safety and efficacy of cyclosporine (0.05% versus 0.09%) in dry eye disease. Is it the strength of cyclosporin that really matters? *Nepal J Ophthalmol*. 2022;14(2):64–77 12/31. doi:10.3126/nepjoph.v14i2.38928.
570. Konstas AG, Boboridis KG, Athanasopoulos GP, et al. Changing from preserved, to preservative-free cyclosporine 0.1% enhanced triple glaucoma therapy: impact on ocular surface disease-a randomized controlled trial. *Eye (Lond)*. 2023;37(17):3666–3674. doi:10.1038/s41433-023-02578-w.
571. Benitez-Del-Castillo JM, López-Pérez MD, Cano-Ortiz A, et al. Efficacy and safety of intense pulsed light of upper and lower eyelids in Meibomian gland dysfunction: a prospective multicentric study. *Eur J Ophthalmol*. 2024;34(3):700–707. doi:10.1177/11206721231199121.
572. Nieto-Bona A, Nombela-Palomo M, Felipe-Márquez G, Teus MA. Tear film osmolarity in response to long-term orthokeratology treatment. *Eye Contact Lens*. 2018;44(2):85–90. doi:10.1097/icl.0000000000000347.
573. Yuksel N, Akcay E, Ayan B, Duru N. Tear-film osmolarity changes following dacryocystorhinostomy in primary acquired nasolacrimal duct obstruction. *Curr Eye Res*. 2017;42(3):348–350. doi:10.1080/02713683.2016.1196706.
574. Blanco-Campoy DG, Graue-Hernandez EO, Quiroz-Casian N, Velez-Cordero JR, Yanez-Soto B. In-vitro evaluation of the evaporation retardation by meibomian lipids in homogeneous and non-homogeneous evaporation. *J Colloid Interface Sci*. 2022;625:210–219. doi:10.1016/j.jcis.2022.06.032.
575. Dursch TJ, Li W, Taraz B, Lin MC, Radke CJ. Tear-film evaporation rate from simultaneous ocular-surface temperature and tear-breakup area. *Optom Vis Sci*. 2018;95(1):5–12. doi:10.1097/OPX.0000000000001156.
576. Svitova TF, MC Lin. Evaporation retardation by model tear-lipid films: the roles of film aging, compositions and interfacial rheological properties. *Colloids Surf B Biointerfaces*. 2021;197:111392. doi:10.1016/j.colsurfb.2020.111392.
577. Xu X, Li G, Zuo YY. Effect of model tear film lipid layer on water evaporation. *Invest Ophthalmol Vis Sci*. 2023;64(1):13. doi:10.1167/iovs.64.1.130.
578. Kim YH, Graham AD, Li W, et al. Tear-film evaporation flux and its relationship to tear properties in symptomatic and asymptomatic soft-contact-lens wearers. *Cont Lens Anterior Eye*. 2023;46(4):101850. doi:10.1016/j.clae.2023.101850.
579. Lin MC, Svitova TF. Effects of model tear proteins and topical ophthalmic formulations on evaporation inhibition and biophysical property of model tear lipid nanofilm in vitro. *JCIS Open*. 2021;4:100028. doi:10.1016/j.jciso.2021.100028.
580. Lee D, Song S, Cho G, et al. Elucidating the molecular interactions between lipids and lysozyme: evaporation resistance and bacterial barriers for dry eye disease. *Nano Lett*. 2023;23(20):9451–9460. doi:10.1021/acs.nanolett.3c02936.
581. Borchman D, Ramakrishnan V, Henry C, Ramasubramanian A. Differences in meibum and tear lipid composition and conformation. *Cornea*. 2020;39(1):122–128. doi:10.1097/ICO.0000000000002095.
582. Nencheva Y, Ramasubramanian A, Eftimov P, Yokoi N, Borchman D, Georgiev GA. Effects of lipid saturation on the surface properties of human meibum films. *Int J Mol Sci*. 2018;19(8):2209. doi:10.3390/ijms19082209.
583. Georgiev GA, Borchman D, Eftimov P, Yokoi N. Lipid saturation and the rheology of human tear lipids. *Int J Mol Sci*. 2019;20(14):3431. doi:10.3390/ijms20143431.
584. Eftimov P, Yokoi N, Tsuji K, et al. Surface chemistry study of normal and diseased human meibum films prior to and after supplementation with tear mimetic eyedrop formulation. *Appl Sci*. 2024;14(8):3339. doi:10.3390/app14083339.
585. Borchman D, Ramasubramanian A, Foulks GN. Human meibum cholesteryl and wax ester variability with age, sex, and meibomian gland dysfunction. *Invest Ophthalmol Vis Sci*. 2019;60(6):2286–2293. doi:10.1167/iovs.19-26812.
586. Hetman ZA, Borchman D. Concentration dependent cholesteryl-ester and wax-ester structural relationships and meibomian gland dysfunction. *Biochem Biophys Rep*. 2020;21:100732. doi:10.1016/j.bbrep.2020.100732.
587. Nagar S, Ajouz L, Nichols KK, et al. Relationship between human meibum lipid composition and the severity of meibomian gland dysfunction: a spectroscopic analysis. *Invest Ophthalmol Vis Sci*. 2023;64(10):22. doi:10.1167/iovs.64.10.22.
588. Ewurum A, Ankem A, Georgiev G, Borchman D. A spectroscopic study of the composition and conformation of cholesteryl and wax esters purified from meibum. *Chem Phys Lipids*. 2021;238:105088. doi:10.1016/j.chemphyslip.2021.105088.
589. Eftimov P, Ewurum A, Ankem A, Borchman D, Georgiev GA. Correlations between bulk and surface properties of meibomian lipids with alteration of wax-to-sterol esters content. *Chem Phys Lipids*. 2022;242:105163. doi:10.1016/j.chemphyslip.2021.105163.
590. Garcia-Queiruga J, Pena-Verdeal H, Sabucedo-Villamarin B, et al. Meibum lipidomic analysis in evaporative dry eye subjects. *Int J Mol Sci*. 2024;25(9):4782. doi:10.3390/ijms25094782.
591. Rantamaki AH, Holopainen JM. The effect of phospholipids on tear film lipid layer surface activity. *Invest*

Ophthalmol Vis Sci. 2017;58(1):149–154. doi:10.1167/iovs.16-20468.
592. Paananen RO, Javanainen M, Holopainen JM, Vattulainen I. Crystalline wax esters regulate the evaporation resistance of tear film lipid layers associated with dry eye syndrome. *J Phys Chem Lett*. 2019;10(14):3893–3898. doi:10.1021/acs.jpclett.9b01187.
593. Viitaja T, Raitanen JE, Hynynen A, et al. On the importance of chain branching in tear film lipid layer wax and cholesteryl esters. *Colloids Surf B Biointerfaces*. 2022;214:112429. doi:10.1016/j.colsurfb.2022.112429.
594. Rubio RG, Guzmán F, Ortega F, Liggieri L. Monolayers of cholesterol and cholesteryl stearate at the water/vapor interface: a physico-chemical study of components of the meibum layer. *Colloids Interfaces*. 2021;5(2):50. doi:10.3390/colloids5020030.
595. Jun I, Kim S, Kim H, et al. Evaluation of meibum lipid composition according to tear interferometric patterns: RRH: meibum composition according to interferometric patterns. *Am J Ophthalmol*. 2022;240:37–50. doi:10.1016/j.ajo.2022.02.010.
596. Viitaja T, Raitanen JE, Moilanen J, Paananen RO, Ekholm FS. Biophysical profiling of synthetic ultralong tear film lipids. *Colloids Surf B Biointerfaces*. 2023;223:113145. doi:10.1016/j.colsurfb.2023.113145.
597. Bland HC, Moilanen JA, Ekholm FS, Paananen RO. Investigating the role of specific tear film lipids connected to dry eye syndrome: a study on *O*-acyl-omega-hydroxy fatty acids and diesters. *Langmuir*. 2019;35(9):3545–3552. doi:10.1021/acs.langmuir.8b04182.
598. Viitaja T, Moilanen J, Svedstrom KJ, Ekholm FS, Paananen RO. Tear film lipid layer structure: self-assembly of *O*-acyl-omega-hydroxy fatty acids and wax esters into evaporation-resistant monolayers. *Nano Lett*. 2021;21(18):7676–7683. doi:10.1021/acs.nanolett.1c02475.
599. Paananen RO, Viitaja T, Olzynska A, Ekholm FS, Moilanen J, Cwiklik L. Interactions of polar lipids with cholesteryl ester multilayers elucidate tear film lipid layer structure. *Ocul Surf*. 2020;18(4):545–553. doi:10.1016/j.jtos.2020.06.001.
600. Viitaja T, Raitanen JE, Moilanen J, Paananen RO, Ekholm FS. The properties and role of *O*-acyl-omega-hydroxy fatty acids and Ttype I-St and Ttype II diesters in the tear film lipid layer revealed by a combined chemistry and biophysics approach. *J Org Chem*. 2021;86(7):4965–4976. doi:10.1021/acs.joc.0c02882.
601. Khanal S, Ngo W, Nichols KK, Wilson L, Barnes S, Nichols JJ. Human meibum and tear film derived (*O*-acyl)-omega-hydroxy fatty acids in meibomian gland dysfunction. *Ocul Surf*. 2021;21:118–128. doi:10.1016/j.jtos.2021.05.009.
602. Khanal S, Bai Y, Ngo W, et al. Human meibum and tear film derived (*O*-acyl)-omega-hydroxy fatty acids as biomarkers of tear film dynamics in meibomian gland dysfunction and dry eye disease. *Invest Ophthalmol Vis Sci*. 2021;62(9):13. doi:10.1167/iovs.62.9.13.
603. Galor A, Sanchez V, Jensen A, et al. Meibum sphingolipid composition is altered in individuals with meibomian gland dysfunction-a side by side comparison of meibum and tear sphingolipids. *Ocul Surf*. 2022;23:87–95. doi:10.1016/j.jtos.2021.11.011.
604. Paranjpe V, Tan J, Nguyen J, et al. Clinical signs of meibomian gland dysfunction (MGD) are associated with changes in meibum sphingolipid composition. *Ocul Surf*. 2019;17(2):318–326. doi:10.1016/j.jtos.2018.12.006.
605. Olzynska A, Cwiklik L. Behavior of sphingomyelin and ceramide in a tear film lipid layer model. *Ann Anat*. 2017;210:128–134. doi:10.1016/j.aanat.2016.10.005.
606. Wizert A, Iskander DR, Cwiklik L. Interaction of lysozyme with a tear film lipid layer model: a molecular dynamics simulation study. *Biochim Biophys Acta Biomembr*. 2017;1859(12):2289–2296. doi:10.1016/j.bbamem.2017.08.015.
607. Miyamoto M, Sassa T, Sawai M, Kihara A. Lipid polarity gradient formed by omega-hydroxy lipids in tear film prevents dry eye disease. *ELife*. 2020;9:e53582. doi:10.7554/eLife.53582.
608. Otsuka K, Sawai-Ogawa M, Kihara A. Formation of fatty alcohols—components of meibum lipids—by the fatty acyl-CoA reductase FAR2 is essential for dry eye prevention. *FASEB J*. 2022;36(4):e22216. doi:10.1096/fj.202101733R.
609. Sassa T, Tadaki M, Kiyonari H, Kihara A. Very long-chain tear film lipids produced by fatty acid elongase ELOVL1 prevent dry eye disease in mice. *FASEB J*. 2018;32(6):2966–2978. doi:10.1096/fj.201700947R.
610. Wilkerson A, Bhat N, Quoc Hai, Pham H, Yuksel S, Butovich I. Physiological effects of inactivation and the roles of Elovl3/ELOVL3 in maintaining ocular homeostasis. *FASEB J*. 2021;35(2):e21327. doi:10.1096/fj.202002323R.
611. Sawai M, Watanabe K, Tanaka K, et al. Diverse meibum lipids produced by Awat1 and Awat2 are important for stabilizing tear film and protecting the ocular surface. *iScience*. 2021;24(5):102478. doi:10.1016/j.isci.2021.102478.
612. Xu X, Wilkerson A, Li G, Butovich IA, Zuo YY. Comparative biophysical study of meibomian lipids of wild type and Soat1-null mice: implications to meibomian gland dysfunction and dry eye disease. *Invest Ophthalmol Vis Sci*. 2023;64(11):20. doi:10.1167/iovs.64.11.20.
613. Inaba T, Tanaka Y, Tamaki S, Ito T, Ntambi JM, Tsubota K. Compensatory increases in tear volume and mucin levels associated with meibomian gland dysfunction caused by stearoyl-CoA desaturase-1 deficiency. *Sci Rep*. 2018;8(1):3358. doi:10.1038/s41598-018-21542-3.
614. Ji C, Guo Y, Liu Y, et al. Inhibition of ceramide de novo synthesis ameliorates meibomian gland dysfunction induced by SCD1 deficiency. *Ocul Surf*. 2021;22:230–241. doi:10.1016/j.jtos.2021.08.016.
615. George CT, Kurien BT, Scofield RH. The potential utility of salivary and tear proteomics to discriminate Sjogren's disease from non-Sjogren's sicca. *Int J Mol Sci*. 2023;24(24):17497. doi:10.3390/ijms242417497.
616. Hsiao YT, Huang YT, Yu HJ, Fang PC, Kuo MT. Tear proteomics approach to distinguishing primary from secondary Sjogren's syndrome for dry eye patients with long-term instillation of eyedrops. *Int J Mol Sci*. 2022;23(23):15239. doi:10.3390/ijms232315239.
617. Das N, Menon NG, de Almeida LGN, et al. Proteomics analysis of tears and saliva from Sjogren's syndrome

patients. *Front Pharmacol*. 2021;12:787193. doi:10.3389/fphar.2021.787193.
618. Akpek EK, Wu HY, Karakus S, Zhang Q, Masli S. Differential diagnosis of Sjogren versus non-Sjogren dry eye through tear film biomarkers. *Cornea*. 2020;39(8):991–997. doi:10.1097/ICO.0000000000002299.
619. Kuo MT, Fang PC, Chao TL, et al. Tear proteomics approach to monitoring Sjogren syndrome or dry eye disease. *Int J Mol Sci*. 2019;20(8):1932. doi:10.3390/ijms20081932.
620. Aqrawi LA, Galtung HK, Guerreiro EM, et al. Proteomic and histopathological characterisation of sicca subjects and primary Sjogren's syndrome patients reveals promising tear, saliva and extracellular vesicle disease biomarkers. *Arthritis Res Ther*. 2019;21(1):181. doi:10.1186/s13075-019-1961-4.
621. Lepine M, Robert MC, Sleno L. Discovery and verification of Sjogren's syndrome protein biomarkers in tears by targeted LC-MRM. *J Proteome Res*. 2024;23(6):2219–2229. doi:10.1021/acs.jproteome.4c00163.
622. Bu J, Liu Y, Zhang R, et al. Potential new target for dry eye disease-oxidative stress. *Antioxidants (Basel)*. 2024;13(4):422. doi:10.3390/antiox13040422.
623. Legatowicz-Koprowska M, Nitek S, Czerwińska J. The complement system in primary Sjögren's syndrome: the expression of certain cascade and regulatory proteins in labial salivary glands - observational study. *Reumatologia*. 2020;58(6):357–366. doi:10.5114/reum.2020.102000.
624. Aljohani S, Jazzar A. Tear cytokine levels in sicca syndrome-related dry eye: a meta-analysis. *Diagnostics (Basel)*. 2023;13(13):2184. doi:10.3390/diagnostics13132184.
625. Shinzawa M, Dogru M, Den S, et al. Epidermal fatty acid-binding protein: a novel marker in the diagnosis of dry eye disease in Sjogren syndrome. *Int J Mol Sci*. 2018;19(11):3463. doi:10.3390/ijms19113463.
626. Gijs M, van de Sande N, Bonnet C, et al. A comprehensive scoping review of methodological approaches and clinical applications of tear fluid biomarkers. *Prog Retin Eye Res*. 2025;106:101338. doi:10.1016/j.preteyeres.2025.101338.
627. Liu C, Lin MT, Lee IXY, et al. Neuropathic corneal pain: tear proteomic and neuromediator profiles, imaging features, and clinical manifestations. *Am J Ophthalmol*. 2024;265:6–20. doi:10.1016/j.ajo.2024.03.015.
628. Uchino Y, Uchino M, Mizuno M, Shigeno Y, Furihata K, Shimazaki J. Morphological alterations in corneal nerves of patients with dry eye and associated biomarkers. *Exp Eye Res*. 2023;230:109438. doi:10.1016/j.exer.2023.109438.
629. Zysset-Burri DC, Schlegel I, Lincke JB, et al. Understanding the interactions between the ocular surface microbiome and the tear proteome. *Invest Ophthalmol Vis Sci*. 2021;62(10):8. doi:10.1167/iovs.62.10.8.
630. Schlegel I, De Gouyon Matignon de Pontourade CMF, Lincke JB, Keller I, Zinkernagel MS, DC Zysset-Burri. The human ocular surface microbiome and its associations with the tear proteome in dry eye disease. *Int J Mol Sci*. 2023;24(18):14091. doi:10.3390/ijms241814091.
631. Li Y, Sang X, Yang L, et al. Low concentration of sodium hyaluronate temporarily elevates the tear film lipid layer thickness in dry eye patients with lipid deficiency. *Int J Ophthalmol*. 2018;11(3):389–394. doi:10.18240/ijo.2018.03.07.
632. Fukuoka S, Arita R. Increase in tear film lipid layer thickness after instillation of 3% diquafosol ophthalmic solution in healthy human eyes. *Ocul Surf*. 2017;15(4):730–735. doi:10.1016/j.jtos.2017.03.005.
633. Eftimov P, Yokoi N, Melo AM, Daull P, Georgiev GA. Interactions of meibum and tears with mucomimetic polymers: a hint towards the interplay between the layers of the tear film. *Int J Mol Sci*. 2021;22(5):2747. doi:10.3390/ijms22052747.
634. Sun YC, Hung KF, Li TY, Chang YA, Yeh PT, Hu FR. Transmembrane mucin 1 blocks fluorescein ingress to corneal epithelium. *Invest Ophthalmol Vis Sci*. 2022;63(2):31. doi:10.1167/iovs.63.2.31.
635. Choi M, Tichenor AA. Regional conjunctival differences in glycocalyx mucin expression in dry eye and normal subjects. *Invest Ophthalmol Vis Sci*. 2024;65(2):20. doi:10.1167/iovs.65.2.20.
636. Choudhury A, Dey M, Dixit HN, Feng JJ. Tear-film breakup: the role of membrane-associated mucin polymers. *Phys Rev E*. 2021;103(1-1):013108. doi:10.1103/PhysRevE.103.013108.
637. Matsuzawa M, Ando T, Fukase S, et al. The protective role of conjunctival goblet cell mucin sialylation. *Nat Commun*. 2023;14(1):1417. doi:10.1038/s41467-023-37101-y.
638. Woodward AM, Senchyna M, Argueso P. Short-term reproducibility of MUC5AC measurement in human tear fluid. *Diagnostics (Basel)*. 2021;11(1):57. doi:10.3390/diagnostics11010057.
639. Botten N, Hodges RR, Li D, et al. Resolvin D2 elevates cAMP to increase intracellular [Ca(2+)] and stimulate secretion from conjunctival goblet cells. *FASEB J*. 2019;33(7):8468–8478. doi:10.1096/fj.201802467R.
640. Kim CE, Kleinman HK, Sosne G, et al. RGN-259 (thymosin beta4) improves clinically important dry eye efficacies in comparison with prescription drugs in a dry eye model. *Sci Rep*. 2018;8(1):10500. doi:10.1038/s41598-018-28861-5.
641. Choo J, Liao CH, Tseng CL, et al. Inhibition of microRNA-328 increases ocular mucin expression and conjunctival goblet cells. *Biomedicines*. 2023;11(2):287. doi:10.3390/biomedicines11020287.
642. ClinicalTrials.gov. Assessment of the Safety and Efficacy of RGN-259 Ophthalmic Solutions for Dry Eye Syndrome: ARISE-2.
643. Altman J, Jones G, Ahmed S, Sharma S, Sharma A. Tear film microRNAs as potential biomarkers: a review. *Int J Mol Sci*. 2023;24(4):3694. doi:10.3390/ijms24043694.
644. Pucker AD, Ngo W, Postnikoff CK, Fortinberry H, Nichols JJ. Tear film miRNAs and their association with human dry eye disease. *Curr Eye Res*. 2022;47(11):1479–1487. doi:10.1080/02713683.2022.2110597.
645. Kim YJ, Yeon Y, Lee WJ, et al. Comparison of microRNA expression in tears of normal subjects and Sjogren syndrome patients. *Invest Ophthalmol Vis Sci*. 2019;60(14):4889–4895. doi:10.1167/iovs.19-27062.
646. Kado Abdalkader R, Chaleckis R, Fujita T, Kamei KI. Modeling dry eye with an air-liquid interface in corneal epithelium-on-a-chip. *Sci Rep*. 2024;14(1):4185. doi:10.1038/s41598-024-54736-z.
647. Nomi K, Hayashi R, Ishikawa Y, et al. Generation of functional conjunctival epithelium, including goblet cells, from

human iPSCs. *Cell Rep*. 2021;34(5):108715. doi:10.1016/j.celrep.2021.108715.
648. Bannier-Helaouet M, Korving J, Ma Z, et al. Human conjunctiva organoids to study ocular surface homeostasis and disease. *Cell Stem Cell*. 2024;31(2):227–243 .e12. doi:10.1016/j.stem.2023.12.008.
649. Madl AC, Liu C, Cirera-Salinas D, Fuller GG, Myung D. A mucin-deficient ocular surface mimetic platform for interrogating drug effects on biolubrication, antiadhesion properties, and barrier functionality. *ACS Appl Mater Interfaces*. 2022;14(16):18016–18030. doi:10.1021/acsami.1c22280.
650. Rabiah NI, Sato Y, Kannan A, Kress W, Straube F, Fuller GG. Understanding the adsorption and potential tear film stability properties of recombinant human lubricin and bovine submaxillary mucins in an in vitro tear film model. *Colloids Surf B Biointerfaces*. 2020;195:111257. doi:10.1016/j.colsurfb.2020.111257.
651. Schicht M, Riedlova K, Kukulka M, et al. The potential role of SP-G as surface tension regulator in tear film: from molecular simulations to experimental observations. *Int J Mol Sci*. 2022;23(10):5783. doi:10.3390/ijms23105783.
652. Storas AM, Fineide F, Magno M, et al. Using machine learning model explanations to identify proteins related to severity of meibomian gland dysfunction. *Sci Rep*. 2023;13(1):22946. doi:10.1038/s41598-023-50342-7.
653. Stern ME, Pflugfelder SC. What we have learned from animal models of dry eye. *Int Ophthalmol Clin*. 2017;57(2):109–118. doi:10.1097/IIO.0000000000000169.
654. Hisey EA, Galor A, Leonard BC. A comparative review of evaporative dry eye disease and meibomian gland dysfunction in dogs and humans. *Vet Ophthalmol*. 2023;26(suppl 1):16–30. doi:10.1111/vop.13066.
655. Harris LW, Purves D. Rapid remodeling of sensory endings in the corneas of living mice. *J Neurosci*. 1989;9(6):2210–2214. doi:10.1523/JNEUROSCI.09-06-02210.1989.
656. Frutos-Rincon L, Gomez-Sanchez JA, Inigo-Portugues A, Acosta MC, Gallar J. An experimental model of neuroimmune interactions in the eye: corneal sensory nerves and resident dendritic cells. *Int J Mol Sci*. 2022;23(6):2997. doi:10.3390/ijms23062997.
657. Patel DV, Tavakoli M, Craig JP, Efron N, McGhee CN. Corneal sensitivity and slit scanning in vivo confocal microscopy of the subbasal nerve plexus of the normal central and peripheral human cornea. *Cornea*. 2009;28(7):735–740. doi:10.1097/ICO.0b013e318193e0e3.
658. Stepp MA, Tadvalkar G, Hakh R. Pal-Ghosh S. Corneal epithelial cells function as surrogate Schwann cells for their sensory nerves. *Glia*. 2017;65(6):851–863. doi:10.1002/glia.23102.
659. Alcalde I, Inigo-Portugues A, Gonzalez-Gonzalez O, et al. Morphological and functional changes in TRPM8-expressing corneal cold thermoreceptor neurons during aging and their impact on tearing in mice. *J Comp Neurol*. 2018;526(11):1859–1874. doi:10.1002/cne.24454.
660. De Felipe C, Belmonte C. c-Jun expression after axotomy of corneal trigeminal ganglion neurons is dependent on the site of injury. *Eur J Neurosci*. 1999;11(3):899–906. doi:10.1046/j.1460-9568.1999.00498.x.
661. Chao C, Golebiowski B, Stapleton F. The role of corneal innervation in LASIK-induced neuropathic dry eye. *Ocul Surf*. 2014;12(1):32–45. doi:10.1016/j.jtos.2013.09.001.
662. Belmonte C, Aracil A, Acosta MC, Luna C, Gallar J. Nerves and sensations from the eye surface. *Ocul Surf*. 2004;2(4):248–253. doi:10.1016/s1542-0124(12)70112-x.
663. Betz J, Behrens H, Harkness BM, et al. Ocular pain after refractive surgery: interim analysis of frequency and risk factors. *Ophthalmology*. 2023;130(7):692–701. doi:10.1016/j.ophtha.2023.02.016.
664. He M, Huang W, Zhong X. Central corneal sensitivity after small incision lenticule extraction versus femtosecond laser-assisted LASIK for myopia: a meta-analysis of comparative studies. *BMC Ophthalmol*. 2015;15:141. doi:10.1186/s12886-015-0129-5.
665. Bech F, Gonzalez-Gonzalez O, Artime E, et al. Functional and morphologic alterations in mechanical, polymodal, and cold sensory nerve fibers of the cornea following photorefractive keratectomy. *Invest Ophthalmol Vis Sci*. 2018;59(6):2281–2292. doi:10.1167/iovs.18-24007.
666. Gallar J, Acosta MC, Moilanen JA, Holopainen JM, Belmonte C, Tervo TM. Recovery of corneal sensitivity to mechanical and chemical stimulation after laser in situ keratomileusis. *J Refract Surg*. 2004;20(3):229–235. doi:10.3928/1081-597X-20040501-06.
667. Niederer RL, Perumal D, Sherwin T, McGhee CN. Corneal innervation and cellular changes after corneal transplantation: an in vivo confocal microscopy study. *Invest Ophthalmol Vis Sci*. 2007;48(2):621–626. doi:10.1167/iovs.06-0538.
668. Rao GN, John T, Ishida N, Aquavella JV. Recovery of corneal sensitivity in grafts following penetrating keratoplasty. *Ophthalmology*. 1985;92(10):1408–1411. doi:10.1016/s0161-6420(85)33857-5.
669. Shimizu T, Hayashi T, Ishida A, et al. Evaluation of corneal nerves and dendritic cells by in vivo confocal microscopy after Descemet's membrane keratoplasty for bullous keratopathy. *Sci Rep*. 2022;12(1):6936. doi:10.1038/s41598-022-10939-w.
670. Kumar RL, Koenig SB, Covert DJ. Corneal sensation after descemet stripping and automated endothelial keratoplasty. *Cornea*. 2010;29(1):13–18. doi:10.1097/ICO.0b013e3181ac052b.
671. Hirayama Y, Satake Y, Hirayama M, Shimazaki-Den S, Konomi K, Shimazaki J. Changes in corneal sensation, epithelial damage, and tear function after descemet stripping automated endothelial keratoplasty. *Cornea*. 2013;32(9):1255–1259. doi:10.1097/ICO.0b013e318299c3b7.
672. Bouheraoua N, Fouquet S, MT Marcos-Almaraz, Karagogeos D, Laroche L, Chedotal A. Genetic analysis of the organization, development, and plasticity of corneal innervation in mice. *J Neurosci*. 2019;39(7):1150–1168. doi:10.1523/JNEUROSCI.1401-18.2018.
673. Jessen KR, Mirsky R. The repair Schwann cell and its function in regenerating nerves. *J Physiol*. 2016;594(13):3521–3531. doi:10.1113/JP270874.
674. Matsuyama A, Takatori S, Sone Y, et al. Effect of nerve growth factor on innervation of perivascular nerves in neovasculatures of mouse cornea. *Biol Pharm Bull*. 2017;40(4):396–401. doi:10.1248/bpb.b16-00583.

675. Bonini S, Lambiase A, Rama P, Caprioglio G, Aloe L. Topical treatment with nerve growth factor for neurotrophic keratitis. *Ophthalmology*. 2000;107(7):1347–1351 discussion 1351-132. doi:10.1016/s0161-6420(00)00163-9.
676. Patel S, Hwang J, Mehra D, Galor A. Corneal nerve abnormalities in ocular and systemic diseases. *Exp Eye Res*. 2021;202:108284. doi:10.1016/j.exer.2020.108284.
677. Ponirakis G, Al-Janahi I, Elgassim E, et al. Sustained corneal nerve loss predicts the development of diabetic neuropathy in type 2 diabetes. *Front Neurosci*. 2024;18:1393105. doi:10.3389/fnins.2024.1393105.
678. Galor A, Hamrah P, Haque S, Attal N, Labetoulle M. Understanding chronic ocular surface pain: an unmet need for targeted drug therapy. *Ocul Surf*. 2022;26:148–156. doi:10.1016/j.jtos.2022.08.005.
679. Dua HS, Said DG, Messmer EM, et al. Neurotrophic keratopathy. *Prog Retin Eye Res*. 2018;66:107–131. doi:10.1016/j.preteyeres.2018.04.003.
680. Guzman M, Miglio M, Keitelman I, et al. Transient tear hyperosmolarity disrupts the neuroimmune homeostasis of the ocular surface and facilitates dry eye onset. *Immunology*. 2020;161(2):148–161. doi:10.1111/imm.13243.
681. Chen M, Seo S, Simmons X, et al. Precise longitudinal monitoring of corneal change through in vivo confocal microscopy in a rat dry eye disease model. *Mol Vis*. 2024;30:150–159.
682. Vereertbrugghen A, Galletti JG. Corneal nerves and their role in dry eye pathophysiology. *Exp Eye Res*. 2022;222:109191. doi:10.1016/j.exer.2022.109191.
683. Maity M, Allay MB, Ali MH, Deshmukh R, Basu S, Singh S. Association of tear osmolarity and corneal nerves structure in dry eye disease: an in vivo study. *Graefes Arch Clin Exp Ophthalmol*. 2025;263(3):753–760. doi:10.1007/s00417-024-06657-w.
684. Corcoran P, Hollander DA, Ousler 3rd GW, et al. Dynamic sensitivity of corneal TRPM8 receptors to menthol instillation in dry eye versus normal subjects. *J Ocul Pharmacol Ther*. 2017;33(9):686–692. doi:10.1089/jop.2017.0050.
685. Talens-Estarelles C, Golebiowski B, Ehrmann K, Garcia-Lazaro S, Cervino A, Stapleton F. Corneal hypersensitivity to cold stimuli in symptomatic computer users. *Optom Vis Sci*. 2024;101(9):571–578. doi:10.1097/OPX.0000000000002183.
686. Situ P, Simpson T, Begley C. Hypersensitivity to cold stimuli in symptomatic contact lens wearers. *Optom Vis Sci*. 2016;93(8):909–916. doi:10.1097/OPX.0000000000000857.
687. Belmonte C. Pain, dryness, and itch sensations in eye surface disorders are defined by a balance between inflammation and sensory nerve injury. *Cornea*. 2019;38(suppl 1):S11–S24. doi:10.1097/ICO.0000000000002116.
688. Situ P, Begley CG, Simpson TL. Effects of tear film instability on sensory responses to corneal cold, mechanical, and chemical stimuli. *Invest Ophthalmol Vis Sci*. 2019;60(8):2935–2941. doi:10.1167/iovs.19-27298.
689. Kovacs I, Luna C, Quirce S, et al. Abnormal activity of corneal cold thermoreceptors underlies the unpleasant sensations in dry eye disease. *Pain*. 2016;157(2):399–417. doi:10.1097/j.pain.0000000000000455.
690. Kovacs I, Dienes L, Perenyi K, et al. Lacosamide diminishes dryness-induced hyperexcitability of corneal cold sensitive nerve terminals. *Eur J Pharmacol*. 2016;787:2–8. doi:10.1016/j.ejphar.2016.05.044.
691. Mecum NE, Russell R, Lee J, Sullivan C, Meng ID. Optogenetic inhibition of Nav1.8 expressing corneal afferents reduces persistent dry eye pain. *Invest Ophthalmol Vis Sci*. Nov 1 2021;62(14):15. doi:10.1167/iovs.62.14.15.
692. De Lott LB, Kaplan C, Harte S, et al. Nociplastic pain among individuals with chronic ocular surface pain: one cause for "pain without stain"? *Surv Ophthalmol*. 2025;70(3):536–543. doi:10.1016/j.survophthal.2025.01.004.
693. Stepp MA, Pal-Ghosh S, Tadvalkar G, Williams AR, Pflugfelder SC, de Paiva CS. Reduced corneal innervation in the CD25 null model of Sjogren syndrome. *Int J Mol Sci*. 2018;19(12):536–543. doi:10.3390/ijms19123821.
694. Shimizu S, Sato S, Taniguchi H, et al. Observation of chronic graft-versus-host disease mouse model cornea with in vivo confocal microscopy. *Diagnostics (Basel)*. 2021;11(8):1515. doi:10.3390/diagnostics11081515.
695. Medeiros CS, Marino GK, Lassance L, Thangavadivel S, Santhiago MR, Wilson SE. The impact of photorefractive keratectomy and mitomycin c on corneal nerves and their regeneration. *J Refract Surg*. 2018;34(12):790–798. doi:10.3928/1081597X-20181112-01.
696. Suanno G, Fonteyne P, Ferrari G. Neurosensory abnormalities and stability of a mouse model of dry eye disease. *Exp Eye Res*. 2023;232:109516. doi:10.1016/j.exer.2023.109516.
697. Kurose M, Meng ID. Dry eye modifies the thermal and menthol responses in rat corneal primary afferent cool cells. *J Neurophysiol*. 2013;110(2):495–504. doi:10.1152/jn.00222.2013.
698. Gyenes A, Tapaszto Z, Quirce S, et al. Cyclosporine A decreases dryness-induced hyperexcitability of corneal cold-sensitive nerve terminals. *Int J Mol Sci*. 2023;24(16):13025. doi:10.3390/ijms241613025.
699. Tran BN, Maass M, Musial G, Stern ME, Gehlsen U, Steven P. Topical application of cannabinoid-ligands ameliorates experimental dry-eye disease. *Ocul Surf*. 2022;23:131–139. doi:10.1016/j.jtos.2021.12.008.
700. Eguchi H, Hiura A, Nakagawa H, Kusaka S, Shimomura Y. Corneal nerve fiber structure, its role in corneal function, and its changes in corneal diseases. *Biomed Res Int*. 2017;2017:3242649. doi:10.1155/2017/3242649.
701. Tepelus TC, Chiu GB, Huang J, et al. Correlation between corneal innervation and inflammation evaluated with confocal microscopy and symptomatology in patients with dry eye syndromes: a preliminary study. *Graefes Arch Clin Exp Ophthalmol*. 2017;255(9):1771–1778. doi:10.1007/s00417-017-3680-3.
702. Li F, Zhang Q, Ying X, et al. Corneal nerve structure in patients with primary Sjogren's syndrome in China. *BMC Ophthalmol*. 2021;21(1):211. doi:10.1186/s12886-021-01967-7.
703. Oliveira-Soto L, Efron N. Morphology of corneal nerves using confocal microscopy. *Cornea*. 2001;20(4):374–384. doi:10.1097/00003226-200105000-00008.
704. Guerrero-Moreno A, Liang H, Moreau N, et al. Corneal nerve abnormalities in painful dry eye disease patients. *Biomedicines*. 2021;9(10):1424. doi:10.3390/biomedicines9101424.

705. Luzu J, Labbe A, Reaux-Le Goazigo A, et al. In vivo confocal microscopic study of corneal innervation in Sjogren's syndrome with or without small fiber neuropathy. *Ocul Surf*. 2022;25:155–162. doi:10.1016/j.jtos.2022.07.003.
706. He J, Ogawa Y, Mukai S, et al. In vivo confocal microscopy evaluation of ocular surface with graft-versus-host disease-related dry eye disease. *Sci Rep*. 2017;7(1):10720. doi:10.1038/s41598-017-10237-w.
707. Chen X, Graham J, Dabbah MA, Petropoulos IN, Tavakoli M, Malik RA. An automatic tool for quantification of nerve fibers in corneal confocal microscopy images. *IEEE Trans Biomed Eng*. 2017;64(4):786–794. doi:10.1109/TBME.2016.2573642.
708. Moein HR, Akhlaq A, Dieckmann G, et al. Visualization of microneuromas by using in vivo confocal microscopy: an objective biomarker for the diagnosis of neuropathic corneal pain? *Ocul Surf*. 2020;18(4):651–656. doi:10.1016/j.jtos.2020.07.004.
709. Kobashi H, Kamiya K, Sambe T, Nakagawa R. Factors influencing subjective symptoms in dry eye disease. *Int J Ophthalmol*. 2018;11(12):1926–1931. doi:10.18240/ijo.2018.12.08.
710. Cox SM, Kheirkhah A, Aggarwal S, et al. Alterations in corneal nerves in different subtypes of dry eye disease: an in vivo confocal microscopy study. *Ocul Surf*. 2021;22:135–142. doi:10.1016/j.jtos.2021.08.004.
711. Giannaccare G, Pellegrini M, Sebastiani S, Moscardelli F, Versura P, Campos EC. In vivo confocal microscopy morphometric analysis of corneal subbasal nerve plexus in dry eye disease using newly developed fully automated system. *Graefes Arch Clin Exp Ophthalmol*. 2019;257(3):583–589. doi:10.1007/s00417-018-04225-7.
712. Kasikci M, Erogul O, Polat O. Evaluation of aqueous-deficient and evaporative dry eye cases with confocal microscopy. *J Fr Ophtalmol*. 2023;46(10):1161–1168. doi:10.1016/j.jfo.2023.05.024.
713. Shetty R, Sethu S, Deshmukh R, et al. Corneal dendritic cell density is associated with subbasal nerve plexus features, ocular surface disease index, and serum vitamin D in evaporative dry eye disease. *Biomed Res Int*. 2016;2016:4369750. doi:10.1155/2016/4369750.
714. Nicolle P, Liang H, Reboussin E, et al. Proinflammatory markers, chemokines, and enkephalin in patients suffering from dry eye disease. *Int J Mol Sci*. 2018;19(4):1221. doi:10.3390/ijms19041221.
715. Dikmetas O, Kocabeyoglu S, Mocan MC. Evaluation of visual acuity outcomes and corneal alterations of new generation hybrid contact lenses in patients with advanced keratoconus. *Cornea*. 2020;39(11):1366–1370. doi:10.1097/ICO.0000000000002438.
716. D'Souza S, Padmanabhan Nair A, Iyappan G, et al. Clinical and molecular outcomes after combined intense pulsed light therapy with low-level light therapy in recalcitrant evaporative dry eye disease with meibomian gland dysfunction. *Cornea*. 2022;41(9):1080–1087. doi:10.1097/ICO.0000000000002954.
717. Jing D, Liu Y, Chou Y, et al. Change patterns in the corneal sub-basal nerve and corneal aberrations in patients with dry eye disease: an artificial intelligence analysis. *Exp Eye Res*. 2022;215:108851. doi:10.1016/j.exer.2021.108851.
718. Zhang Y, Wu Y, Li W, Huang X. Semiautomated and automated quantitative analysis of corneal sub-basal nerves in patients with DED with ocular pain using IVCM. *Front Med (Lausanne)*. 2022;9:831307. doi:10.3389/fmed.2022.831307.
719. Chinnery HR, Rajan R, Jiao H, et al. Identification of presumed corneal neuromas and microneuromas using laser-scanning in vivo confocal microscopy: a systematic review. *Br J Ophthalmol*. 2022;106(6):765–771. doi:10.1136/bjophthalmol-2020-318156.
720. Stepp MA, Pal-Ghosh S, Downie LE, et al. Corneal epithelial "neuromas": a case of mistaken identity? *Cornea*. 2020;39(7):930–934. doi:10.1097/ICO.0000000000002294.
721. D'Souza S, Shetty R, Nair AP, et al. Corneal confocal microscopy features and tear molecular profile in study participants with discordance between ocular surface disease clinical signs and discomfort. *J Clin Med*. 2022;11(9):2407. doi:10.3390/jcm11092407.
722. Dermer H, Hwang J, Mittal R, Cohen AK, Galor A. Corneal sub-basal nerve plexus microneuromas in individuals with and without dry eye. *Br J Ophthalmol*. 2022;106(5):616–622. doi:10.1136/bjophthalmol-2020-317891.
723. Wei S, Shi F, Wang Y, Chou Y, Li X. A deep learning model for automated sub-basal corneal nerve segmentation and evaluation using in vivo confocal microscopy. *Transl Vis Sci Technol*. 2020;9(2):32. doi:10.1167/tvst.9.2.32.
724. Tuisku IS, Konttinen YT, Konttinen LM, Tervo TM. Alterations in corneal sensitivity and nerve morphology in patients with primary Sjogren's syndrome. *Exp Eye Res*. 2008;86(6):879–885. doi:10.1016/j.exer.2008.03.002.
725. Situ P, Simpson TL, Fonn D, Jones LW. Conjunctival and corneal pneumatic sensitivity is associated with signs and symptoms of ocular dryness. *Invest Ophthalmol Vis Sci*. 2008;49(7):2971–2976. doi:10.1167/iovs.08-1734.
726. Rahman EZ, Lam PK, Chu CK, Moore Q, Pflugfelder SC. Corneal sensitivity in tear dysfunction and its correlation with clinical parameters and blink rate. *Am J Ophthalmol*. 2015;160(5):858–866 .e5. doi:10.1016/j.ajo.2015.08.005.
727. Bourcier T, Acosta MC, Borderie V, et al. Decreased corneal sensitivity in patients with dry eye. *Invest Ophthalmol Vis Sci*. 2005;46(7):2341–2345. doi:10.1167/iovs.04-1426.
728. Galor A, Felix ER, Feuer W, Levitt RC, Sarantopoulos CD. Corneal nerve pathway function in individuals with dry eye symptoms. *Ophthalmology*. 2021;128(4):619–621. doi:10.1016/j.ophtha.2020.07.061.
729. Golebiowski B, Papas E, Stapleton F. Assessing the sensory function of the ocular surface: implications of use of a non-contact air jet aesthesiometer versus the Cochet-Bonnet aesthesiometer. *Exp Eye Res*. 2011;92(5):408–413. doi:10.1016/j.exer.2011.02.016.
730. Spierer O, Felix ER, McClellan AL, et al. Corneal mechanical thresholds negatively associate with dry eye and ocular pain symptoms. *Invest Ophthalmol Vis Sci*. 2016;57(2):617–625. doi:10.1167/iovs.15-18133.
731. Tagawa Y, Noda K, Ohguchi T, Tagawa Y, Ishida S, Kitaichi N. Corneal hyperalgesia in patients with short tear film break-up time dry eye. *Ocul Surf*. 2019;17(1):55–59. doi:10.1016/j.jtos.2018.08.004.
732. Carreno-Galeano JT, Johns LK, Dana R, Yin J. Autologous

serum tears improve corneal nerve density and sensitivity in patients with ocular graft-versus-host disease-associated dry eye disease. *Ocul Surf*. 2023;28:37–39. doi:10.1016/j.jtos.2023.01.004.
733. Levy O, Labbe A, Borderie V, et al. Increased corneal sub-basal nerve density in patients with Sjogren syndrome treated with topical cyclosporine A. *Clin Exp Ophthalmol*. 2017;45(5):455–463. doi:10.1111/ceo.12898.
734. Kaido M, Kawashima M, Shigeno Y, Yamada Y, Tsubota K. Randomized controlled study to investigate the effect of topical diquafosol tetrasodium on corneal sensitivity in short tear break-up time dry eye. *Adv Ther*. 2018;35(5):697–706. doi:10.1007/s12325-018-0685-1.
735. Ayoubi M, Cabrera K, Locatelli EV, Felix ER, Galor A. Associations between corneal nerve structure and function in a veteran population. *J Clin Med*. 2024;13(9):2513. doi:10.3390/jcm13092513.
736. Qin G, Chen J, Li L, et al. Relationship between ocular surface pain and corneal nerve loss in dry eye diabetics: a cross-sectional study in Shenyang, China. *BMJ Open*. 2023;13(9):e076932. doi:10.1136/bmjopen-2023-076932.
737. Wang MTM, Meyer JJ, Xue AL, Power B, Craig JP. Predictive performance of corneal and lid margin sensitivity for dry eye disease: an investigator-masked, prospective, prognostic accuracy study. *Ocul Surf*. 2024;33:11–15. doi:10.1016/j.jtos.2024.03.010.
738. Crabtree JR, Tannir S, Tran K, Boente CS, Ali A, Borschel GH. Corneal nerve assessment by aesthesiometry: history, advancements, and future directions. *Vision (Basel)*. 2024;8(2):34. doi:10.3390/vision8020034.
739. Nosch DS, Kaser E, Bracher T, Joos RE. Clinical application of the Swiss liquid jet aesthesiometer for corneal sensitivity measurement. *Clin Exp Optom*. 2024;107(1):14–22. doi:10.1080/08164622.2023.2191782.
740. Ehrmann K, Talens-Estarelles C, Stapleton F, Truong B, Chen J, Golebiowski B. A new method to measure ocular surface sensitivity: repeatability and reproducibility of the liquid jet esthesiometer. *Eye Contact Lens*. 2024. doi:10.1097/ICL.0000000000001146.
741. Giovannetti F, Sacchetti M, Marenco M, et al. New disposable esthesiometer (KeraSense®) to improve diagnosis and management of neurotrophic keratitis. *Ocul Surf*. 2024;32:192–197. doi:10.1016/j.jtos.2024.03.009.
742. Dieckmann G, Goyal S, Hamrah P. Neuropathic corneal pain: approaches for management. *Ophthalmology*. 2017;124(11S):S34–S47. doi:10.1016/j.ophtha.2017.08.004.
743. Goyal S, Hamrah P. Understanding neuropathic corneal pain—gaps and current therapeutic approaches. *Semin Ophthalmol*. 2016;31(1-2):59–70. doi:10.3109/08820538.2015.1114853.
744. Rosenthal P, Borsook D. Ocular neuropathic pain. *Br J Ophthalmol*. 2016;100(1):128–134. doi:10.1136/bjophthalmol-2014-306280.
745. Crane AM, Feuer W, Felix ER, et al. Evidence of central sensitisation in those with dry eye symptoms and neuropathic-like ocular pain complaints: incomplete response to topical anaesthesia and generalised heightened sensitivity to evoked pain. *Br J Ophthalmol*. 2017;101(9):1238–1243. doi:10.1136/bjophthalmol-2016-309658.
746. Kim J, Yoon HJ, You IC, Ko BY, Yoon KC. Clinical characteristics of dry eye with ocular neuropathic pain features: comparison according to the types of sensitization based on the Ocular Pain Assessment Survey. *BMC Ophthalmol*. 2020;20(1):455. doi:10.1186/s12886-020-01733-1.
747. Galor A, Levitt RC, McManus KT, et al. Assessment of somatosensory function in patients with idiopathic dry eye symptoms. *JAMA Ophthalmol*. 2016;134(11):1290–1298. doi:10.1001/jamaophthalmol.2016.3642.
748. Shtein RM, Harper DE, Pallazola V, et al. Discordant dry eye disease (An American Ophthalmological Society Thesis). *Trans Am Ophthalmol Soc*. 2016;114:T4.
749. Ong ES, Felix ER, Levitt RC, Feuer WJ, Sarantopoulos CD, Galor A. Epidemiology of discordance between symptoms and signs of dry eye. *Br J Ophthalmol*. 2018;102(5):674–679. doi:10.1136/bjophthalmol-2017-310633.
750. Levitt AE, Galor A, Small L, Feuer W, Felix ER. Pain sensitivity and autonomic nervous system parameters as predictors of dry eye symptoms after LASIK. *Ocul Surf*. 2021;19:275–281. doi:10.1016/j.jtos.2020.10.004.
751. Rodriguez DA, Galor A, Felix ER. Self-report of severity of ocular pain due to light as a predictor of altered central nociceptive system processing in individuals with symptoms of dry eye disease. *J Pain*. 2022;23(5):784–795. doi:10.1016/j.jpain.2021.11.010.
752. Choudhury A, Reyes N, Galor A, Mehra D, Felix E, Moulton EA. Clinical neuroimaging of photophobia in individuals with chronic ocular surface pain. *Am J Ophthalmol*. 2023;246:20–30. doi:10.1016/j.ajo.2022.09.020.
753. Holmes S, Reyes N, Huang JJ, et al. Disentangling the neurological basis of chronic ocular pain using clinical, self-report, and brain imaging data: use of K-means clustering to explore patient phenotypes. *Front Neurol*. 2023;14:1265082. doi:10.3389/fneur.2023.1265082.
754. Glover GH. Overview of functional magnetic resonance imaging. *Neurosurg Clin N Am*. 2011;22(2):133–139 vii. doi:10.1016/j.nec.2010.11.001.
755. Reyes N, Huang JJ, Choudhury A, et al. Botulinum toxin A decreases neural activity in pain-related brain regions in individuals with chronic ocular pain and photophobia. *Front Neurosci*. 2023;17:1202341. doi:10.3389/fnins.2023.1202341.
756. Reyes N, Huang JJ, Choudhury A, et al. FL-41 Tint reduces activation of neural pathways of photophobia in patients with chronic ocular pain. *Am J Ophthalmol*. 2024;259:172–184. doi:10.1016/j.ajo.2023.12.004.
757. Stapleton F, Alves M, Bunya VY, et al. TFOS DEWS II epidemiology report. *Ocul Surf*. 2017;15(3):334–365.
758. Van Went C, Brasnu E, Hamard P, Baudouin C, Labbe A. Influence des pathologies de la surface oculaire sur le traitement du glaucome. [The influence of ocular surface diseases in the management of glaucoma]. *J Fr Ophtalmol*. 2011;34(4):230–237. doi:10.1016/j.jfo.2010.11.010.
759. Jaenen N, Baudouin C, Pouliquen P, Manni G, Figueiredo A, Zeyen T. Ocular symptoms and signs with preserved and preservative-free glaucoma medications. *Eur J Ophthalmol*. 2007;17(3):341–349. doi:10.1177/112067210701700311.
760. Sahlu M, Giorgis AT. Dry eye disease among glaucoma patients on topical hypotensive medications, in a tertiary hos-

pital, Ethiopia. *BMC Ophthalmol*. 2021;21(1):155. doi:10.1186/s12886-021-01917-3.
761. Leung EW, Medeiros FA, Weinreb RN. Prevalence of ocular surface disease in glaucoma patients. *J Glaucoma*. 2008;17(5):350–355. doi:10.1097/IJG.0b013e31815c5f4f.
762. Erb C, Gast U, Schremmer D. German register for glaucoma patients with dry eye. I. Basic outcome with respect to dry eye. *Graefes Arch Clin Exp Ophthalmol*. 2008;246(11):1593–1601. doi:10.1007/s00417-008-0881-9.
763. Pisella PJ, Pouliquen P, Baudouin C. Prevalence of ocular symptoms and signs with preserved and preservative free glaucoma medication. *Br J Ophthalmol*. 2002;86(4):418–423. doi:10.1136/bjo.86.4.418.
764. Nagstrup AH. The use of benzalkonium chloride in topical glaucoma treatment: an investigation of the efficacy and safety of benzalkonium chloride-preserved intraocular pressure-lowering eye drops and their effect on conjunctival goblet cells. *Acta Ophthalmol*. 2023;101(suppl 278):3–21. doi:10.1111/aos.15808.
765. Rossi GC, Pasinetti GM, Scudeller L, Bianchi PE. Ocular surface disease and glaucoma: how to evaluate impact on quality of life. *J Ocul Pharmacol Ther*. 2013;29(4):390–394. doi:10.1089/jop.2011.0159.
766. Tirpack AR, Vanner E, Parrish JM, Galor A, Hua HU, Wellik SR. Dry eye symptoms and ocular pain in veterans with glaucoma. *J Clin Med*. 2019;8(7):1076. doi:10.3390/jcm8071076.
767. Vitoux MA, Kessal K, Melik Parsadaniantz S, et al. Benzalkonium chloride-induced direct and indirect toxicity on corneal epithelial and trigeminal neuronal cells: proinflammatory and apoptotic responses in vitro. *Toxicol Lett*. 2020;319:74–84. doi:10.1016/j.toxlet.2019.10.014.
768. Rolando M, Barabino S, Mingari C, Moretti S, Giuffrida S, Calabria G. Distribution of conjunctival HLA-DR expression and the pathogenesis of damage in early dry eyes. *Cornea*. 2005;24(8):951–954. doi:10.1097/01.ico.0000157421.93522.00.
769. Zhang R, Park M, Richardson A, et al. Dose-dependent benzalkonium chloride toxicity imparts ocular surface epithelial changes with features of dry eye disease. *Ocul Surf*. Jan 2020;18(1):158–169. doi:10.1016/j.jtos.2019.11.006.
770. Swamynathan SK, Wells A. Conjunctival goblet cells: Ocular surface functions, disorders that affect them, and the potential for their regeneration. *Ocul Surf*. Jan 2020;18(1):19–26. doi:10.1016/j.jtos.2019.11.005.
771. Chen W, Li Z, Hu J, et al. Corneal alternations induced by topical application of benzalkonium chloride in rabbit. *PLoS One*. 2011;6(10):e26103. doi:10.1371/journal.pone.0026103.
772. Izzotti A, La Maestra S, Micale RT, Longobardi MG, Sacca SC. Genomic and post-genomic effects of antiglaucoma drugs preservatives in trabecular meshwork. *Mutat Res*. 2015;772:1–9. doi:10.1016/j.mrfmmm.2014.11.006.
773. Lou Q, Pan L, Xiang S, et al. Suppression of NLRP3/caspase-1/GSDMD mediated corneal epithelium pyroptosis using melatonin-loaded liposomes to inhibit benzalkonium chloride-induced dry eye disease. *Int J Nanomedicine*. 2023;18:2447–2463. doi:10.2147/IJN.S403337.
774. Sarkar J, Chaudhary S, Namavari A, et al. Corneal neurotoxicity due to topical benzalkonium chloride. *Invest Ophthalmol Vis Sci*. 2012;53(4):1792–1802. doi:10.1167/iovs.11-8775.
775. Kahook MY, Rapuano CJ, Messmer EM, Radcliffe NM, Galor A, Baudouin C. Preservatives and ocular surface disease: A review. *Ocul Surf*. 2024;34:213–224. doi:10.1016/j.jtos.2024.08.001.
776. Skov AG, Rives AS, Freiberg J, Virgili G, Azuara-Blanco A, Kolko M. Comparative efficacy and safety of preserved versus preservative-free beta-blockers in patients with glaucoma or ocular hypertension: a systematic review. *Acta Ophthalmol*. 2022;100(3):253–261. doi:10.1111/aos.14926.
777. Jee D, Park SH, Kim MS, Kim EC. Antioxidant and inflammatory cytokine in tears of patients with dry eye syndrome treated with preservative-free versus preserved eye drops. *Invest Ophthalmol Vis Sci*. Jul 3 2014;55(8):5081–5089. doi:10.1167/iovs.14-14483.
778. Nasser L, Rozycka M, Gomez Rendon G, Navas A. Real-life results of switching from preserved to preservative-free artificial tears containing hyaluronate in patients with dry eye disease. *Clin Ophthalmol*. 2018;12:1519–1525. doi:10.2147/OPTH.S160053.
779. Bastelica P, Renard JP, Aptel F, et al. The PRAMOS study: PRostaglandin Analogues Monotherapy-awareness survey on Ocular Surface involvement. *Ophthalmol Ther*. 2024;13(6):1537–1551. doi:10.1007/s40123-024-00936-9.
780. Jandrokovic S, Vidas Pauk S, Lesin Gacina D, et al. Tolerability in glaucoma patients switched from preserved to preservative-free prostaglandin-timolol combination: a prospective real-life study. *Clin Ophthalmol*. 2022;16:3181–3192. doi:10.2147/OPTH.S382497.
781. Kim DW, Shin J, Lee CK, Kim M, Lee S, Rho S. Comparison of ocular surface assessment and adherence between preserved and preservative-free latanoprost in glaucoma: a parallel-grouped randomized trial. *Sci Rep*. 2021;11(1):14971. doi:10.1038/s41598-021-94574-x.
782. Liang H, Baudouin C, Daull P, Garrigue JS. Brignole-Baudouin F. In vitro corneal and conjunctival wound-healing assays as a tool for antiglaucoma prostaglandin formulation characterization. *Front Biosci (Landmark Ed)*. 2022;27(5):147. doi:10.31083/j.fbl2705147.
783. Gazzard G, Konstantakopoulou E, Garway-Heath D, et al. Laser in Glaucoma and Ocular Hypertension (LiGHT) trial. A multicentre, randomised controlled trial: design and methodology. *Br J Ophthalmol*. 2018;102(5):593–598. doi:10.1136/bjophthalmol-2017-310877.
784. Wong J, Lan W, Ong LM, Tong L. Non-hormonal systemic medications and dry eye. *Ocul Surf*. 2011;9(4):212–226. doi:10.1016/s1542-0124(11)70034-9.
785. Schein OD, Hochberg MC, Munoz B, et al. Dry eye and dry mouth in the elderly: a population-based assessment. *Arch Intern Med*. 1999;159(12):1359–1363. doi:10.1001/archinte.159.12.1359.
786. Guo M, Diaz GM, Yu Y, et al. Association between systemic medication use and severity of dry eye signs and symptoms in the DRy Eye Assessment and Management (DREAM) study. *Ocul Surf*. 2024;32:112–119. doi:10.1016/j.jtos.2024.01.009.
787. Dana R, Bradley JL, Guerin A, Pivneva I, Evans AM, Stillman IO. Comorbidities and prescribed medications in patients with or without dry eye disease: a population-based

study. *Am J Ophthalmol*. 2019;198:181–192. doi:10.1016/j.ajo.2018.10.001.
788. Ghossein N, Kang M, Lakhkar AD. *StatPearls [Internet]*. Anticholinergic medications. StatPearls Publishing; 2020.
789. Wellington K, Jarvis B. Cetirizine/pseudoephedrine. *Drugs*. 2001;61(15):2231–2240 discussion 2241-2242. doi:10.2165/00003495-200161150-00009.
790. Vardy MD, Mitcheson HD, Samuels TA, et al. Effects of solifenacin on overactive bladder symptoms, symptom bother and other patient-reported outcomes: results from VIBRANT - a double-blind, placebo-controlled trial. *Int J Clin Pract*. 2009;63(12):1702–1714. doi:10.1111/j.1742-1241.2009.02209.x.
791. Ousler GW, Wilcox KA, Gupta G, Abelson MB. An evaluation of the ocular drying effects of 2 systemic antihistamines: loratadine and cetirizine hydrochloride. *Ann Allergy Asthma Immunol*. 2004;93(5):460–464. doi:10.1016/s1081-1206(10)61413-5.
792. Kam WR, Sullivan DA. Neurotransmitter influence on human meibomian gland epithelial cells. *Invest Ophthalmol Vis Sci*. 2011;52(12):8543–8548. doi:10.1167/iovs.11-8113.
793. Vallerand IA, Lewinson RT, Farris MS, et al. Efficacy and adverse events of oral isotretinoin for acne: a systematic review. *Br J Dermatol*. 2018;178(1):76–85. doi:10.1111/bjd.15668.
794. Fouladgar N, Khabazkhoob M, Hanifnia AR, Yekta A, Mirzajani A. Evaluation of the effects of isotretinoin for treatment of acne on corneal sensitivity. *J Curr Ophthalmol*. 2018;30(4):326–329. doi:10.1016/j.joco.2018.06.005.
795. Caglar C, Senel E, Sabancilar E, Durmus M. Reduced ocular surface disease index (OSDI) scores in patients with isotretinoin treatment. *Int Ophthalmol*. 2017;37(1):197–202. doi:10.1007/s10792-016-0263-y.
796. Duzgun E, Ozkur E. The effect of oral isotretinoin therapy on meibomian gland morphology and dry eye tests. *J Dermatolog Treat*. 2022;33(2):762–768. doi:10.1080/09546634.2020.1774041.
797. Zhang P, Tian L, Bao J, et al. Isotretinoin impairs the secretory function of meibomian gland via the PPARγ signaling pathway. *Invest Ophthalmol Vis Sci*. 2022;63(3):29. doi:10.1167/iovs.63.3.29.
798. Aslan Bayhan S, Bayhan HA, Çölgeçen E, Gürdal C. Effects of topical acne treatment on the ocular surface in patients with acne vulgaris. *Cont Lens Anterior Eye*. 2016;39(6):431–434. doi:10.1016/j.clae.2016.06.009.
799. Santaella RM, Fraunfelder FW. Ocular adverse effects associated with systemic medications: recognition and management. *Drugs*. 2007;67(1):75–93. doi:10.2165/00003495-200767010-00006.
800. Wang SQ, Zhang LW, Wei P, Hua H. Is hydroxychloroquine effective in treating primary Sjogren's syndrome: a systematic review and meta-analysis. *BMC Musculoskelet Disord*. 2017;18(1):186. doi:10.1186/s12891-017-1543-z.
801. Chu LL, Cui K, Pope JE. Meta-analysis of treatment for primary Sjogren's syndrome. *Arthritis Care Res (Hoboken)*. 2020;72(7):1011–1021. doi:10.1002/acr.23917.
802. Gottenberg JE, Ravaud P, Puechal X, et al. Effects of hydroxychloroquine on symptomatic improvement in primary Sjogren syndrome: the JOQUER randomized clinical trial. *JAMA*. 2014;312(3):249–258. doi:10.1001/jama.2014.7682.
803. Fraunfelder FW, Solomon J, Mehelas TJ. Ocular adverse effects associated with cyclooxygenase-2 inhibitors. *Arch Ophthalmol*. 2006;124(2):277–279. doi:10.1001/archopht.124.2.277.
804. Yazici A, Sari E, Ayhan E, et al. The effect of low-dose aspirin on dry eye parameters and ocular surface disease index questionnaire. *J Ocul Pharmacol Ther*. 2018;34(3):256–259. doi:10.1089/jop.2017.0064.
805. Moss SE, Klein R, Klein BE. Prevalence of and risk factors for dry eye syndrome. *Arch Ophthalmol*. 2000;118(9):1264–1268. doi:10.1001/archopht.118.9.1264.
806. Liu SH, Saldanha IJ, Abraham AG, et al. Topical corticosteroids for dry eye. *Cochrane Database Syst Rev*. 2022;10(10):CD015070. doi:10.1002/14651858.CD015070.pub2.
807. Valenzuela CV, Liu JC, Vila PM, Simon L, Doering M, Lieu JEC. Intranasal corticosteroids do not lead to ocular changes: a systematic review and meta-analysis. *Laryngoscope*. 2019;129(1):6–12. doi:10.1002/lary.27209.
808. Schlatter A, Hommer N, Kallab M, et al. Effect of treatment with topical azithromycin or oral doxycycline on tear film thickness in patients with meibomian gland dysfunction: a randomized controlled trial. *J Ocul Pharmacol Ther*. 2023;39(6):371–378. doi:10.1089/jop.2022.0186.
809. Ben Ephraim Noyman D, Chan CC, Mimouni M, Safir M. Systemic antibiotic treatment for meibomian gland dysfunction—a systematic review and meta-analysis. *Acta Ophthalmol*. 2024;102(1):e1–e10. doi:10.1111/aos.15681.
810. Pejcic AV. Stevens-Johnson syndrome and toxic epidermal necrolysis associated with the use of macrolide antibiotics: a review of published cases. *Int J Dermatol*. 2021;60(1):12–24. doi:10.1111/ijd.15144.
811. Nappe TM, Goren-Garcia SL, Jacoby JL. Stevens-Johnson syndrome after treatment with azithromycin: an uncommon culprit. *Am J Emerg Med*. 2016;34(3):676.e1–676.e3. doi:10.1016/j.ajem.2015.06.039.
812. Rakofsky JJ, Rakofsky SI, Dunlop BW. Dry those crying eyes: the role of depression and antidepressants in dry eye disease. *J Clin Psychopharmacol*. 2021;41(3):295–303. doi:10.1097/JCP.0000000000001382.
813. Isik-Ulusoy S, MO Ulusoy. Influence of different antidepressants on ocular surface in patients with major depressive disorder. *J Clin Psychopharmacol*. 2021;41(1):49–52. doi:10.1097/JCP.0000000000001325.
814. Zhang X, Yin Y, Yue L, Gong L. Selective serotonin reuptake inhibitors aggravate depression-associated dry eye via activating the NF-kappaB pathway. *Invest Ophthalmol Vis Sci*. 2019;60(1):407–419. doi:10.1167/iovs.18-25572.
815. Dixitha V, Abhilash B, Bhavya HU, Babu Mahesh, Sudhakar NA. Quantitative analysis of tear film in patients on atypical antipsychotics. *Ocul Immunol Inflamm*. 2024;32(5):566–571. doi:10.1080/09273948.2023.2177175.
816. Acan D, Kurtgoz P. Influence of selective serotonin reuptake inhibitors on ocular surface. *Clin Exp Optom*. 2017;100(1):83–86. doi:10.1111/cxo.12415.
817. Ismayilov AS, Celikel G. Effects of tricyclic antidepressants, selective serotonin reuptake inhibitors, and selective serotonin-norepinephrine reuptake inhibitors on the ocular surface. *Arq Bras Oftalmol*. 2022;86(5):e20230068. doi:10.5935/0004-2749.20230068.

818. Park JSY, Sharma RA, Sharma V. Ophthalmic adverse effects of lithium. *Int Clin Psychopharmacol*. 2020;35(2):69–73. doi:10.1097/YIC.0000000000000295.
819. Schaumberg DA, Buring JE, Sullivan DA, Dana MR. Hormone replacement therapy and dry eye syndrome. *JAMA*. 2001;286(17):2114–2119. doi:10.1001/jama.286.17.2114.
820. Nanavaty MA, Long M, Malhotra R. Transdermal androgen patches in evaporative dry eye syndrome with androgen deficiency: a pilot study. *Br J Ophthalmol*. 2014;98(4):567–569. doi:10.1136/bjophthalmol-2013-304637.
821. Schiffman RM, Bradford R, Bunnell B, Lai F, Bernstein P, Whitcup SW. A multi-center, double-masked, randomized, vehicle-controlled, parallel group study to evaluate the safety and efficacy of testosterone ophthalmic solution in patients with meibomian gland dysfunction. *Invest Ophthalmol Vis Sci*. 2006;47(13) 5608-5608.
822. Morgan PB, Murphy PJ, Gifford KL, et al. BCLA CLEAR—effect of contact lens materials and designs on the anatomy and physiology of the eye. *Cont Lens Anterior Eye*. 2021;44(2):192–219. doi:10.1016/j.clae.2021.02.006.
823. Stapleton F, Bakkar M, Carnt N, et al. CLEAR—contact lens complications. *Cont Lens Anterior Eye*. 2021;44(2):330–367. doi:10.1016/j.clae.2021.02.010.
824. Acimovic L, Stanojlovic S, Kalezic T. Dacic Krnjaja B. Evaluation of dry eye symptoms and risk factors among medical students in Serbia. *PLoS One*. 2022;17(10):e0275624. doi:10.1371/journal.pone.0275624.
825. Alamri A, Amer KA, Aldosari AA, et al. Assessment of dry eye syndrome among contact lens users in Asir Region, Saudi Arabia. *Cureus*. 2022;14(1):e21526. doi:10.7759/cureus.21526.
826. Fan Z, Du Y, Tang C, et al. Awareness, prevalence, and knowledge of dry eye among internet professionals: a cross-sectional study in China. *Eye Contact Lens*. 2023;49(3):92–97. doi:10.1097/icl.0000000000000968.
827. Supiyaphun C, Jongkhajornpong P, Rattanasiri S, Lekhanont K. Prevalence and risk factors of dry eye disease among University Students in Bangkok, Thailand. *PLoS One*. 2021;16(10):e0258217. doi:10.1371/journal.pone.0258217.
828. Schornack M, Nau C, Nau A, Harthan J, Fogt J, Shorter E. Visual and physiological outcomes of scleral lens wear. *Cont Lens Anterior Eye*. 2019;42(1):3–8. doi:10.1016/j.clae.2018.07.007.
829. Schornack MM, Pyle J, Patel SV. Scleral lenses in the management of ocular surface disease. *Ophthalmology*. 2014;121(7):1398–1405. doi:10.1016/j.ophtha.2014.01.028.
830. Li W, Sun X, Wang Z, Zhang Y. A survey of contact lens-related complications in a tertiary hospital in China. *Cont Lens Anterior Eye*. 2018;41(2):201–204. doi:10.1016/j.clae.2017.10.007.
831. Yang TT, Ma BK, Liu RJ, Qi H. Research advances in contact lens-associated dry eye. Article in Chinese. *Zhonghua Yan Ke Za Zhi*. 2022;58(2):149–154. doi:10.3760/cma.j.cn112142-20210525-00256.
832. Harbiyeli II, Bozkurt B, Erdem E, et al. Associations with meibomian gland loss in soft and rigid contact lens wearers. *Cont Lens Anterior Eye*. 2022;45(1):101400. doi:10.1016/j.clae.2020.12.005.
833. Pucker AD, Jones-Jordan LA, Marx S, et al. Clinical factors associated with contact lens dropout. *Cont Lens Anterior Eye*. 2019;42(3):318–324. doi:10.1016/j.clae.2018.12.002.
834. Shiraishi A, Yamaguchi M, Ohashi Y. Prevalence of upper- and lower-lid-wiper epitheliopathy in contact lens wearers and non-wearers. *Eye Contact Lens*. 2014;40(4):220–224. doi:10.1097/ICL.0000000000000040.
835. McMonnies CW. Incomplete blinking: exposure keratopathy, lid wiper epitheliopathy, dry eye, refractive surgery, and dry contact lenses. *Cont Lens Anterior Eye*. 2007;30(1):37–51. doi:10.1016/j.clae.2006.12.002.
836. Muntz A, Subbaraman LN, Craig JP, Jones L. Cytomorphological assessment of the lid margin in relation to symptoms, contact lens wear and lid wiper epitheliopathy. *Ocul Surf*. 2020;18(2):214–220. doi:10.1016/j.jtos.2019.12.001.
837. Pult H, Murphy PJ, Purslow C. A novel method to predict the dry eye symptoms in new contact lens wearers. *Optom Vis Sci*. 2009;86(9):E1042–E1050. doi:10.1097/OPX.0b013e3181b598cd.
838. Kojima T. Contact lens-associated dry eye disease: recent advances worldwide and in Japan. *Invest Ophthalmol Vis Sci*. 2018;59(14):DES102–DES108. doi:10.1167/iovs.17-23685.
839. Chinese expert consensus on the diagnosis and treatment of contact lens-associated dry eye (2024). Article in Chinese. *Zhonghua Yan Ke Za Zhi*. 2024;60(2):120–126. doi:10.3760/cma.j.cn112142-20231121-00243.
840. Yang L, Zhang L, Jian Hu R, Yu PP, Jin X. The influence of overnight orthokeratology on ocular surface and dry eye-related cytokines IL-17A, IL-6, and PGE2 in children. *Cont Lens Anterior Eye*. 2021;44(1):81–88. doi:10.1016/j.clae.2020.04.001.
841. Liu Q, Xu Z, Xu Y, et al. Changes in corneal dendritic cell and sub-basal nerve in long-term contact lens wearers with dry eye. *Eye Contact Lens*. 2020;46(4):238–244. doi:10.1097/icl.0000000000000691.
842. Lum E, Golebiowski B, Swarbrick HA. Reduced corneal sensitivity and sub-basal nerve density in long-term orthokeratology lens wear. *Eye Contact Lens*. 2017;43(4):218–224. doi:10.1097/icl.0000000000000285.
843. Nombela-Palomo M, Felipe-Marquez G, Teus MA, Hernandez-Verdejo JL, Nieto-Bona A. Long-term impacts of orthokeratology treatment on sub-basal nerve plexus and corneal sensitivity responses and their reversibility. *Eye Contact Lens*. 2018;44(2):91–96. doi:10.1097/icl.0000000000000386.
844. Jin W, Ye J, Zhang J, et al. Short-term effects of overnight orthokeratology on corneal sensitivity in Chinese children and adolescents. *J Ophthalmol*. 2018;2018:6185919. doi:10.1155/2018/6185919.
845. Stapleton F, Marfurt C, Golebiowski B, et al. The TFOS International Workshop on Contact Lens Discomfort: report of the subcommittee on neurobiology. *Invest Ophthalmol Vis Sci*. 2013;54(11):TFOS71–TFOS97. doi:10.1167/iovs.13-13226.
846. Situ P, Begley C, Simpson T. The roles of neural adaptation and sensitization in contact lens discomfort. *Ocul Surf*. 2024;34:132–139. doi:10.1016/j.jtos.2024.07.003.
847. Mobeen R, Stapleton F, Chao C, et al. Epithelial immune cell response to initial soft contact lens wear in the human

corneal and conjunctival epithelium. *Invest Ophthalmol Vis Sci*. 2023;64(15):18. doi:10.1167/iovs.64.15.18.
848. Saliman NH, Morgan PB, MacDonald AS, Maldonado-Codina C. Subclinical inflammation of the ocular surface in soft contact lens wear. *Cornea*. 2020;39(2):146–154. doi:10.1097/ICO.0000000000002192.
849. Society of Contact Lens Safety Monitoring & Vision Health of Chinese Health AssociationExpert consensus on diagnosis and treatment of adverse reactions of contact lens wear in China (2021). Article in Chinese. *Zhonghua Yan Ke Za Zhi*. 2021;57(8):573–579. doi:10.3760/cma.j.cn112142-20210520-00243.
850. Wolffsohn JS, Dumbleton K, Huntjens B, et al. CLEAR - Evidence-based contact lens practice. *Cont Lens Anterior Eye*. Apr 2021;44(2):368–397. doi:10.1016/j.clae.2021.02.008.
851. Igarashi T, Kobayashi M, Yaguchi C, Fujimoto C, Suzuki H, Takahashi H. Efficacy of rebamipide instillation for contact lens discomfort with dry eye. *Eye Contact Lens*. 2018;44(Suppl 2):S137–S142. doi:10.1097/icl.0000000000000438.
852. Siddireddy JS, Tan J, Vijay AK, Willcox MDP. The effect of microblepharon exfoliation on clinical correlates of contact lens discomfort. *Optom Vis Sci*. 2019;96(3):187–199. doi:10.1097/OPX.0000000000001354.
853. Blackie CA, Coleman CA, Nichols KK, et al. A single vectored thermal pulsation treatment for meibomian gland dysfunction increases mean comfortable contact lens wearing time by approximately 4 hours per day. *Clin Ophthalmol*. 2018;12:169–183. doi:10.2147/OPTH.S153297.
854. Pavan Michielon E, Gheller P, Piñero DP, Stanco L. Contact lens discomfort (CLD) treatment with MY MASK light modulation LED mask. 2024-12-31 doi:10.15626/sjovs.v17i2.4212
855. Stapleton F, Jia T, DePuy V, Bosworth C, Gleeson M, Tan J. The effect of a biweekly novel selenium sulfide-containing topical treatment in symptomatic contact lens wearers: an exploratory study. *Ocul Surf*. 2025;36:190–197. doi:10.1016/j.jtos.2025.01.015.
856. Stapleton F, Hinds M, Tan J, et al. AZR-MD-001 0.5% selenium sulfide ophthalmic ointment for the treatment of contact lens discomfort: a vehicle-controlled, randomized, clinical trial. *Ocul Surf*. 2024 S1542-0124(24)00144-7. doi:10.1016/j.jtos.2024.12.009.
857. Choi EW, Yeom DJ, Jang SY. Botulinum toxin A injection for the treatment of intractable dry eye disease. *Medicina (Kaunas)*. 2021;57(3):247. doi:10.3390/medicina57030247.
858. Ziahosseini K, Al-Abbadi Z, Malhotra R. Botulinum toxin injection for the treatment of epiphora in lacrimal outflow obstruction. *Eye (Lond)*. 2015;29(5):656–661. doi:10.1038/eye.2015.18.
859. Ho MC, Hsu WC, Hsieh YT. Botulinum toxin type A injection for lateral canthal rhytids: effect on tear film stability and tear production. *JAMA Ophthalmol*. 2014;132(3):332–337. doi:10.1001/jamaophthalmol.2013.6243.
860. Žiak P, Halička J, Kapitánová K, Mojžiš P. Effect of botulinum toxin A application in neuro-ophthalmologic indications on Schirmers test and tears osmolarity. Vplyv aplikácie botulotoxínu A v neurooftalmologických indikáciách na výsledky Schirmerovho testu a osmolarity. Article in Czech. *Cesk Slov Oftalmol*. 2019;75(2):74–77. doi:10.31348/2019/2/3.
861. Kaynak P, Karabulut GO, Ozturker C, et al. Comparison of botulinum toxin-A injection in lacrimal gland and conjunctivodacryocystorhinostomy for treatment of epiphora due to proximal lacrimal system obstruction. *Eye (Lond)*. 2016;30(8):1056–1062. doi:10.1038/eye.2016.88.
862. Alsuhaibani AH, Eid SA. Botulinum toxin injection and tear production. *Curr Opin Ophthalmol*. 2018;29(5):428–433. doi:10.1097/icu.0000000000000506.
863. Lipham WJ, Tawfik HA, Dutton JJ. A histologic analysis and three-dimensional reconstruction of the muscle of Riolan. *Ophthalmic Plast Reconstr Surg*. 2002;18(2):93–98. doi:10.1097/00002341-200203000-00002.
864. Ho RW, Fang PC, Chang CH, Liu YP, Kuo MT. A review of periocular botulinum neurotoxin on the tear film homeostasis and the ocular surface change. *Toxins (Basel)*. 2019;11(2):66. doi:10.3390/toxins11020066.
865. Ozgur OK, Murariu D, Parsa AA, Parsa FD. Dry eye syndrome due to botulinum toxin type-A injection: guideline for prevention. *Hawaii J Med Public Health*. 2012;71(5):120–123.
866. Wollensak G, Spoerl E, Seiler T. Riboflavin/ultraviolet-a-induced collagen crosslinking for the treatment of keratoconus. *Am J Ophthalmol*. 2003;135(5):620–627. doi:10.1016/s0002-9394(02)02220-1.
867. Ashwin PT, McDonnell PJ. Collagen cross-linkage: a comprehensive review and directions for future research. *Br J Ophthalmol*. 2010;94(8):965–970. doi:10.1136/bjo.2009.164228.
868. Wang X, Bai X, Jin C, Zhao L, Wei R. Analysis of tear function outcomes following collagen cross-linking treatment in ectatic corneas. *J Ophthalmol*. 2022;2022:1910607. doi:10.1155/2022/1910607.
869. Voronin GV, Bubnova IA, Averich VV, Sarkisova KG. Sostoyanie prekorneal'noi sleznoi plenki posle krosslinkinga rogovichnogo kollagena pri keratokonuse (predvaritel'noe soobshchenie). Precorneal tear film after corneal collagen cross-linking in keratoconus (preliminary report). Article in Russian. *Vestn Oftalmol*. 2021;137(5. Vyp. 2):224–230. doi:10.17116/oftalma2021137052224.
870. Recalde JI, Acera A, Rodríguez-Agirretxe I, Sánchez-Tena MA, San-Cristóbal J, Durán JA. Ocular surface disease parameters after collagen cross-linking for keratoconus. *Cornea*. 2017;36(2):148–152. doi:10.1097/ico.0000000000001085.
871. Bubnova IA, Surnina ZV, Averich VV, Sarkisova KG. The effects of corneal collagen cross-linking on the corneal structure in keratoconus. Vliyanie krosslinkinga rogovichnogo kollagena na strukturu rogovitsy pri keratokonuse. Article in Russian. *Vestn Oftalmol*. 2020;136(5. Vyp. 2):268–276. doi:10.17116/oftalma2020136052268.
872. Akgöz H, Fındık H, Aslan MG. Evaluation of tear parameters and meibomian gland morphology in keratoconus patients after epithelial-on corneal cross-linking. *Eur J Ophthalmol*. 2022:11206721221118740. doi:10.1177/11206721221118740.
873. Smith TM, Suzuki S, Sabat N, Rayner CL, Harkin DG, Chirila TV. Further investigations on the crosslinking of tarsal collagen as a treatment for eyelid laxity: optimizing the procedure in animal tissue. *Ophthalmic*

Plast Reconstr Surg. 2019;35(6):600–603. doi:10.1097/iop.0000000000001413.
874. Uysal BS, Akcay E, Kilicarslan A, et al. Tear function and ocular surface changes following corneal collagen cross-linking treatment in keratoconus patients: 18-month results. *Int Ophthalmol*. 2020;40(1):169–177. doi:10.1007/s10792-019-01161-1.
875. Kontadakis GA, Kymionis GD, Kankariya VP, Pallikaris AI. Effect of corneal collagen cross-linking on corneal innervation, corneal sensitivity, and tear function of patients with keratoconus. *Ophthalmology*. 2013;120(5):917–922. doi:10.1016/j.ophtha.2012.10.012.
876. Mazzotta C, Traversi C, Baiocchi S, et al. Corneal healing after riboflavin ultraviolet—A collagen cross-linking determined by confocal laser scanning microscopy in vivo: early and late modifications. *Am J Ophthalmol*. 2008;146(4):527–533. doi:10.1016/j.ajo.2008.05.042.
877. Bubnova IA, Sarkisova KG. Izmenenie sostoyaniya prerogovichnoi sleznoi plenki i glaznoi poverkhnosti posle krosslinkinga rogovichnogo kollagena pri keratokonuse. Changes in precorneal tear film and ocular surface in keratoconic patients after corneal collagen cross-linking. Article in Russian. *Vestn Oftalmol*. 2022;138(3):118–123. doi:10.17116/oftalma2022138031118.
878. El Ameen A, Majzoub S, Vandermeer G, Pisella PJ. Influence of cataract surgery on Meibomian gland dysfunction. *J Fr Ophtalmol*. 2018;41(5):e173–e180. doi:10.1016/j.jfo.2018.03.001.
879. Liu L, Fukuyama T, Han T, et al. Observation and assessment of the immediate use of a silicon hydrogel contact lens after transepithelial corneal cross linking: a prospective study. *BMC Ophthalmol*. 2023;23(1):471. doi:10.1186/s12886-023-03217-4.
880. Ondes Yilmaz F, Kepez Yildiz B, Tunc U, Kandemir Besek N, Yildirim Y, Demirok A. Comparison of topical omega-3 fatty acids with topical sodium hyaluronate after corneal crosslinking: short term results. *Ocul Immunol Inflamm*. 2022;30(4):959–965. doi:10.1080/09273948.2020.1858117.
881. Bikbov MM, Khalimov AR, Usubov EL. Ultraviolet corneal crosslinking. Article in Russian. *Vestn Ross Akad Med Nauk*. 2016;71(3):224–232. doi:10.15690/vramn562.
882. Knutsson KA, Genovese PN, Paganoni G, et al. Evaluation of a post-operative therapy protocol after epithelium-off corneal cross-linking in patients affected by keratoconus. *J Clin Med*. 2022;11(23):7093. doi:10.3390/jcm11237093.
883. Recalde JI, Duran JA, Rodriguez-Agirretxe I, et al. Changes in tear biomarker levels in keratoconus after corneal collagen crosslinking. *Mol Vis*. 2019;25:12–21.
884. Jeganathan VS, Wirth A, MacManus MP. Ocular risks from orbital and periorbital radiation therapy: a critical review. *Int J Radiat Oncol Biol Phys*. 2011;79(3):650–659. doi:10.1016/j.ijrobp.2010.09.056.
885. Bhandare N, Moiseenko V, Song WY, Morris CG, Bhatti MT, Mendenhall WM. Severe dry eye syndrome after radiotherapy for head-and-neck tumors. *Int J Radiat Oncol Biol Phys*. 2012;82(4):1501–1508. doi:10.1016/j.ijrobp.2011.05.026.
886. Durkin SR, Roos D, Higgs B, Casson RJ, Selva D. Ophthalmic and adnexal complications of radiotherapy. *Acta Ophthalmol Scand*. 2007;85(3):240–250. doi:10.1111/j.1600-0420.2006.00822.x.
887. Gordon KB, Char DH, Sagerman RH. Late effects of radiation on the eye and ocular adnexa. *Int J Radiat Oncol Biol Phys*. 1995;31(5):1123–1139. doi:10.1016/0360-3016(95)00062-4.
888. Soni M, Walia S, Jain P. Dry eye disease in head and neck cancer patients undergoing radiotherapy. *Indian J Ophthalmol*. 2023;71(4):1556–1560. doi:10.4103/ijo.Ijo_2673_22.
889. Thariat J, Maschi C, Lanteri S, et al. Dry eye syndrome after proton therapy of ocular melanomas. *Int J Radiat Oncol Biol Phys*. 2017;98(1):142–151. doi:10.1016/j.ijrobp.2017.01.199.
890. Parsons JT, Bova FJ, Fitzgerald CR, Mendenhall WM, Million RR. Severe dry-eye syndrome following external beam irradiation. *Int J Radiat Oncol Biol Phys*. 1994;30(4):775–780. doi:10.1016/0360-3016(94)90348-4.
891. Bessell EM, Henk JM, Whitelocke RA, Wright JE. Ocular morbidity after radiotherapy of orbital and conjunctival lymphoma. *Eye (Lond)*. 1987;1(pt 1):90–96. doi:10.1038/eye.1987.14.
892. Groenewald C, Konstantinidis L, Damato B. Effects of radiotherapy on uveal melanomas and adjacent tissues. *Eye (Lond)*. 2013;27(2):163–171. doi:10.1038/eye.2012.249.
893. Konstantinidis L, Roberts D, Errington RD, Kacperek A, Heimann H, Damato B. Transpalpebral proton beam radiotherapy of choroidal melanoma. *Br J Ophthalmol*. 2015;99(2):232–235. doi:10.1136/bjophthalmol-2014-305313.
894. Tiwari S, Bhatt A, Nagamodi J, et al. Aqueous deficient dry eye syndrome post orbital radiotherapy: a 10-year retrospective study. *Transl Vis Sci Technol*. 2017;6(3):19. doi:10.1167/tvst.6.3.19.
895. Sia S, Harper C, McAllister I, Perry A. Iodine-I25 episcleral plaque therapy in uveal melanoma. *Clin Exp Ophthalmol*. 2000;28(6):409–413. doi:10.1046/j.1442-9071.2000.00354.x.
896. Karp LA, Streeten BW, Cogan DG. Radiation-induced atrophy of the meibomian gland. *Arch Ophthalmol*. 1979;97(2):303–305. doi:10.1001/archopht.1979.01020010155013.
897. Parsons JT, Bova FJ, Mendenhall WM, Million RR, Fitzgerald CR. Response of the normal eye to high dose radiotherapy. *Oncology (Williston Park)*. 1996;10(6):837–847 discussion 847-848, 851-852.
898. Chen X, Badian RA, Hynne H, et al. Alterations in meibomian glands in patients treated with intensity-modulated radiotherapy for head and neck cancer. *Sci Rep*. 2021;11(1):22419. doi:10.1038/s41598-021-01844-9.
899. Ferrara JL, Levine JE, Reddy P, Holler E. Graft-versus-host disease. *Lancet*. 2009;373(9674):1550–1561. doi:10.1016/s0140-6736(09)60237-3.
900. Franklin RM, Kenyon KR, Tutschka PJ, Saral R, Green WR, Santos GW. Ocular manifestations of graft-vs-host disease. *Ophthalmology*. 1983;90(1):4–13. doi:10.1016/s0161-6420(83)34604-2.
901. Hirst LW, Jabs DA, Tutschka PJ, Green WR, Santos GW. The eye in bone marrow transplantation. I. Clinical study. *Arch Ophthalmol*. 1983;101(4):580–584. doi:10.1001/archopht.1983.01040010580010.

902. Westeneng AC, Hettinga Y, Lokhorst H, Verdonck L, van Dorp S, Rothova A. Ocular graft-versus-host disease after allogeneic stem cell transplantation. *Cornea*. 2010;29(7):758–763. doi:10.1097/ICO.0b013e3181ca321c.
903. Ogawa Y, Yamazaki K, Kuwana M, et al. A significant role of stromal fibroblasts in rapidly progressive dry eye in patients with chronic GVHD. *Invest Ophthalmol Vis Sci*. 2001;42(1):111–119.
904. Ogawa Y, Kuwana M, Yamazaki K, et al. Periductal area as the primary site for T-cell activation in lacrimal gland chronic graft-versus-host disease. *Invest Ophthalmol Vis Sci*. 2003;44(5):1888–1896. doi:10.1167/iovs.02-0699.
905. Yaguchi S, Ogawa Y, Shimmura S, et al. Presence and physiologic function of the renin-angiotensin system in mouse lacrimal gland. *Invest Ophthalmol Vis Sci*. 2012;53(9):5416–5425. doi:10.1167/iovs.12-9891.
906. Yaguchi S, Ogawa Y, Kawakita T, Shimmura S, Tsubota K. Tissue renin-angiotensin system in lacrimal gland fibrosis in a murine model of chronic graft-versus-host disease. *Cornea*. 2015;34(suppl 11):S142–S152. doi:10.1097/ico.0000000000000586.
907. Kheirkhah A, Rahimi Darabad R, Cruzat A, et al. Corneal epithelial immune dendritic cell alterations in subtypes of dry eye disease: a pilot in vivo confocal microscopic study. *Invest Ophthalmol Vis Sci*. 2015;56(12):7179–7185. doi:10.1167/iovs.15-17433.
908. Chatterjee D. A brief account on ocular graft versus host disease. *Indian J Ophthalmol*. 2023;71(4):1115–1122. doi:10.4103/ijo.Ijo_2839_22.
909. Yamane M, Sato S, Shimizu E, et al. Senescence-associated secretory phenotype promotes chronic ocular graft-vs-host disease in mice and humans. *FASEB J*. 2020;34(8):10778–10800. doi:10.1096/fj.201900218R.
910. Rodier F, Coppé JP, Patil CK, et al. Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. *Nat Cell Biol*. 2009;11(8):973–979. doi:10.1038/ncb1909.
911. Yang F, Hayashi I, Sato S, et al. Eyelid blood vessel and meibomian gland changes in a sclerodermatous chronic GVHD mouse model. *Ocul Surf*. 2022;26:328–341. doi:10.1016/j.jtos.2021.10.006.
912. Ban Y, Ogawa Y, Ibrahim OM, et al. Morphologic evaluation of meibomian glands in chronic graft-versus-host disease using in vivo laser confocal microscopy. *Mol Vis*. 2011;17:2533–2543.
913. Sinha S, Singh RB, Dohlman TH, Taketani Y, Yin J, Dana R. Prevalence and risk factors associated with corneal perforation in chronic ocular graft-versus-host-disease. *Cornea*. 2021;40(7):877–882. doi:10.1097/ico.0000000000002526.
914. Lee GA, Chen SX. Autologous serum in the management of recalcitrant dry eye syndrome. *Clin Exp Ophthalmol*. 2008;36(2):119–122. doi:10.1111/j.1442-9071.2008.01680.x.
915. Wolff D, Gerbitz A, Ayuk F, et al. Consensus conference on clinical practice in chronic graft-versus-host disease (GVHD): first-line and topical treatment of chronic GVHD. *Biol Blood Marrow Transplant*. 2010;16(12):1611–1628. doi:10.1016/j.bbmt.2010.06.015.
916. Dausch D, Lee S, Dausch S, Kim JC, Schwert G, Michelson W. Comparative study of treatment of the dry eye syndrome due to disturbances of the tear film lipid layer with lipid-containing tear substitutes. Vergleichende Studie zur Therapie des Trockenen Auges bedingt durch Lipidphasenstörungen mit lipidhaltigen Tränenpräparaten. Article in German. . Klin Monbl Augenheilkd. 2006;223(12):974–983. doi:10.1055/s-2006-927266.
917. Pflugfelder SC. Antiinflammatory therapy for dry eye. *Am J Ophthalmol*. 2004;137(2):337–342. doi:10.1016/j.ajo.2003.10.036.
918. Sabbe LJ, Van De Merwe D, Schouls L, Bergmans A, Vaneechoutte M, Vandamme P. Clinical spectrum of infections due to the newly described Actinomyces species A. *turicensis*, *A. radingae*, and i. *J Clin Microbiol*. 1999;37(1):8–13. doi:10.1128/jcm.37.1.8-13.1999.
919. Ogawa Y, Okamoto S, Kuwana M, et al. Successful treatment of dry eye in two patients with chronic graft-versus-host disease with systemic administration of FK506 and corticosteroids. *Cornea*. 2001;20(4):430–434. doi:10.1097/00003226-200105000-00020.
920. Noble BA, Loh RS, MacLennan S, et al. Comparison of autologous serum eye drops with conventional therapy in a randomised controlled crossover trial for ocular surface disease. *Br J Ophthalmol*. 2004;88(5):647–652. doi:10.1136/bjo.2003.026211.
921. Ogawa Y, Okamoto S, Mori T, et al. Autologous serum eye drops for the treatment of severe dry eye in patients with chronic graft-versus-host disease. *Bone Marrow Transplant*. 2003;31(7):579–583. doi:10.1038/sj.bmt.1703862.
922. Pezzotta S, Del Fante C, Scudeller L, et al. Long-term safety and efficacy of autologous platelet lysate drops for treatment of ocular GvHD. *Bone Marrow Transplant*. 2017;52(1):101–106. doi:10.1038/bmt.2016.221.
923. Tarnawska D, Wylegała E. Corneal grafting and aggressive medication for corneal defects in graft-versus-host disease following bone marrow transplantation. *Eye (Lond)*. 2007;21(12):1493–1500. doi:10.1038/sj.eye.6702589.
924. ICH. ICH guidelines. https://www.ich.org/page/ich-guidelines
925. Dry Eye: Developing Drugs for Treatment Guidance for Industry. Center for Drug Evaluation and Research; 2020.
926. Cui D, Saldanha IJ, Li G, Mathews PM, Lin MX, Akpek EK. United States regulatory approval of topical treatments for dry eye. *Am J Ophthalmol*. 2024;258:14–21. doi:10.1016/j.ajo.2023.09.024.
927. Akpek EK, Wirta DL, Downing JE, et al. Efficacy and safety of a water-free topical cyclosporine, 0.1%, solution for the treatment of moderate to severe dry eye disease: the ESSENCE-2 randomized clinical trial. *JAMA Ophthalmol*. 2023;141(5):459–466. doi:10.1001/jamaophthalmol.2023.0709.
928. Kinoshita S, Oshiden K, Awamura S, et al. A randomized, multicenter phase 3 study comparing 2% rebamipide (OPC-12759) with 0.1% sodium hyaluronate in the treatment of dry eye. *Ophthalmology*. 2013;120(6):1158–1165. doi:10.1016/j.ophtha.2012.12.022.
929. Novack GD, Asbell P, Barabino S, et al. TFOS DEWS II clinical trial design report. *Ocul Surf*. 2017;15(3):629–649. doi:10.1016/j.jtos.2017.05.009.

930. Wirta D, Torkildsen GL, Boehmer B, et al. ONSET-1 phase 2b randomized trial to evaluate the safety and efficacy of OC-01 (varenicline solution) nasal spray on signs and symptoms of dry eye disease. *Cornea*. 2022;41(10):1207–1216. doi:10.1097/ICO.0000000000002941.
931. Korenfeld M, Nichols KK, Goldberg D, et al. Safety of KPI-121 ophthalmic suspension 0.25% in patients with dry eye disease: a pooled analysis of 4 multicenter, randomized, vehicle-controlled studies. *Cornea*. 2021;40(5):564–570. doi:10.1097/ICO.0000000000002452.
932. Tauber J, Schechter BA, Bacharach J, et al. A phase II/III, randomized, double-masked, vehicle-controlled, dose-ranging study of the safety and efficacy of OTX-101 in the treatment of dry eye disease. *Clin Ophthalmol*. 2018;12:1921–1929. doi:10.2147/OPTH.S175065.
933. Goldberg DF, Malhotra RP, Schechter BA, Justice A, Weiss SL, Sheppard JD. A phase 3, randomized, double-masked study of OTX-101 ophthalmic solution 0.09% in the treatment of dry eye disease. *Ophthalmology*. 2019;126(9):1230–1237. doi:10.1016/j.ophtha.2019.03.050.
934. Takamura E, Tsubota K, Watanabe H, Ohashi Y. Diquafosol Ophthalmic Solution Phase 3 Study G. A randomised, double-masked comparison study of diquafosol versus sodium hyaluronate ophthalmic solutions in dry eye patients. *Br J Ophthalmol*. 2012;96(10):1310–1315. doi:10.1136/bjophthalmol-2011-301448.
935. White DE, Zhao Y, Ogundele A, et al. Real-world treatment patterns of cyclosporine ophthalmic emulsion and lifitegrast ophthalmic solution among patients with dry eye. *Clin Ophthalmol*. 2019;13:2285–2292. doi:10.2147/OPTH.S226168.
936. Mah F, Milner M, Yiu S, Donnenfeld E, Conway TM, Hollander DA. PERSIST: physician's evaluation of restasis((R)) satisfaction in second trial of topical cyclosporine ophthalmic emulsion 0.05% for dry eye: a retrospective review. *Clin Ophthalmol*. 2012;6:1971–1976. doi:10.2147/OPTH.S30261.
937. DEWS Clinical TrialsDesign and conduct of clinical trials: report of the Clinical Trials Subcommittee of the International Dry Eye WorkShop (2007). *Ocul Surf*. 2007;5(2):153–162. doi:10.1016/s1542-0124(12)70084-8.
938. Holland EJ, Luchs J, Karpecki PM, et al. Lifitegrast for the treatment of dry eye disease: results of a phase III, randomized, double-masked, placebo-controlled trial (OPUS-3). *Ophthalmology*. 2017;124(1):53–60. doi:10.1016/j.ophtha.2016.09.025.
939. Sall K, Stevenson OD, Mundorf TK, Reis BL. Two multicenter, randomized studies of the efficacy and safety of cyclosporine ophthalmic emulsion in moderate to severe dry eye disease. CsA Phase 3 Study Group. *Ophthalmology*. 2000;107(4):631–639. doi:10.1016/s0161-6420(99)00176-1.
940. Semba CP, Torkildsen GL, Lonsdale JD, et al. A phase 2 randomized, double-masked, placebo-controlled study of a novel integrin antagonist (SAR 1118) for the treatment of dry eye. *Am J Ophthalmol*. 2012;153(6):1050–1060 .e1. doi:10.1016/j.ajo.2011.11.003.
941. Sheppard JD, Torkildsen GL, Lonsdale JD, et al. Lifitegrast ophthalmic solution 5.0% for treatment of dry eye disease: results of the OPUS-1 phase 3 study. *Ophthalmology*. 2014;121(2):475–483. doi:10.1016/j.ophtha.2013.09.015.
942. Tauber J, Karpecki P, Latkany R, et al. Lifitegrast ophthalmic solution 5.0% versus placebo for treatment of dry eye disease: results of the randomized phase III OPUS-2 study. *Ophthalmology*. 2015;122(12):2423–2431. doi:10.1016/j.ophtha.2015.08.001.
943. Morgan P, Van Der Graaf PH, Arrowsmith J, et al. Can the flow of medicines be improved? Fundamental pharmacokinetic and pharmacological principles toward improving Phase II survival. *Drug Discov Today*. 2012;17(9-10):419–424. doi:10.1016/j.drudis.2011.12.020.
944. Craig JP, Nichols KK, Akpek EK, et al. TFOS DEWS II definition and classification report. *Ocul Surf*. 2017;15(3):276–283. doi:10.1016/j.jtos.2017.05.008.
945. Asbell PA, Stapleton FJ, Wickstrom K, et al. The International Workshop on Meibomian Gland Dysfunction: report of the clinical trials subcommittee. *Invest Ophthalmol Vis Sci*. 2011;52(4):2065–2085. doi:10.1167/iovs.10-6997h.
946. Foulks G, Chalmers R, Keir N, et al. The TFOS International Workshop on Contact Lens Discomfort: report of the subcommittee on clinical trial design and outcomes. *Invest Ophthalmol Vis Sci*. 2013;54(11) TFOS157-83. doi:10.1167/iovs.13-13189.
947. Wirta DL, Torkildsen GL, Moreira HR, et al. A clinical phase II study to assess efficacy, safety, and tolerability of waterfree cyclosporine formulation for treatment of dry eye disease. *Ophthalmology*. 2019;126(6):792–800. doi:10.1016/j.ophtha.2019.01.024.
948. Sheppard JD, Wirta DL, McLaurin E, et al. A waterfree 0.1% cyclosporine a solution for treatment of dry eye disease: results of the randomized phase 2B/3 ESSENCE study. *Cornea*. 2021;40(10):1290–1297. doi:10.1097/ICO.0000000000002633.
949. Sheppard JD, Nichols KK. Dry eye disease associated with meibomian gland dysfunction: focus on tear film characteristics and the therapeutic landscape. *Ophthalmol Ther*. 2023;12(3):1397–1418. doi:10.1007/s40123-023-00669-1.
950. Tauber J, Berdy GJ, Wirta DL, Krosser S, Vittitow JL, Group GS. NOV03 for dry eye disease associated with meibomian gland dysfunction: results of the randomized phase 3 GOBI Study. *Ophthalmology*. 2023;130(5):516–524. doi:10.1016/j.ophtha.2022.12.021.
951. Watson SL, Jones LW, Stapleton F, et al. Efficacy and safety of AZR-MD-001 selenium sulfide ophthalmic ointment in adults with meibomian gland dysfunction: a vehicle-controlled, randomized clinical trial. *Ocul Surf*. 2023;29:537–546. doi:10.1016/j.jtos.2023.07.002.
952. Fahmy AM, Harthan JS, Evans DG, et al. Perfluorohexyloctane ophthalmic solution for dry eye disease: pooled analysis of two phase 3 clinical trials. *Front Ophthalmol (Lausanne)*. 2024;4:1452422. doi:10.3389/fopht.2024.1452422.
953. Protzko EE, Segal BA, Korenfeld MS, Krosser S, Vittitow JL. Long-term safety and efficacy of perfluorohexyloctane ophthalmic solution for the treatment of patients with dry eye disease: the KALAHARI study. *Cornea*. 2024;43(9):1100–1107. doi:10.1097/ICO.0000000000003418.
954. Downie LE, Craig JP, Stapleton F, et al. Efficacy and safety of AZR-MD-001 selenium sulfide ophthalmic ointment in adults with meibomian gland dysfunction over six months

of treatment: a phase 2, vehicle-controlled, randomized extension trial. *Ocul Surf*. 2025;35:15–24. doi:10.1016/j.jtos.2024.11.008.
955. Wirta D, Vollmer P, Paauw J, et al. Efficacy and safety of OC-01 (varenicline solution) nasal spray on signs and symptoms of dry eye disease: the ONSET-2 phase 3 randomized trial. *Ophthalmology*. 2022;129(4):379–387. doi:10.1016/j.ophtha.2021.11.004.
956. Hynnekleiv L, Magno M, Vernhardsdottir RR, et al. Hyaluronic acid in the treatment of dry eye disease. *Acta Ophthalmol*. 2022;100(8):844–860. doi:10.1111/aos.15159.
957. Watanabe H. Medical treatment for dry eye in Japan. *Invest Ophthalmol Vis Sci*. 2018;59(14):DES116–DES120. doi:10.1167/iovs.18-24130.
958. Greaves MW. Anti-inflammatory action of corticosteroids. *Postgrad Med J*. 1976;52(612):631–633. doi:10.1136/pgmj.52.612.631.
959. Coutinho AE, Chapman KE. The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. *Mol Cell Endocrinol*. 2011;335(1):2–13. doi:10.1016/j.mce.2010.04.005.
960. Tomlinson A, Bron AJ, Korb DR, et al. The international workshop on meibomian gland dysfunction: report of the diagnosis subcommittee. *Invest Ophthalmol Vis Sci*. 2011;52(4):2006–2049. doi:10.1167/iovs.10-6997f.
961. Geerling G, Tauber J, Baudouin C, et al. The International Workshop on Meibomian Gland Dysfunction: report of the subcommittee on management and treatment of meibomian gland dysfunction. *Invest Ophthalmol Vis Sci*. 2011;52(4):2050–2064. doi:10.1167/iovs.10-6997g.
962. Kent C. Devices for treating the meibomian glands. *Review of Ophthalmology*. September 18, 2018.
963. Valencia-Nieto L, Novo-Diez A, Blanco-Vazquez M, Lopez-Miguel A. Therapeutic instruments targeting meibomian gland dysfunction. *Ophthalmol Ther*. 2020;9(4):797–807. doi:10.1007/s40123-020-00304-3.
964. Tauber J, Owen J, Bloomenstein M, Hovanesian J, Bullimore MA. Comparison of the iLUX and the LipiFlow for the treatment of meibomian gland dysfunction and symptoms: a randomized clinical trial. *Clin Ophthalmol*. 2020;14:405–418. doi:10.2147/OPTH.S234008.
965. Li S, Yang K, Wang J, et al. Effect of a novel thermostatic device on meibomian gland dysfunction: a randomized con0trolled trial in Chinese patients. *Ophthalmol Ther*. 2022;11(1):261–270. doi:10.1007/s40123-021-00431-5.
966. Laufenbock C. Thermo-Pulsations-System (LipiFLow(R)) zur Behandlung der Meibom-Drusen-Dysfunktion (MDD) aus der Sicht eines niedergelassenen Augenfacharztes. Thermal pulsation system (LipiFlow(R)) for treatment of meibomian gland dysfunction (MGD) from the perspective of an ophthalmologist in private practice. Article in German. *Ophthalmologie*. 2022;119(6):605–610. doi:10.1007/s00347-021-01541-y.
967. Finis D, Konig C, Hayajneh J, Borrelli M, Schrader S, Geerling G. Six-month effects of a thermodynamic treatment for MGD and implications of meibomian gland atrophy. *Cornea*. 2014;33(12):1265–1270. doi:10.1097/ICO.0000000000000273.
968. Ballesteros-Sanchez A, Gargallo-Martinez B, Gutierrez-Ortega R, Sanchez-Gonzalez JM. Eyelid exfoliation treatment efficacy and safety in dry eye disease, blepharitis, and contact lens discomfort patients: a systematic review. *Asia Pac J Ophthalmol (Phila)*. 2023;12(3):315–325. doi:10.1097/APO.0000000000000607.
969. Badawi D. A novel system, TearCare((R)), for the treatment of the signs and symptoms of dry eye disease. *Clin Ophthalmol*. 2018;12:683–694. doi:10.2147/OPTH.S160403.
970. Lane SS, DuBiner HB, Epstein RJ, et al. A new system, the LipiFlow, for the treatment of meibomian gland dysfunction. *Cornea*. 2012;31(4):396–404. doi:10.1097/ICO.0b013e318239aaea.
971. Galperin G, Berra M, Marquez MI, Mandaradoni M, Tau J, Berra A. Impact of environmental pollution on the ocular surface of Sjogren's syndrome patients. *Arq Bras Oftalmol*. 2018;81(6):481–489. doi:10.5935/0004-2749.20180091.
972. Gonzalez-Garcia MJ, Gonzalez-Saiz A, de la Fuente B, et al. Exposure to a controlled adverse environment impairs the ocular surface of subjects with minimally symptomatic dry eye. *Invest Ophthalmol Vis Sci*. 2007;48(9):4026–4032. doi:10.1167/iovs.06-0817.
973. Youssouf H, Liousse C, Roblou L, et al. Non-accidental health impacts of wildfire smoke. *Int J Environ Res Public Health*. 2014;11(11):11772–11804. doi:10.3390/ijerph111111772.
974. Calonge M, Labetoulle M, Messmer EM, et al. Controlled adverse environment chambers in dry eye research. *Curr Eye Res*. 2018;43(4):445–450. doi:10.1080/02713683.2017.1420197.
975. Ousler 3rd GW, Rimmer D, Smith LM, Abelson MB. Use of the controlled adverse environment (CAE) in clinical research: a review. *Ophthalmol Ther*. 2017;6(2):263–276. doi:10.1007/s40123-017-0110-x.
976. Meerovitch K, Torkildsen G, Lonsdale J, et al. Safety and efficacy of MIM-D3 ophthalmic solutions in a randomized, placebo-controlled Phase 2 clinical trial in patients with dry eye. *Clin Ophthalmol*. 2013;7:1275–1285. doi:10.2147/OPTH.S44688.
977. Liew SH, Nichols KK, Klamerus KJ, Li JZ, Zhang M, Foulks GN. Tofacitinib (CP-690,550), a Janus kinase inhibitor for dry eye disease: results from a phase 1/2 trial. *Ophthalmology*. 2012;119(7):1328–1335. doi:10.1016/j.ophtha.2012.01.028.
978. Montecchi-Palmer M, Wu M, Rolando M, Lau C, Perez Quinones VL, Dana R. Possible strategies to mitigate placebo or vehicle response in dry eye disease trials: a narrative review. *Ophthalmol Ther*. 2023;12(4):1827–1849. doi:10.1007/s40123-023-00720-1.
979. Stapleton F, Velez FG, Lau C, Wolffsohn JS. Dry eye disease in the young: a narrative review. *Ocul Surf*. 2024;31:11–20. doi:10.1016/j.jtos.2023.12.001.
980. Wickström K. Dry Eyes – Regulatory perspectives. EU Regulatory Workshop – Ophthalmology Läkemedelsverket Medical Products Agency; 2011.
981. Withdrawal of application for the marketing authorisation of Xiidra (lifitegrast) (2020).
982. European Medicines Agency EPAR Report Ikervis January 2015. (https://www.ema.europa.eu/en/medicines/human/EPAR/ikervis#:~:text=Further%20information%20can%20be%20found,Ikervis%20on%2019%20March%202015.) Accessed March 2025.
983. Baudouin C, de la Maza MS, Amrane M, et al. One-year efficacy and safety of 0.1% cyclosporine a cationic

emulsion in the treatment of severe dry eye disease. *Eur J Ophthalmol*. 2017;27(6):678–685. doi:10.5301/ejo.5001002.
984. Baudouin C, Figueiredo FC, Messmer EM, et al. A randomized study of the efficacy and safety of 0.1% cyclosporine A cationic emulsion in treatment of moderate to severe dry eye. *Eur J Ophthalmol*. 2017;27(5):520–530. doi:10.5301/EJO.5000952.
985. Chapin M, Matsuda H, Kawaba T. Considerations for development in Japan. *Review of Ophthalmology*. 2017. https://www.reviewofophthalmology.com/article/considerations-for-development-in-japan.
986. Ministry of Health LaW. *Pharmaceutical Evaluation Division PSB*. [Basic principles for conducting phase 1 studies in Japanese prior to initiating multi-regional clinical trials including Japan for drugs in which early clinical development is preceding outside Japan]. Ministry of Health, Labour and Welfare; 2023.
987. Ministry of Health, Labour and Welfare. Q&A for basic principles for conducting phase 1 studies in Japanese prior to initiating multi-regional clinical trials including Japan for drugs in which early clinical development is preceding outside Japan. 2023.
988. Keating GM. Diquafosol ophthalmic solution 3%: a review of its use in dry eye. *Drugs*. 2015;75(8):911–922. doi:10.1007/s40265-015-0409-7.
989. Matsumoto Y, Ohashi Y, Watanabe H, Tsubota K. Diquafosol Ophthalmic Solution Phase 2 Study G. Efficacy and safety of diquafosol ophthalmic solution in patients with dry eye syndrome: a Japanese phase 2 clinical trial. *Ophthalmology*. 2012;119(10):1954–1960. doi:10.1016/j.ophtha.2012.04.010.
990. Yokoi N, Kato H, Kinoshita S. Facilitation of tear fluid secretion by 3% diquafosol ophthalmic solution in normal human eyes. *Am J Ophthalmol*. 2014;157(1):85–92 .e1. doi:10.1016/j.ajo.2013.09.009.
991. Kinoshita S, Awamura S, Oshiden K, et al. Rebamipide (OPC-12759) in the treatment of dry eye: a randomized, double-masked, multicenter, placebo-controlled phase II study. *Ophthalmology*. 2012;119(12):2471–2478. doi:10.1016/j.ophtha.2012.06.052.
992. Hori Y, Oka K, Inai M. Efficacy and safety of the long-acting diquafosol ophthalmic solution de-089c in patients with dry eye: a randomized, double-masked, placebo-controlled phase 3 study. *Adv Ther*. 2022;39(8):3654–3667. doi:10.1007/s12325-022-02194-2.
993. Arita R, Fukuoka S, Kaido M. Tolerability of diquas LX on tear film and meibomian glands findings in a real clinical scenario. *PLoS One*. 2024;19(9):e0305020. doi:10.1371/journal.pone.0305020.
994. Fukuoka S, Arita R. Tear film lipid layer increase after diquafosol instillation in dry eye patients with meibomian gland dysfunction: a randomized clinical study. *Sci Rep*. 2019;9(1):9091. doi:10.1038/s41598-019-45475-7.
995. Shimazaki J. Definition and diagnostic criteria of dry eye disease: historical overview and future directions. *Invest Ophthalmol Vis Sci*. 2018;59(14):DES7–DES12. doi:10.1167/iovs.17-23475.
996. Novack GD. The development of drugs vs devices. *Ocul Surf*. 2011;9(1):56–57. doi:10.1016/s1542-0124(11)70010-6.
997. Van Norman GA. Drugs, devices, and the FDA: part 2: an overview of approval processes: FDA approval of medical devices. *JACC Basic Transl Sci*. 2016;1(4):277–287. doi:10.1016/j.jacbts.2016.03.009.
998. Van Norman GA. Drugs, devices, and the FDA: Part 1: an overview of approval processes for drugs. *JACC Basic Transl Sci*. 2016;1(3):170–179. doi:10.1016/j.jacbts.2016.03.002.
999. Konishi A, Isobe S, Sato D. New regulatory framework for medical devices in Japan: current regulatory considerations regarding clinical studies. *J Vasc Interv Radiol*. 2018;29(5):657–660. doi:10.1016/j.jvir.2017.12.022.
1000. Kathuria A, Shamloo K, Jhanji V, Sharma A. Categorization of marketed artificial tear formulations based on their ingredients: a rational approach for their use. *J Clin Med*. 2021;10(6):1289. doi:10.3390/jcm10061289.
1001. Ren Y, Chen J, Zheng Q, Chen W. Short-term effect of a developed warming moist chamber goggle for video display terminal-associated dry eye. *BMC Ophthalmol*. 2018;18(1):33. doi:10.1186/s12886-018-0700-y.
1002. Roy NS, Wei Y, Kuklinski E, Asbell PA. The growing need for validated biomarkers and endpoints for dry eye clinical research. *Invest Ophthalmol Vis Sci*. 2017;58(6):BIO1–BIO19. doi:10.1167/iovs.17-21709.
1003. Lanza NL, Valenzuela F, Perez VL, Galor A. The matrix metalloproteinase 9 point-of-care test in dry eye. *Ocul Surf*. 2016;14(2):189–195. doi:10.1016/j.jtos.2015.10.004.
1004. Leonardi A, Messmer EM, Labetoulle M, et al. Efficacy and safety of 0.1% ciclosporin A cationic emulsion in dry eye disease: a pooled analysis of two double-masked, randomised, vehicle-controlled phase III clinical studies. *Br J Ophthalmol*. 2019;103(1):125–131. doi:10.1136/bjophthalmol-2017-311801.
1005. Donnenfeld E, Baudouin C, Galor A, Weissgerber G, He Y, Perez VL. Pharmacogenomic analysis of response to topical tumor necrosis factor alpha antagonist licaminlimab (OCS-02) in dry eye disease [RETRACTED]. *Cornea*. 2024;43(10):e36–e42. doi:10.1097/ICO.0000000000003510.
1006. Senn S, Weir J, Hua TA, Berlin C, Branson M, Glimm E. Creating a suite of macros for meta-analysis in SAS®: A case study in collaboration. *Statistics & Probability Letters*. 2011;81(7):842–851. doi:10.1016/j.spl.2011.02.010.
1007. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Controlled Clinical Trials*. 1986;7:177–188. doi:10.1016/0197-2456(86)90046-2.