Minireview

The microbiome and ophthalmic disease

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Impact statement

This review describes a growing body of research on relationships between the microbiome and eye disease. Several groups have investigated the microbiota of the ocular surface; dysregulation of this delicate ecosystem has been associated with a variety of pro-inflammatory states. Other research has explored the effects of the gastrointestinal microbiota on ophthalmic diseases. Characterizing the ways these microbiotas influence ophthalmic homeostasis and pathogenesis may lead to research on new techniques for managing ophthalmic disease.

Abstract

Progress in microbiome research has accelerated in recent years. Through the use of 16S rRNA assays and other genomic sequencing techniques, researchers have provided new insights about the communities of microorganisms that inhabit human and animal hosts. There is mounting evidence about the importance of these 'microbiotas' in a wide variety of disease states, suggesting potential targets for preventative and therapeutic interventions. Until recently, however, the microbiome received relatively little attention in ophthalmology. This review explores emerging research on the roles that ocular and extraocular microbiotas may play in the pathogenesis and treatment of ophthalmic diseases. These include diseases of the ocular surface as well as autoimmune uveitis, age-related macular degeneration, and primary open angle glaucoma. Many questions remain about the potential impacts of microbiome research on the diagnosis, treatment, and prevention of ophthalmic disease. In light of current findings, we suggest directions for future study as this exciting area of research continues to expand.

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Introduction

The past decade has seen a surge of interest in the microbes that colonize the human body, as new research elucidates how these organisms – which form communities termed 'microbiotas' – influence states of health and disease. Key to this research has been the development of high-throughput sequencing techniques that enable large-scale cataloging of microbiota reference genomes.[1] The term 'microbiome' denotes these genomic catalogs, although it has since become a popular label for microbiotas themselves.[2]

The Human Microbiome Project, an initiative launched in 2007 and funded largely by the National Institutes of Health, sought to improve these reference genomes and describe the microbiota of healthy human hosts.[3,4] Subsequent advances in assays and bioinformatics have yielded increasingly detailed pictures of the microbiotas found in humans and laboratory animals, as well as those residing in human-built and natural environments.[1,5,6] In particular, 16S rRNA sequencing has become a powerful tool for determining the composition of a microbiota; these assays take advantage of the fact that 16S rRNA genes are highly conserved with variable regions that distinguish bacterial genera from each other.[1] Experimental repertoires have expanded to track shifts in microbiota constituents through time and now include functional measures of their transcriptional activity, protein expression, or metabolic by-products.[7]

Impact of microbiotas on non-ophthalmic disease

The bulk of microbiome research has focused on the gastrointestinal tract, with disturbances of the gut microbiota now implicated in a wide range of disease states including irritable bowel syndrome,[8] inflammatory bowel disease,[9] carcinogenesis,[10] obesity and cardiometabolic diseases,[11] multiple sclerosis,[12] rheumatoid arthritis,[13] graft-versus-host disease,[14] mood disorders,[15] and neurodegenerative

diseases.[16] Several groups have demonstrated that gut commensals and dysbiosis may be involved in the pathogenesis of type 1 and type 2 diabetes, both of which are associated with serious ophthalmic sequelae.[17–20] The gut microbiota has been shown to modulate adaptive immune responses, notably through induction of IgA class switching, promotion of T_H17 cell differentiation, and stimulation of regulatory T-cell populations.[21] There is evidence that the gut microbiota influences innate immune responses at the gastrointestinal mucosa as well.[22] The gut microbiota has provided investigators with mechanistic links that explain how environmental factors potentiate certain disease states. For example, antibiotics are known to set the stage for *Clostridium difficile*-associated diarrhea by disturbing the balance of gastrointestinal commensals in exposed individuals.[23] Another interesting area of research explores how the gut microbiota may mediate well-established associations between high-fat diet and metabolic or immune disorders. For instance, high-fat diets have been shown to increase circulating levels of lipopolysaccharide and other pro-inflammatory bacterial by-products.[11,24,25]These findings suggest that disturbances in microbiotas contribute to pathogenesis in their own right and are not simply epiphenomena of disease. Germ-free and gnotobiotic animal models have become especially powerful tools for demonstrating causality, although further work is needed to apply animal studies to humans.[1]

Despite the focus of microbiome research on the gut microbiota, the composition and pathogenic significance of microbiotas outside the gut have also been explored. For instance, analysis of the oral microbiome has generated new perspectives on the pathogenesis of tooth decay and oral cancer.[26] The skin microbiota is thought to be a significant modulator of cutaneous immune responses, with animal and human studies demonstrating involvement of *Staphylococcus aureus* in the pathogenesis of atopic dermatitis.[27,28]

There is some evidence to suggest that targeted manipulation of microbiotas may help prevent or treat disease. Oral probiotics containing *Bifidobacterium* spp. and *Lactobacillus* spp. have been widely studied, but these formulations are difficult to investigate and implement in clinical practice due to variations in the strains they contain.[29] Although controversy persists about the efficacy of probiotics, there is evidence to suggest their utility in some applications; these include the prevention of necrotizing enterocolitis in preterm infants,[30] prevention of antibiotic-associated diarrhea,[31] and treatment of irritable bowel syndrome.[32] Some groups suggest administering oral probiotics before hematopoietic stem cell transplantation to reduce the risk of graft-versus-host disease, but current evidence is mixed.[33,34] Fecal microbiota transplantation has shown promise in treating recurrent *C. difficile* infection; when normal gut commensals are reestablished, these microorganisms have been shown to compete with pathogenic bacteria and modulate host immune responses to counteract infection.[35] Efforts to engineer modified strains of commensals have also been underway, with one group recently reporting the use of inducible promoters in *Bacteroides* spp. to regulate gene expression.[36] Techniques for modifying and controlling microbiotas may one day prove useful in clinical applications.

In the following section, we review research that has sought to characterize the ocular surface microbiota as well as its possible effects on local immune responses and pathogenesis. We then explore recent insights about the roles of extraocular microbiotas in a variety of ophthalmic diseases, with emphasis on autoimmune uveitis, age-related macular degeneration (AMD), and primary open angle glaucoma (POAG). Finally, we conclude by considering the implications of these findings and suggest future directions for microbiome research in ophthalmology.

Ocular surface microbiota

Composition

Despite constant exposure to the environment, the conjunctiva, lid margins, and tears of healthy individuals feature a unique population of microorganisms compared to those of the facial skin and oral mucosa.[37,38] Before the widespread use of genetic assays, researchers attempting to characterize the ocular surface microbiota noted that swabs of healthy conjunctiva yielded sparse growth when cultured; the most frequently cultivated organisms were coagulase-negative staphylococci, *Propionibacterium* spp., and *Corynebacterium* spp.[38]

One advantage of 16S rRNA assays and other microbiome-based techniques is that they permit efficient identification of microbiota constituents that are otherwise difficult to cultivate, due to their low abundance or poor response to culture media. A 2016 study by Doan *et al*.[37] found that *Corynebacterium* spp. followed by *Propionibacterium* spp. and then coagulase-negative staphylococci were most abundant in conjunctival samples; these are the same organisms that predominate in conventional cultures, albeit with a different order of frequency. This study also confirmed that the ocular surface harbors a distinct bacterial community compared to facial skin and oral mucosa with 150- to 200-fold fewer bacteria than these sites, possibly due to antimicrobial compounds in the tear film.[39] Another recent study found that bacteria in conjunctival samples display more phylogenetic diversity than those isolated from the skin under the eye.[40] These findings suggest that ocular surface assays are not simply detecting microorganisms from adjacent sites, although the risk of contamination is indeed high and samples must be collected with care.[41]

Aside from contamination, other aspects of the collection process can affect microbiota surveys. One study found that 'deep' conjunctival swabs taken with firm pressure yielded a different profile of microorganisms than swabs taken with light pressure, perhaps indicating that bacteria are vertically stratified on the ocular surface.[42] Another study found that use of topical proparacaine before conjunctival swabbing decreased the range of organisms detected, possibly by diluting or rinsing away bacteria.[40]

Given that 16S rRNA exists only in prokaryotes, there has been interest in next-generation sequencing assays that

can also identify viruses, fungi, and parasites. Doan *et al.*[37] have developed one such assay and found that torque teno virus may be a constituent of the normal ocular surface microbiota. Another study found that torque teno virus was uniformly present in aqueous or vitreous samples taken from patients with culture-negative endophthalmitis, while vitreous samples taken from controls had no evidence of the virus.[43] It is still unclear whether torque teno virus may be involved in the pathogenesis of endophthalmitis or whether the virus is a non-specific marker of intraocular inflammation.

In addition to only detecting bacteria, a further limitation of 16S rRNA assays is their inability to evaluate the functional status of microbiotas. This is a major shortcoming when studying the ocular surface, where antimicrobial stresses such as tear lysozyme may dramatically curtail the lifespan of microorganisms detected in these assays. Questions remain about whether these microorganisms thrive at the ocular surface as a durable population or whether they are transiently introduced and inactivated after a short time. After correcting for potential contaminants, Ozkan *et al.*[44] found that healthy individuals maintained a relatively stable microbiota on repeat 16S rRNA assays during a three-month period. The most frequently isolated genus in these assays was *Corynebacterium*, although inconsistencies across individuals led the authors to question whether the ocular surface is home to a 'core' microbiota akin to the gut and other mucosal sites. Transcriptional assays may help answer these questions by measuring the activity of whole microbiotas instead of merely detecting the presence or absence of particular microbes.[41,45] The use of new assays that identify organisms by species or strain will also provide valuable information, as the taxonomic resolution of conventional 16S rRNA techniques typically stops at the level of genus.[45]

Responses to host and environmental factors

Studies of healthy individuals provide us with crucial background information, but it is important to remember that microbiotas are not static communities. Rather, the ocular surface is a dynamic ecosystem where microbes must respond to a range of environmental influences. For instance, the use of contact lenses has been associated with an altered ocular surface microbiota.[46,47] One study found that the conjunctival microbiota of contact lens wearers was enriched with skin-associated bacterial genera such as *Pseudomonas*, *Acinetobacter*, and *Methylobacterium* when compared to the microbiota of non-wearers; in turn, contact lens wearers had reduced levels of genera that are typical of the healthy ocular surface, such as *Staphylococcus* and *Corynebacterium*. It is unclear if these differences are caused by inoculation of the ocular surface during lens insertion, or if the presence of contact lenses creates a selective pressure that favors skin commensals.[40] Another recent study found that contact lens wear did not affect the overall diversity of the ocular microbiota, although modest differences were observed in the abundance of particular bacterial genera.[48]

In eyes implanted with the Boston type 1 keratoprosthesis, Jassim *et al.*[49] identified wider microbial diversity compared to contralateral healthy eyes. Owji and Khalili[50] found that among patients with nasolacrimal duct obstruction, conjunctival cultures of affected eyes displayed higher colony counts than fellow eyes but featured significantly less growth of *Staphylococcus epidermidis*. This study found that when continuity between the conjunctiva and nasopharyngeal space was restored with dacryocystorhinostomy, colony counts normalized in the affected eyes within eight weeks of surgery. These findings raise new questions about how ocular microbes respond to therapies that limit tear drainage through the nasolacrimal duct, such as punctal plugs used in the treatment of dry eye. The effects of topical antibiotics on the ocular surface microbiota have been explored as well, providing information not only about patterns of antimicrobial resistance, but also about the influence these agents have on commensal organisms.[51–54]

There is evidence that other topical agents may affect the profile of microorganisms at the ocular surface. In a small prospective study, Ohtani *et al.*[55] found that use of topical latanoprost in glaucoma patients was associated with greater frequency of methicillin-resistant *S. epidermidis* in conjunctival cultures compared to patients using another prostaglandin analog (travoprost) and healthy controls; however, there were no significant differences in the bacterial species isolated from these groups. The authors attribute variations in methicillin resistance to a difference in the preservatives used within the two drug formulations, with latanoprost containing benzalkonium chloride and travoprost containing an ionic buffer. The study suggests that preservatives may influence the ocular surface microbiota and illustrates the need for future research to distinguish the effects of preservatives from those of medications themselves.

Several other host factors have been found to influence the composition and metabolic activity of the ocular surface microbiota. Aging may be one such factor, with Wen *et al.*[56] reporting that the microbiota of older adults exhibits significant alterations in carbohydrate and lipid metabolism as well as enrichment of antibiotic resistance genes. Multiple studies have also attempted to characterize the ocular microbial populations associated with systemic diseases, such as diabetes mellitus[57–59] and HIV.[60] For instance, Suto *et al.*[57] found greater frequency of methicillin-resistant coagulase-negative staphylococci in diabetic patients compared to controls, along with increased rates of resistance to levofloxacin and tobramycin. Determining the organisms that predominate in specific patient subgroups could provide valuable guidance for antibiotic selection, especially when perioperative prophylaxis is indicated.

Roles in pathogenesis

Aside from guiding antibiotic selection, defining the profiles and responses of the ocular surface microbiota is essential for understanding how it might influence the pathogenesis of ophthalmic diseases. Like the gut

mucosa, the ocular surface maintains its integrity and homeostasis through the careful regulation of immune responses. Pathways for antigen surveillance, response, and tolerance have been described at the ocular surface; microbiotas have been shown to influence these mechanisms at other mucosal sites, and there is some evidence that the ocular surface microbiota may serve a similar function.[61–63] A recent study by St Leger *et al.*[64] demonstrated that *Corynebacterium mastitidis* stably colonizes the ocular surface and also described a cellular mechanism by which this organism evokes an interleukin-17 response from $\gamma\delta$ T-cells in the ocular mucosa; this, in turn, promoted neutrophil recruitment and the release of tear antimicrobials that protected against *Candida albicans* or *Pseudomonas aeruginosa* infection. Another study by Kugadas *et al.*[65] found that depletion of ocular surface commensals increased susceptibility to *P. aeruginosa* keratitis in mice, and also found that these microorganisms – especially coagulase-negative staphylococci – regulate the recruitment of neutrophils to ocular tissues. The use of topical probiotics to modulate immune responses at the ocular surface has been proposed, but scant research exists on this topic. In a small pilot study, patients with vernal keratoconjunctivitis receiving a topical *Lactobacillus acidophilus* formulation showed clinical improvement after four weeks of use.[66] Research on topical probiotics faces obstacles comparable to studies of other probiotic applications, including the challenge of standardizing probiotic therapies across multiple research groups; without standardization of the strains used in these therapies, it is more difficult to build consensus about their efficacy as new evidence becomes available.

Other groups have explored the role of the ocular surface microbiota in dry eye and related diseases. Although many studies have found that patients with dry eye exhibit higher bacterial loads than healthy subjects, evidence is mixed as to whether their microbiotas are comprised of significantly different bacterial taxa.[67–71] One study employing 16S rRNA assays found that tear samples from blepharitis patients contained decreased levels of *Propionibacterium* spp., leading the authors to speculate whether members of this genus might be protective against blepharitis. The authors identified *Propionibacterium*, *Staphylococcus*, *Streptophyta*, *Corynebacterium*, and *Enhydrobacter* as the most common genera at the ocular surface in both healthy individuals and patients with blepharitis, although the tears of blepharitis patients demonstrated higher levels of *Staphylococcus*, *Streptophyta*, *Corynebacterium*, and *Enhydrobacter* compared to healthy subjects.[72] These findings suggest a possible role for dysbiosis in the pathogenesis of blepharitis. While a later study found no significant differences in bacteria cultured from patients with blepharitis when compared to healthy controls, the poor cultivability of microbes at the ocular surface suggests 16S rRNA assays should be favored over culture-based techniques.[73] Where changes have been observed in the ocular surface microbiota of dry eye patients, it remains unclear whether these shifts contribute to pathogenesis or are consequences of an altered mucosal ecosystem.[68]

Extraocular microbiotas and ophthalmic disease

As evidence accumulates about the ways microbiotas regulate immune and metabolic homeostasis across multiple organ systems, several groups have identified links between ophthalmic diseases and microbiotas in the gut or oral cavity. Most of this research has concentrated on autoimmune uveitis, AMD, and POAG. We explore current evidence regarding these three conditions in greater detail, followed by a brief discussion about other ophthalmic diseases that have been associated with extraocular microbiotas.

Autoimmune uveitis

Evidence from animal studies has suggested that gut commensals are involved in the pathogenesis of autoimmune uveitis. In one of the earliest studies on the topic, Lin *et al.*[74] compared the cecal microbiota of rats transgenic for HLA-B27 and human β2-microglobulin to the microbiota of wild-type controls using a technique known as 'biome representational in situ karyotyping' in conjunction with 16S rRNA gene sequencing. The authors found increased abundance of *Paraprevotella* spp. and *Bacteroides vulgatus* in these transgenic rats compared to controls, as well as decreased abundance of the family Rikenellaceae. Although these findings suggested that the gut microbiota may be involved in the development of autoimmune uveitis, the mechanisms underlying this potential involvement remained unclear. Early hypotheses proposed that inflammation at the intestinal mucosa may increase gut permeability and facilitate the translocation of microbes (or microbial by-products) that incite ocular inflammation, either through direct effects on the eyes or indirectly via molecular mimicry and immune sensitization.[75]

Subsequent research has provided intriguing clues about the immunomodulatory effects of the gut microbiota and their relationship to autoimmune uveitis. Employing a transgenic mouse model for autoimmune uveitis where T-cells are sensitized to a known uveitogenic ocular antigen, Horai *et al.*[76] found that germ-free transgenic mice and transgenic mice treated with a broad-spectrum oral antibiotic cocktail had delayed onset of disease; however, introduction of germ-free animals to a conventional environment was observed to cause uveitis.[77] Injection of T-cells cultured from these transgenic mice into wild-type mice caused uveitis, but this effect was only observed if the T-cells were cultured in the presence of intestinal extracts. Although the study did not implicate specific microbes or microbial by-products in the activation of cross-reactive T-cells, a later report by the same group suggests that multiple populations of microbes may be providing these pro-inflammatory signals, as partial ablation of the gut microbiota with single antibiotic agents yielded smaller reductions in disease severity compared to mice treated with the broader-spectrum cocktail.[78]

Reports from other research groups have complemented these findings. For instance, Heissigerova *et al.*[79] found that germ-free mice were protected from experimentally

induced autoimmune uveitis, as were mice treated with oral antibiotics one week before induction of uveitis was attempted. Similarly, Nakamura *et al.*[80] found that mice with induced autoimmune uveitis had different profiles of gut commensals compared to controls, with differences becoming more prominent as the uveitis continued; they also found that oral antibiotics reduced the severity of uveitis by increasing regulatory T-cell presence in the retina and decreasing levels of both effector T-cells and cytokines in peripheral lymphoid tissue. In a later report, the same group employed transgenic mice carrying a fluorescent protein marker to demonstrate for the first time that lymphocytes migrate between the gut and eye in uveitis.[81] Interestingly, the authors also report that supplementation with exogenously produced short chain fatty acids – which are known metabolites of gut bacteria – reduced disease severity in mice with uveitis, potentially through the induction of regulatory T-cells in the gut and the suppression of effector T-cells.[81] This finding suggests that loss of certain commensal organisms from the intestinal microbiota may promote uveitis, as they are no longer secreting anti-inflammatory metabolites.

Work is currently underway to expand upon findings from animal models and explore whether the gut microbiota influences the development of autoimmune uveitis in humans. Reporting preliminary data from an ongoing study, one group found that the overall diversity of intestinal microbes was not significantly different between human patients with uveitis (predominantly posterior uveitis) and healthy controls; patients did show increased fractions of *Fusobacterium* spp. and members of the family Enterobacteriaceae compared to controls, while one genus detectable in over half of the control subjects, *Prevotella*, was undetectable in the rectal fluids of patients.[82,83] These findings diverge from earlier reports that rats transgenic for HLA-B27 had increased levels of *Paraprevotella*, a genus related to *Prevotella*.[74] Another recent study by Huang *et al.*[84] found a similar composition of gut microbiota in human patients with acute anterior uveitis and healthy controls, although differences in the microbial metabolites isolated from fecal samples suggest a possible association between uveitis and the metabolic phenotype of the microbiota. Specifically, the authors note increased levels of linoleic acid and azelaic acid in rectal samples taken from uveitis patients; in contrast to animal data reported by Nakamura *et al.*,[81] no association was identified between uveitis and short chain fatty acids. With these human studies, it is important to bear in mind that patients are often on immunosuppressive regimens and that differences in microbiota may represent the effects of treatment.[83]

These findings suggest that strong associations exist between the gut microbiota and autoimmune uveitis, although much work remains to characterize the precise mechanisms responsible for those associations. As with other disease states that have been linked to the gut microbiota, future research may lead to therapeutic approaches that act directly on commensal microorganisms such as antibiotics, probiotics, dietary modifications, or perhaps even microbiota transplantation.[82,85,86]

AMD

The influence of extraocular microbiotas on AMD pathogenesis is another promising area of research that has grown in recent years, although early reports lack consensus as to specific microbes (or patterns of microbial activity) that may be implicated in this disease. One study by Zinkernagel *et al.*[87] found that patients with neovascular AMD had gut microbiotas that were enriched with *Anaerotruncus* spp., *Oscillibacter* spp., *Ruminococcus torques*, and *Eubacterium ventriosum*, while the microbiotas of healthy controls were enriched with *Bacteroides eggerthii*, which may be protective against immune-mediated disease.[88] This study also found differences in bacterial genes related to a variety of metabolic pathways, including a decreased proportion of genes involved in fatty acid elongation and enrichment of genes related to L-alanine fermentation, glutamate degradation, and arginine biosynthesis. The authors acknowledge that functional assays of microbial transcription or metabolism are needed to confirm these findings and explore their implications for AMD.

Reporting preliminary data from another case–control study, Lin's group found increased abundance of *Prevotella* spp. and decreased abundance of the family Rikenellaceae in the gut microbiota of AMD patients compared to controls; in contrast to Zinkernagel *et al.*,[87] the authors also report that the family Ruminococcaceae had decreased abundance in AMD patients.[82] These findings mirror the group's earlier report of increased Paraprevotella and Rikenellaceae in rats with experimentally induced uveitis.[74] Lin's[82] group also identified several alterations of microbial metabolic pathways that may be involved in AMD pathogenesis, and they report shifts in bacterial abundance following administration of a vitamin cocktail that is widely used for the management of AMD.[89–91] Further research is needed to determine the significance of these metabolic pathways in AMD and evaluate whether manipulation of the gut microbiota could be a useful addition to established therapies.

Beyond the use of vitamins in treating AMD, there has been broader interest in the role of nutrition in the development and control of this disease.[92–94] The possibility that gut commensals may mediate some of these associations was suggested by early research with germ-free mice, which showed altered retinal lipid metabolism compared to conventional controls.[95] To date, two studies have investigated how connections between diet and AMD may be mediated by the gut microbiota. One study by Andriessen *et al.*[96] found that mice with choroidal neovascularization (CNV) exhibited greater disease severity when fed a high-fat diet; however, oral treatment with a broad-spectrum, non-gut permeable antibiotic reduced the rate of CNV to the level seen in regular diet controls. Transplant of the gut microbiota from regular diet mice into high-fat diet mice also reduced the rate of CNV. These findings are consistent with research by Skondra *et al.*[97] demonstrating that high-fat diet worsens the severity of CNV and dry AMD features in mice, especially in the presence of genetic predisposition.

Another study by Rowan *et al.*[98] observed that mice fed a high-glycemic-index diet were more prone to develop histologic features of dry AMD than those fed a low-glycemic-index diet and found that both the composition and metabolic activity of the gut microbiota were significantly different between these two groups. The authors report that serotonin, a known microbial metabolite, was negatively associated with retinal damage scores; several other microbial metabolites were elevated in low-glycemic-index mice and showed negative associations with AMD features, lending support to the possibility of a 'gut–retina axis' that underlies relationships between diet and AMD.[99] Interestingly, crossover of mice from a high- to low-glycemic-index diet reduced the prevalence of AMD features and restored the gut microbiota to the population observed in low-glycemic-index mice, suggesting this dietary intervention ought to receive further study in human patients. Continued investigation of the gut microbiome could reveal unknown aspects of AMD pathogenesis and potentially yield new therapeutic targets and strategies. As a prelude to human studies, gnotobiotic animal models would be especially valuable for researchers attempting to identify whether certain profiles of microbiota promote or protect against AMD.

Although most research on the role of microbes in AMD pathogenesis has focused on the intestinal microbiota, some groups have turned their attention to other anatomical sites. A recent case–control study by Ho *et al.*[100] suggests that oropharyngeal microbes may be involved in this disease, with genomic assays of throat swabs taken from patients with advanced AMD showing enrichment of *Streptococcus* spp. and *Gemella* spp. when compared to healthy controls; the genus *Prevotella* exhibited decreased abundance in patients with advanced AMD, contrasting against reports that this genus is enriched in the gut microbiota of AMD patients.[82] The relative abundances of bacterial taxa in early AMD patients were more similar to controls, leading the authors to propose that the extent of oropharyngeal dysbiosis may correlate with disease progression. As with studies of the intestinal microbiota, a need exists for prospective, longitudinal studies that can test these preliminary findings.

POAG

A few groups have explored the possible influence of the oral microbiota on POAG. One study found that patients with glaucoma had higher levels of bacterial 16S rRNA in oral samples; the authors also found that peripheral injection of bacterial lipopolysaccharide increased axonal degeneration and neuronal loss in two separate animal models of glaucoma, likely through upregulation of TLR4 and complement signaling that in turn induce microglial activation in the retina and optic nerve head.[101] A prospective cohort study found that tooth loss within the preceding two years, particularly in the setting of severe periodontal disease, was associated with a transiently increased risk of POAG.[102] A subsequent case–control study found an inverse association between POAG and the number of natural teeth, and noted that *Streptococci* 16S rRNA levels were higher in the saliva of POAG patients compared to controls – although these levels were not significantly correlated with visual field damage.[103] Taken together, these studies suggest that the oral microbiota may be involved in glaucoma pathogenesis. Additional research is needed to understand the clinical implications and exact mechanisms of this relationship.

Although much of the research on POAG and other glaucoma variants has focused on the oral cavity, microorganisms found throughout the gastrointestinal tract have been proposed as potential modulators of these complex disease states. Gupta,[104] for instance, has conjectured whether the gut microbiota could affect the production of neuroprotective factors that in turn promote the survival of retinal ganglion cells. Research from several groups also suggests a possible relationship between glaucoma and *Helicobacter pylori*, a non-commensal colonizer of the gastrointestinal tract.[105,106] An early study by Kountouras *et al.*[107] found that successful *H. pylori* eradication improved intraocular pressure and visual field measurements in patients with chronic open-angle glaucoma. A more recent meta-analysis by Zeng *et al.*[108] identified that *H. pylori* carriage was associated with increased risk of open-angle glaucoma and normal tension glaucoma, but was not associated with glaucoma secondary to pseudoexfoliation syndrome. Several mechanisms for these associations have been proposed, including remote effects of reactive oxygen species and inflammatory cytokines that may travel from the gastric mucosa to the optic disc or trabecular meshwork,[105,109] possible cross-reactivity of *H. pylori* IgG antibodies with ocular tissues,[110] and even intraocular *H. pylori* colonization as suggested by the intriguing presence of this microbe on histology of trabeculectomy specimens.[111] Nevertheless, the involvement of *H. pylori* in glaucoma pathogenesis remains controversial with wide variability of diagnostic criteria among existing studies as well as other groups reporting no significant association with either pathogenic or non-pathogenic strains of this organism.[112]

Other conditions

Several other ophthalmic disease states have been linked to the extraocular microbiota. Some of this research has focused on diseases of the ocular surface. Using a mouse model for Sjögren's syndrome, one study found an inverse association between disease severity and the diversity of the fecal microbiota.[70] Kugadas *et al.*[65] found that depletion of gut commensals (without alteration of the ocular surface microbiota) was associated with decreased neutrophil response to *P. aeruginosa* as well as increased susceptibility to *P. aeruginosa* keratitis. Other research has explored possible associations between *H. pylori* and a range of ophthalmic diseases beyond glaucoma.[105] A study by Sacca *et al.*[113] found that individuals with blepharitis were more likely to carry *H. pylori* than healthy controls; blepharitis also improved in about half of patients undergoing *H. pylori* eradication, although the authors caution that the antibiotics themselves may have mitigated blepharitis via their intrinsic anti-inflammatory properties or effects on the ocular surface microbiota.

Despite abundant evidence that the gastrointestinal microbiota is involved in diabetes pathogenesis at a systemic level, a longstanding gap existed in research on relationships between gut commensals and diabetic retinopathy.[114] A recent study by Beli *et al.*[115] marks a breakthrough in the field with the first report of a concrete association between the gut microbiota and this disease. Using a mouse model for type 2 diabetes, the authors compared retinal histology of mice placed on an intermittent fasting diet to those fed *ad libitum*; mice on the *ad libitum* diet developed classic features of diabetic retinopathy, such as acellular capillaries and infiltration of inflammatory cells, while intermittent fasting mice did not experience these changes and exhibited retinal histology similar to non-diabetic controls. These effects were observed even though intermittent fasting mice had glycated hemoglobin levels identical to those fed an *ad libitum* diet. Intermittent fasting mice also displayed restructured gut microbiota composition, with an increased ratio of Firmicutes to Bacteroidetes on 16S rRNA assays. Furthermore, intermittent fasting mice showed alterations in bacterial metabolites including a significant increase in levels of taurochenodeoxycholate (TUDCA), a bile acid metabolite with known anti-inflammatory effects.[116] TUDCA is an activator of TGR5, a widely expressed G protein-coupled receptor that the authors found to be expressed in the retinal ganglion cell layer. Although neither diabetes nor diet was associated with changed TGR5 expression, intermittent fasting mice did exhibit decreased levels of TNF-α (a downstream target of TGR5) and were protected from diabetic retinopathy when TGR5 was pharmacologically activated. These experiments provide strong evidence that intermittent fasting prevented diabetic retinopathy through alteration of the gastrointestinal microbiota and bacterial metabolism. Further research is needed to distinguish these effects from other ways that intermittent fasting may protect against diabetic retinopathy, such as the reduction of oxidative stress.[117]

Summary and future directions

We are presently at an important juncture in microbiome research, both within ophthalmology and beyond. Building on refinements in experimental technique over the past decade, researchers have been able to characterize the composition and function of microbiotas with increasingly fine detail. New assays and bioinformatics methods allow researchers to analyze host–microbiota interactions at deeper levels, and large-scale longitudinal studies enable more robust claims about the influence of microbiotas on pathogenesis. Clinical trials have been crucial as researchers explore microbiotas as potential biomarkers and test hypotheses about manipulating microbiotas to prevent or treat disease.[118]

As we review here, many provisional findings have been made about the roles that microbiotas may play in ophthalmic disease. Nevertheless, relative to other fields, investigators in ophthalmology have just begun to study the microbiome. Echoing prior reviews on this topic, we believe much work remains to develop these early insights into clinically relevant interventions.[41,45,119]

A key challenge for investigators in ophthalmology will be to harness methods from the wider field of microbiome research. For instance, what can we learn by profiling the transcriptional activity, protein expression, and metabolic by-products of microbiotas? Functional measures are especially important for studying the ocular surface: given the antimicrobial stresses that exist at this site, these techniques may help establish whether organisms are transiently present and quickly inactivated or whether they persist and form stable, active communities. In studying any site – ocular or extraocular – functional measures can help clarify how microbial metabolites and other by-products contribute to host biology. Gnotobiotic animal models have also been important tools in microbiome research,[1] yet they are currently underutilized by ophthalmology investigators; incorporating these models into future studies would be valuable as researchers build upon recent findings. By exploring new experimental techniques, investigators in ophthalmology will continue to build a foundation of basic science and observational research that could support the design of larger scale clinical trials.

In addition to the conditions that have been investigated thus far, it is possible that many other ophthalmic diseases may be associated with changes in ocular and extraocular microbiotas. There are also many ophthalmic interventions that may have the potential to affect the ocular surface microbiota, but that have not received study. For example, what effects might intravitreal medications such as anti-vascular endothelial growth factor agents have on microbes at the ocular surface? How do these organisms respond to topical immunomodulating agents such as cyclosporine and to systemic immunomodulation? The responses of the ocular surface microbiota to these and other common ophthalmic therapies will be important for future investigators to characterize. Finally, unconfirmed reports that intraocular bacteria have been detected in patients with glaucoma[111] and AMD[120] bear further scrutiny. While it is premature to infer the existence of a distinct intraocular microbiota from such reports, their substantiation would raise further questions about the mechanisms of entry and pathogenic significance of these organisms.

This is a moment of great opportunity and excitement for microbiome research in ophthalmology. The work we review here provides compelling arguments that ocular and extraocular microbiotas contribute to common ophthalmic pathologies; although the mechanisms of these associations are just now coming into focus, the groundwork has been laid for future exploration into the diagnostic, therapeutic, and preventative significance of these microbial communities. Continued progress in this field may lead to a new era in ophthalmology by providing us with novel ways of understanding and managing ophthalmic disease.

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DS critically reviewed the manuscript and revised the paper before submission.

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DECLARATION OF CONFLICTING INTERESTS

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