The gut-eye axis represents the ability of gut bacteria presence and activity to affect eye function. The presence of gut microbiota has been suggested to play a role in influencing several retinal diseases, including glaucoma. Regular cues, originating from microbiota, are essential to the normal development of the central nervous system. For instance, brain formation and differentiation during development require long-distance cell migration, a process that is influenced by cues from the gut microbiota; dietary components are known to directly interact with the developing brain and impact its function. Gut microbiota changes in response to external environmental cues. When factors, such as age and unhealthy diet, cause shifts in the microbiomes, immune signals and metabolic products, including butyrate and short-chain fatty acids (SCFAs), are altered. To better understand the pathogenesis of glaucoma, immune signal changes are an important focus. Alongside increased intraocular pressure (IOP), higher levels of proinflammatory cytokines and chemokines have been shown in patients with glaucoma throughout several studies. In tracing the metabolites and signals produced by bacteria, especially in the context of glaucoma pathogenesis, scientists can examine the pathways and mechanisms that lead to major retinal disease, providing opportunities for developing novel therapeutic interventions. Through

MINI-REVIEW

The Role of Gut Microbiota in Glaucoma Progression and Other Retinal Diseases

From the Department of Ophthalmology, Schepens Eye Research Institute of Massachusetts Eye and Ear, Harvard Medical School, Boston, Massachusetts

As a rapidly growing field, microbiota research offers novel approaches to promoting ocular health and treating major retinal diseases, such as glaucoma. Gut microbiota changes throughout life; however, certain patterns of population changes have been increasingly associated with specific diseases. It has been well established that disrupted microbiome contributes to central nervous system diseases, including Alzheimer disease, Parkinson disease, multiple sclerosis, and glioma, suggesting that it could play a prominent role in neurodegenerative diseases. This review summarizes the progress in identifying significant microbe changes in patients with glaucoma by compiling studies completed in insulation that underlies the association between the microbiota and disease progression. Among pathways of interest, the finding of increased Firmicutes/Bacteroidetes ratio in patients with primary open-angled glaucoma links the increased taurocholic acid and decreased glutathione to a negative impact on retinal ganglion cell survival. Connecting these microbes to specific metabolites sheds light on the pathogenic mechanism and novel treatment strategies. In summary, the current review is intended to synthesize the findings of several studies investigating the effects of shifting bacterial population in retinal diseases, particularly glaucoma, to identify the current direction of treatment development and help direct future endeavors. (Am J Pathol 2023; 183: 1–7; https://doi.org/10.1016/j.ajpath.2023.06.015)
establishing the relationship between gut bacteria and retinal eye diseases, several possible approaches can be considered. Probiotics and the introduction of healthy microbiota are several methods being explored to increase prevention or alleviate glaucoma and several other retinal diseases.7

Microbiome and Retinal Aging

An individual’s gut microbiota is inherited from the maternal parent and then changes as it is exposed to the surrounding environment. Although the gut microbiota population is expected to fluctuate and change throughout a lifetime, certain disruptions have detrimental impacts on health. The most dramatic changes in microbiota occur during infancy and old age when the immune system is at its weakest, demonstrating a connection between microbiota, the immune system, and aging.8 Mouse studies have shown that aging populations have increased Clostridia relative to Bacteroidales and decreased Lactobacillus.9,10 Human studies also show a shift in microglia population in older adults, in whom Bacteroides and Bifidobacteria decrease and Fusobacteria, Clostridia, and Eubacteria increase.10 In both mouse and human studies, the trends of Clostridia and Bacteroidales populations in aging populations seem to correlate. These shifting populations of microorganisms lead to fewer SCFA producers and more opportunistic pathogens, contributing to inflammation.11 A key risk factor for retinal diseases is aging.12 The decreased bacterial diversity and increase in bacterial population found in older adults can trigger immune response and dysregulated para-inflammation,11 which is detrimental to retinal health.12

The inflammatory process is a fundamental component underlying several age-related eye diseases, such as glaucoma and age-related macular degeneration (AMD), two of the leading causes of vision loss in elderly individuals. In exploring the intersection of eye disease, aging, and shifting microbiota bacterial populations, we can better understand disease pathways and uncover novel interventions. Scientists are already exploring specific retinal diseases and the influence of microbiota in their progression. Dysbiosis of gut microbiome is reported to lead to systemic inflammation and even induce the onset of many diseases.13 The normal human gut microbiota consists primarily of two bacterial phyla, Firmicutes and Bacteroidetes.14 Inflammation contributes to neurodegeneration in glaucoma.15 Thus, inflammation caused by dysbiosis (disruption in gut microbiota) and a leaky gut-vessel barrier indicates the potential role of the gut microbiota in retinal disease progression.16 Increased inflammatory signals are found in glaucomatous eyes.17 Obesity, which is linked with gut dysbiosis, and an increased risk of glaucoma development indicate a connection between the microbiome and glaucoma. Further evidence of the role of bacteria in retinal disease is demonstrated by dietary supplementation, which has been proven an effective treatment to slow AMD disease progression.18 Because the retinal pigment epithelium is located in an environment with high oxygen partial pressure, it is especially sensitive to oxidative stress. Thus, the anti-oxidative capability of bacteria that produce anti-oxidants, especially as this protective capability decreases in aging retinal pigment epithelium cells.19 Beneficial bacteria significantly benefit the production of anti-oxidants, and there is also increasing evidence of a direct relationship between damaged flora and oxidative stress, suggesting that gut dysbiosis leads to age-related retinal degeneration.

Gut microbiota can also exert a beneficial effect when certain macromolecule intake is decreased. The impact of both high-fat diet and high glucose on microbiota population and the resulting metabolic effects has been extensively studied and established.20 Metabolic products produced by gut bacteria as a result of macromolecular breakdown mediate crosstalk between the gut and immune systems.21 Furthermore, the composition of these metabolites affects bacterial composition. High-fat diet generally leads to increased bacterial Firmicutes alongside a decreased number of Bacteroidetes. This bacterial population imbalance results in enhanced inflammation.22,23 In a significant study, a diet emulating the Western diet was fed to mice, which then presented with an AMD-like disease state. Altering the Western diet, even among older ages in mice, halted the progression of the retinal disease.24 Furthermore, a study including microbiota analysis confirmed that mice fed a diet of high glucose showed disease-like features in the retina and an increased population of Firmicutes, whereas those with the low-glucose diet did not develop disease-like symptoms and possessed greater populations of Bacteroidales and Erysipelothrici.25 This finding strengthens the connection between microbiota composition and retinal health, implicating that gut microbiota imbalance contributes to retinal disease development or progression. Examining bacterial populations of interest in AMD may help define their actions in the pathogenesis of glaucoma or other retinal diseases. AMD and glaucoma share similar vascular pathophysiological pathways.26 Moreover, changes in metabolite concentration within the blood stemming from microbiota alterations seem like a promising area of study, where the pathogenesis of glaucoma and AMD, and potentially other retinal diseases, may overlap.

Gut Microbiota and Glaucoma

Glaucoma, the second leading cause of blindness after cataract in the world, has been shown to be impacted by altered microbiota.27 The hallmarks of glaucoma pathogenesis include progressive degeneration of retinal ganglion cells and their axons that results in irreversible vision loss. It affects >70 million people worldwide.27 Mere administration of heat shock protein 27 has been shown to activate
T cells, inducing an inflammatory response that leads to neurodegeneration resembling the pathology of glaucoma. A significant study found that germ-free mice could not evoke the heat shock protein—specific CD4+ T-cell responses nor did they develop the glaucomatous neurodegeneration under elevated IOP; however, a milder form of glaucomatous degeneration was detected in altered Schae-dler flora Swiss Webster mice that were colonized by eight defined gut bacteria. Thus, the presence of a specific microbiota population may be related to the initiation or perpetuation of neurodegenerative T-cell responses. Taken together, these animal studies suggest an association between the presence of microbiota and the progression of neuron loss in glaucoma.

Gut microbiota and its changing composition underlie the presentation as well as the progression of important retinal diseases. To further understand the changes of gut microbiome, accumulating studies showed the changes of gut microbiome profiles in animal models of glaucoma or in patients with glaucoma. At the phyla level, Zhang et al showed that there is significantly higher Firmicutes/Bacte-rioidetes (F/B) ratio, as well as an increased Verruomi-nae bacterium, accumulating studies showed the changes of gut microbiome population in humans, Firmicutes (65%) and Bacteroides (23%) are major bacterial phyla. Dysbiosis of oral microbiome may affect glaucoma progression. A cohort study showed severe periodontal disease was associated with the risk of primary open-angle glaucoma (POAG). POAG is considered the most common type in America and western Europe and specifically includes the presenta-
tion of increased IOP. Zeng et al reported, in a meta-
analysis study, that oral microbiome Helicobacter pylori was associated with POAG and normal-tension glaucoma. Kountouras et al demonstrated that eradication of H. pylori improved IOP level and visual field defect in patients with POAG. Nevertheless, the association of H. pylori in glaucoma is still controversial, and a different study was unable to find a significant association of H. pylori with the occurrence of glaucoma. In addition, Streptococcus load was higher in the saliva of patients with POAG than in the controls. Astafurov et al showed that altered commensal microbiome induced changes in cytokine and complement activation. However, independent studies showed changes of oral microbiome had no correlation to visual field decline. Further cohort studies of association of oral microbiome profiling and glaucoma are needed.

Recently, more studies were reported to associate the specific profiles of gut microbiome and neurodegenerative diseases. Yoon et al reported an increased Faecalibacteriu-m load in patients with glaucoma. Faecalibacterium modulates butyrate metabolite in the gut that is correlated to anti-inflammatory effect; in addition, the shift of Faecalibacterium population is reduced in patients with Alzheimer and Parkinson diseases. In addition, extensive studies demonstrated the linkage between gut microbiota and neurodegenerative disease, such as Alzheimer disease. This further supports a possible association of glaucoma and Alzheimer disease. Recently, accumulating studies began to explore any correlation between gut microbiota and glaucoma.

Normal human gut microbiota consists primarily of two bacterial phyla, Firmicutes and Bacteroidetes. Different research groups reported various shifting approaches of gut microbiota profiles or related changes in patients with glaucoma. In 2014, Ma et al showed mitochondrial DNA variants m.15784T>C and m.16390G>A were associated with Firmicutes and Proteobacteria phyla, respectively. These variants were enriched in patients with POAG, which was later shown by Collins et al. Recently, Gong et al reported increased abundance of family Prevotellaceae, Enterobacteriaceae, and Escherichia coli and decreased loads of Megamonas and Bacteroides plebeius in patients with glaucoma compared with the controls. Remarkably, they detected a negative correlation between Megamonas and visual acuity, visual field mean defect, and retinal nerve fiber thickness. In contrast, a positive correlation of Streptococcus with retinal nerve fiber layer thickness and a negative correlation of Faecalibacteriu-m with visual field mean deviation were observed. Recently, Chen et al analyzed the gut microbiome diversity between patients with POAG and healthy patients. The data showed that family Dysgonamonadaceae was enriched in patients with POAG, whereas Barnesiellaceae was enriched in healthy subjects, suggesting a shift in specific gut bacteria, such as Lactobacillus, which may contribute to neuroinflammation and, hence, the pathogenesis of glaucoma (Table 1).

**Gut Microbiota-Derived Metabolites in the Development and Progression of Glaucoma**

It is challenging to design a therapeutic approach for neurodegenerative diseases by modulating microbiota. Thus, understanding the role of gut microbiome-releasing factors or metabolites is crucial. It may be a simpler approach to target the progression of neurodegenerative diseases. Bacterial colonies secrete signals influencing immune regulation. In the retina, several specific immune cell functions are altered by gut microbiota, especially through their metabolite by-products. The impact of the microbiome is especially apparent in glaucoma, notably when examining the F/B ratio, a widely accepted indicator of intestinal homeostasis. Zhang et al did a cecal metabolomic profiling study in rats with glaucoma. They observed a positive correlation between an increased F/B ratio and...
Chen et al

**Table 1** Population Changes of Microbes in Glaucoma

<table>
<thead>
<tr>
<th>Gut microbe</th>
<th>Changes in glaucoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Firmicutes</td>
<td>Increased in rats with glaucoma and patients with POAG</td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>Decreased in rats with glaucoma and patients with POAG</td>
</tr>
<tr>
<td>Verrucomicrobia</td>
<td>Increased in rats with glaucoma</td>
</tr>
<tr>
<td>Romboutsia</td>
<td>Increased in rats with glaucoma</td>
</tr>
<tr>
<td>Akkermansia</td>
<td>Increased in rats with glaucoma</td>
</tr>
<tr>
<td>Bacteroides</td>
<td>Increased in rats; decreased in patients with glaucoma</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>Decreased in patients with POAG</td>
</tr>
<tr>
<td>Helicobacter pylori</td>
<td>Increased in oral cavity of patients with POAG; removal of it improves IOP and visual defect in POAG</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>Increased in oral cavity of patients with POAG</td>
</tr>
<tr>
<td>Faecalibacterium</td>
<td>Increased in patients with glaucoma; negatively correlated to VF MD</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>Increased in patients with POAG</td>
</tr>
<tr>
<td>Prevotellaceae</td>
<td>Increased in patients with POAG</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>Increased in patients with POAG</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Increased in patients with POAG</td>
</tr>
<tr>
<td>Megamonas</td>
<td>Decreased in patients with POAG; negative correlation to RNFT, VA, and VF MD</td>
</tr>
<tr>
<td>Bacteroides plebeius</td>
<td>Decreased in patients with POAG</td>
</tr>
<tr>
<td>Dysgonomonadaceae</td>
<td>Increased in patients with POAG</td>
</tr>
<tr>
<td>Barnesiellaceae</td>
<td>Decreased in patients with POAG</td>
</tr>
</tbody>
</table>

IOP, intraocular pressure; POAG, primary open-angle glaucoma; RNFT, retinal nerve fiber thickness; VA, visual acuity; VF MD, visual field mean deviation.

taurocholic acid, which is linked to decreased RGC survival. Conversely, they noted a negative correlation between F/B ratio and glutathione, an antioxidant associated with enhanced RGC survival. Spermidine, a polyamine that shows a property of preserving mitochondrial function, is also reported to be negatively correlated with F/B ratio. Other microbes, such as Bacteroides and Romboutsia, are known to negatively impact glutathione or spermidine. In line with a role for gut microbiota in glaucomatous degeneration, Chen et al52 showed that RGC loss is abolished in germ-free mice with elevated IOP.

Gas chromatography/mass spectrometry is a powerful tool to analyze gut microbiota metabolites in patients or animal models of neurodegeneration, including POAG.28 Gong et al48 detected a total of 35 metabolites that differed between the patient population: 20 increased significantly, whereas 15 decreased significantly. Megamonas and Enterobacteriaceae are decreased in patients with POAG. Moreover, the study assessed that Megamonas was positively correlated with citric acids and negatively correlated with L-γ-glutamyl-L-alanine, hypoxanthine, and 3-methoxy-4-hydroxyphenylglycol. On the other hand, unidentified Enterobacteriaceae were negatively correlated with citric acids and positively correlated with 3-methoxy-4-hydroxyphenylglycol. The opposing bacterial effects on metabolites suggest that metabolites play a key role in disease progression. Studies showed that Gram-negative bacteria, which were significantly increased in patients with POAG, could induce prostaglandin E2 synthesis and lead to nitric oxide and proinflammatory cytokine productions.48,53 Furthermore, the increased Escherichia coli in patients with POAG suggests that gut dysbiosis participates in disease pathogenesis. Gram-negative bacteria, such as E. coli, produce lipopolysaccharide, which can generate strong immune responses and increase proinflammatory cytokine, nitric oxide, and eicosanoid secretion.54 An elevated and prolonged state of inflammation would then contribute to POAG pathogenesis. Another difference in microbiota population was the increase in Prevotellaceae, butyrate producers, in patients with POAG.48 SCFA butyrate has been shown to exhibit an anti-inflammatory effect.55,56 A possible mechanism of anti-inflammation is mediated by suppression of NF-κB activation.57 Butyrate enhances regulatory T-cell differentiation, linking Prevotellaceae to pathogenic inflammation.60 Evaluation of several publications concerning POAG noted differences in six common metabolites (glutamate, creatine, glycine, lysine, alanine, and hydroxyproline) between controls and patients.29

Recently, Chen59 demonstrated that gut metabolite SCFAs, including propionate, isovalerate, and caproate, were significantly higher in patients with POAG. SCFAs also increase in patients with Alzheimer disease and mediate neuroinflammation, suggesting dysbiosis-induced up-regulation of SCFAs may be a common biomarker in neurodegenerative diseases.50 Chen59 later showed that administration of SCFAs further enhanced the neuro-inflammation and retinal degeneration in a mouse model of glaucoma by up-regulation of miR-122-5p and proinflammatory cytokines, such as tumor necrosis factor-α and IL-1β. The consistency and prevalence of these altered byproducts of metabolism suggest that gut microbiota has long-ranging effects that can contribute to glaucoma development.29

Alongside glaucoma, other retinal diseases are affected by gut bacteria population metabolites. The study by Rowan et al57 examined the metabolites produced by protective
bacteria, establishing the pathway that changing gut microbiota affects the retina. As opposed to the high-glucose diet, AMD-like disease presenting mice treated with low-glucose diet showed a high abundance of metabolites, such as serotonin, trimethylamine, hippurate, and tyrosine. These differences in metabolite levels stemmed from a different microbial population and offered a protective effect. For example, serotonin operates by signaling several receptors, such as 5-HT1A, SIRT-1, and SIRT-2, that activate neuroprotection against neuropathy. Increasing these neuroprotective metabolites could benefit treatment of glaucoma because one of its hallmarks is the progressive neuropathy of the optic nerve.

In several retinal diseases, studies have consistently shown the impact of diverging bacteria on metabolites. Thus, the microbiome may affect its host’s retinal health at least in part through secretion of metabolites.

### Targeting Microbiota to Mediate Retinal Inflammation

Considering the association between bacteria populations, metabolites, and inflammatory pathways in retinal diseases, numerous therapeutic approaches may be viable. Methods, such as increasing advantageous bacteria, decreasing harmful bacteria, or introducing beneficial metabolites, offer potential targets for pharmaceutical intervention.

Bacteria that promote homeostatic function or influence T-cell regulation can positively impact a host’s physiology during retinal disease progression. The therapeutic properties of probiotic bacteria have been explored in many immune and inflammatory diseases. More recent studies have begun to examine probiotic bacteria’s potential in eye diseases that act through inflammatory pathways. For example, the mouse study by Verma et al used the bacteria, lactobacillus, to deliver human angiotensin-converting enzyme 2, a protein with anti-inflammatory properties and capable of reducing oxidative stress. The result of introducing this probiotic anaerobe to two different mice models with retinal neuropathy was decreased acellular capillaries, blocked retinal ganglion cell loss, and reduced cytokine expression. In uveitis models, CD8+IL-17hi and CD8+ interferon-γhi cells were significantly reduced. Several studies support that probiotics can modulate immune-related retinal diseases, indicating their potential as a therapeutic approach. In glaucoma specifically, the reduction of inflammation and specifically interferon-γ through probiotics could have potential benefits in treatment of this disease.

Introduction of beneficial metabolites is another viable method to decrease the incidence of retinal diseases in cases of disrupted microbiota. Several studies have demonstrated that enriching a diet with ω-3 long-chain polyunsaturated fatty acids (PUFAs) has proven to suppress pathogenic angiogenesis. PUFAs are naturally found in both the vascular and the neural tissues of the retina. They beneficially act by modulating the activation and potency of bioactive molecules active in retinal diseases. However, microbiota affects one’s PUFAs levels. When conventionally raised mice were compared with germ-free mice, conventionally raised mice had a diminished level of lens phosphatidylcholines, a major type of PUFAs. In mice, supplementation increased regrowth of retinal vessels following injury, decreasing the stimulus for neovascularization and the resulting avascular area. This finding suggests a possible association between microbiota and retinal diseases through modulation of PUFAs levels. The effects of PUFAs were further supported by a significant study evaluating ω-3 polyunsaturated acids in glaucoma. The study reported a neuroprotective effect of supplementing ω-3 polyunsaturated acids on retinal neurons in hereditary glaucoma mouse models through controlling inflammation. Thus, beneficial metabolites seem to hold potential in protecting the retina through multiple pathways.

### Concluding Remarks

The loss of sight or decreased ability to see in those with glaucoma has severely impacted millions of people. Lack of established or completely curative treatments has necessitated research in alternative fields. Gut dysbiosis is suggested to be a contributing factor to glaucoma development. Exploring how disrupted microbiota contributes to disease pathways in the retina, especially glaucoma, is critical to expanding the current understanding of major retinal disease pathogenesis as well as developing novel treatments. Although the feasibility of long-term microbiota correction in humans is still being explored, actively resolving imbalanced microbiota offers a different approach that could complement and enhance currently proposed treatments. Studies that have examined the contrasting physiological differences between germ-free and wild-type mice illustrate the powerful impact that microbiota can have in glaucoma. Although improved outcomes have been achieved in major retinal diseases, such as glaucoma, through probiotics and metabolites, further studies should address how microbiota can benefit health through other systems than solely the immune system. 16S ribosomal DNA sequencing has allowed researchers to isolate which bacterial populations on a species level are contributing to pathogenicity or benefiting their host. Metabolites or signals induced by specific bacteria can then be traced to cytokine production, T-cell regulation, and other modifications, deepening our understanding of retinal disease pathways. The field of microbiota research has rapidly expanded. Potentially harmful fluctuations in microbiota occur on a regular basis because of diet, age, or changing environments. The underlying mechanisms leading to the adverse imbalance of gut microbiota remain unclear. Identification of beneficial bacteria and resolving deviations...
from a homeostatic state through investigations of eye diseases can aid in therapeutic development for autoimmune and inflammatory diseases of other systems in the body.

References


