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 a disrupted microbiome contributes to central nervous system diseases, including Alzheimer disease, Parkinson disease, multiple sclerosis, and glioma, suggesting a prominent role of microbiome in neurodegenerative diseases. This review summarizes the progress in identifying significant changes in the microbial composition of patients with glaucoma by compiling studies on the association between microbiota and disease progression. Of interest is the relationship between increased Firmicutes/Bacteroidetes ratio in patients with primary open-angle glaucoma, increased taurocholic acid, decreased glutathione, and a reduction in 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 synthesizes the findings of several studies investigating the effects of shifting bacterial population in retinal diseases, particularly glaucoma, with the aim to identify the current direction of treatment and help direct future endeavors. (Am J Pathol 2023, 193: 1662–1668; https://doi.org/10.1016/j.ajpath.2023.06.015)
healthy microbiota are some of the methods being explored to prevent or alleviate glaucoma and other retinal diseases.6

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.7 Mouse studies have shown that aging populations have increased Clostridia relative to Bacteriodales and decreased Lactobacillus.8,9 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.8 A key risk factor for retinal diseases is aging.6 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. Studying the relationship between eye disease, aging, and shifting microbiota bacterial populations can help better understand disease pathways and uncover novel interventions. Studies of retinal diseases and the influence of microbiota are already underway. The normal human gut microbiota consists primarily of two bacterial phyla, Firmicutes and Bacteroidetes.13 Dysbiosis (disruption in microbiota) of gut microbiome leads to systemic inflammation and induces the onset of many diseases.14 Inflammation contributes to neurodegeneration in glaucoma.15 Thus, inflammation caused by dysbiosis 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 (linked to gut dysbiosis), and an increased risk of glaucoma, indicate a connection between the microbiome and glaucoma. Further evidence of the role of bacteria in retinal disease is demonstrated by dietary supplantation, 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 retinal pigment epithelium is crucial and helps explain the benefits of bacteria that produce antioxidants, especially as this protective capability decreases in aging retinal pigment epithelium cells.19 Beneficial bacteria benefit from the production of antioxidants. 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 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 system.21 Furthermore, the composition of these metabolites affects bacterial composition. High-fat diet generally leads to increased bacterial Firmicutes and decreased Bacteroidetes. This bacterial population imbalance results in enhanced inflammation.22,23 In a seminal study, mice fed a diet emulating the Western diet presented with an AMD-like disease state. Altering the Western diet, even among older mice, halted the progression of the retinal disease.24 A study analyzing microbiota confirmed that mice fed a high glucose diet showed disease-like features in the retina and an increased population of Firmicutes, whereas those fed a low-glucose diet did not develop disease-like symptoms and possessed larger populations of Bacteroidales and Erysipelotrichi.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 since AMD and glaucoma share similar vascular pathophysiological pathways.26 Changes in metabolite concentration within the blood stemming from microbiota alterations is 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, is impacted by altered microbiota.6,27 The hallmarks of glaucoma pathogenesis include progressive degeneration of retinal ganglion cells and their axons which 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.28 A seminal 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 Schaedler flora Swiss Webster mice that were colonized by eight defined gut bacteria. \(^{29}\) 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 neuronal loss in glaucoma.

Gut microbiota and their changing composition underlie the presentation as well as the progression of several retinal diseases. Several studies show changes in gut microbiome profiles in animal models of glaucoma or in patients with glaucoma. At the phyla level, Zhang et al \(^ {30}\) showed a significantly higher Firmicutes/Bacteroidetes (F/B) ratio, as well as an increased Verrucomicrobia load, in a rat with glaucoma. At the genus level, Ruminococcus, Akkermansia, and Bacteroides were significantly higher in a rat with glaucoma, but Lactobacillus load was higher. \(^ {30}\)

The gut microbiome population in humans consists of Firmicutes (65%) and Bacteroidetes (23%) as major bacterial phyla. \(^ {13}\) Approximately 5% of gut microbiome composition is reflective of oral microbiome. \(^ {31}\) Dysbiosis of oral microbiome may affect glaucoma progression. A cohort study showed an association between severe periodontal disease and a risk of primary open-angle glaucoma (POAG). \(^ {32}\) POAG is the most common glaucoma type in America and western Europe and specifically includes the presentation of increased IOP. \(^ {33,34}\) Zeng et al \(^ {35}\) reported, in a meta-analysis study, that oral microbiome Helicobacter pylori were associated with POAG and normal-tension glaucoma. Kountouras et al \(^ {36}\) demonstrated that eradication of H. pylori improved IOP and visual field defect in patients with POAG. Nevertheless, the association of H. pylori with glaucoma is still controversial, and a different study was unable to find a significant association between H. pylori and the occurrence of glaucoma. \(^ {37}\) In addition, Streptococcus load was higher in the saliva of patients with POAG than in the controls. Astafurov et al \(^ {38}\) showed that altered commensal microbiome induced changes in cytokine and complement activation. However, independent studies showed that changes in oral microbiome had no correlation to visual field decline. \(^ {38,39}\) Further cohort studies of association between oral microbiome profiling and glaucoma are needed.

Recent studies report an association between the specific profiles of gut microbiome and neurodegenerative diseases. Yoon et al \(^ {40}\) reported an increased Faecalibacterium load in patients with glaucoma. Faecalibacterium modulates butyrate metabolite in the gut that is correlated to anti-inflammatory effect. Additionally, Faecalibacterium population is reduced in patients with Alzheimer and Parkinson diseases. \(^ {40}\) Multiple studies demonstrate a link between gut microbiota and neurodegenerative disease, such as Alzheimer disease. \(^ {41-42}\) This further supports a possible association between glaucoma and Alzheimer disease. \(^ {43-45}\) Recent studies have started to explore correlation between gut microbiota and glaucoma.

Normal human gut microbiota consists primarily of two bacterial phyla, Firmicutes and Bacteroidetes. \(^ {13}\) Different studies report different changes in the gut microbiota of patients with glaucoma. In 2014, Ma et al \(^ {46}\) showed an association between mitochondrial DNA variants m.15784T>C and m.16390G>A and Firmicutes and Proteobacteria phyla, respectively. \(^ {14}\) Collins et al \(^ {47}\) later showed that these variants were enriched in patients with POAG. Recently, Gong et al \(^ {48}\) reported increased abundance of family Prevotellaceae, Enterobacteriaceae, and Escherichia coli and decreased loads of Megamonas and Bacteroides plebeius in patients with glaucoma compared with controls. Remarkably, they detected a negative correlation between Megamonas and visual acuity, visual field mean defect, and retinal nerve fiber thickness. In contrast, they observed a positive correlation of Streptococcus with retinal nerve fiber layer thickness and a negative correlation of Faecalibacterium with visual field mean deviation. Recently, Chen et al \(^ {49}\) analyzed the gut microbiome diversity between patients with POAG and healthy subjects. Family Dysgonamonadaceae was enriched in patients with POAG, whereas Bacteroidellaceae was enriched in healthy subjects, suggesting a shift in specific gut bacteria or microbiome patterns that 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 more feasible to target the progression of neurodegenerative diseases. Bacterial colonies secrete signals influencing immune regulation. \(^ {50}\) In the retina, several specific immune cell functions are altered by gut microbiota, especially through their metabolite by-products. \(^ {51}\) 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 \(^ {50}\) did a cecal metabolomic profiling study in rats with glaucoma. They observed a positive correlation between an increased F/B ratio and taurocholic acid, which is linked to decreased retinal ganglion cell survival. Conversely, they noted a negative correlation between F/B ratio and glutathione, an antioxidant associated with enhanced retinal ganglion cell survival. \(^ {50}\) Spermidine, a polyamine involved in preserving mitochondrial function, is also negatively correlated with F/B ratio. Other microbes, such as Bacteroides and Romboutsia, negatively impact glutathione or spermidine. In line with a role for gut microbiota in glaucomatous degeneration, Chen et al \(^ {52}\)
shown that retinal ganglion cell 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. Gong et al. detected a total of 35 metabolites that differed between the patient population: 20 increased significantly, whereas 15 decreased significantly. *Megamonas* and *Enterobacteriaceae* were decreased in patients with POAG. *Megamonas* was positively correlated with citric acids and negatively correlated with 1,γ-glutamyl-1-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. Gram-negative bacteria, which are significantly increased in patients with POAG, induce prostaglandin E2 synthesis and lead to nitric oxide and proinflammatory cytokine production. Furthermore, the increased *Escherichia coli* in patients with POAG suggest 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. An elevated and prolonged state of inflammation would then contribute to POAG pathogenesis. Another difference in microbiota population is the increase in *Prevotellaceae*, butyrate producers, in patients with POAG. SCFA butyrate exhibits an anti-inflammatory effect. A possible mechanism of anti-inflammation is mediated by suppression of NF-κB activation. Butyrate enhances regulatory T-cell differentiation, linking *Prevotellaceae* to pathogenic inflammation. Literature review of POAG indicated differences in six common metabolites (glutamine, creatine, glycine, lysine, alanine, and hydroxyproline) between controls and patients. Recently, Chen 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. Chen later showed that administration of SCFAs further enhanced the neuroinflammation 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 by-products of metabolism suggest that gut microbiota has long-ranging effects that can contribute to glaucoma development.

Alongside glaucoma, other retinal diseases are affected by gut bacteria metabolites. The study by Rowan et al. examined the metabolites produced by protective bacteria, establishing the pathway through which 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

### 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>Verruomicrobia</td>
<td>Increased in rats with glaucoma</td>
</tr>
<tr>
<td>Rомнoutsia</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>Streptococci</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 plebeus</td>
<td>Decreased in patients with POAG</td>
</tr>
<tr>
<td>Dysgonomonadaceae</td>
<td>Increased in patients with POAG</td>
</tr>
<tr>
<td>Banesiellaceae</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.
several receptors, such as 5-hydroxytryptamine 1A (5-HT1A), sirtuin (SIRT)-1, and SIRT-2, that activate neuro-protection against neuropathy.\textsuperscript{25,61,62} Increasing these neuropeptidergic metabolites could benefit treatment of glaucoma because one of its hallmarks is the progressive neuropathy of the optic nerve.\textsuperscript{63}

Studies on several retinal diseases 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**

Given 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.\textsuperscript{64} The therapeutic properties of probiotic bacteria have been explored in many immune and inflammatory diseases.\textsuperscript{65} Recent studies have begun to examine the potential of probiotic bacteria in eye diseases occurring as a result of inflammation. For example, a mouse study by Verma et al\textsuperscript{66} 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.\textsuperscript{66} In uveitis models, CD8\textsuperscript{+}IL-17\textsuperscript{hi} and CD8\textsuperscript{+} interferon-γ\textsuperscript{hi} cells were significantly reduced. Several studies support that probiotics can modulate immune-related retinal diseases, indicating their potential in therapy. Reduction of inflammation, specifically interferon-γ, through probiotics, may have potential benefits in the treatment of glaucoma.\textsuperscript{67}

Introduction of beneficial metabolites is another viable method to decrease the incidence of retinal diseases in cases of disrupted microbiota. Enriching a diet with ω-3 long-chain polyunsaturated fatty acids (PUFAs) suppresses pathogenic angiogenesis.\textsuperscript{68} PUFAs are naturally found in both the vascular and the neural tissues of the retina.\textsuperscript{68} They modulate the activation and potency of bioactive molecules in retinal diseases.\textsuperscript{67} However, microbiota affects one’s PUFA levels. Conventionally raised mice had a diminished level of lens phosphatidylcholines, a major type of PUFA, compared to germ-free mice.\textsuperscript{69} In mice, supplementation increased regrowth of retinal vessels following injury, decreasing the stimulus for neovascularization and the resulting avascular area.\textsuperscript{68} This suggests a possible association between microbiota and retinal diseases through modulation of PUFA levels. The effects of PUFA are further supported by a seminal 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.\textsuperscript{69} Thus, beneficial metabolites appear to protect 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 a potential contributing factor for 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 pathogeneses as well as developing novel treatments. Although the feasibility of long-term microbiota correction in humans is still being explored,\textsuperscript{70} actively resolving imbalanced microbiota offers a unique approach that could complement and enhance current treatments. Studies examining the physiological differences between germ-free and wild-type mice illustrate the powerful impact that microbiota can have on glaucoma.\textsuperscript{52,56} 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 systems other than just the immune system. 16S ribosomal DNA sequencing has allowed for the isolation of bacterial populations at a species level that are contributing to pathogenicity or benefiting their host.\textsuperscript{59} Metabolites or signals induced by specific bacteria can then be traced to cytokine production, T-cell regulation, and other modifications, deepening the 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 the therapeutic development for autoimmune and inflammatory diseases of multiple other systems in the body.

**References**


