The Emerging Role of Gut Microbiota in Age-Related Macular Degeneration

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Age-related macular degeneration (AMD) is the leading cause of irreversible blindness in the industrialized world. With a global prevalence of 196 million, which is expected to increase to 288 million by 2040, the disease remains a major public health concern with immense implications on socioeconomic inequality.

The progressive neurodegenerative disease stems from an imbalanced accumulation of macular cell debris originating from the retinal pigment epithelium (RPE) and collecting in Bruch membrane. These include intracellular lipofuscin from the RPE as well as extracellular drusen. Although periodic hard drusen is considered a normal finding with aging, the presence of diffuse, soft drusen is a significant risk factor and marker for AMD. This accumulation of damaged cellular components, alongside pathologic inflammation and activation of the complement cascade, eventually leads to nutrient obstruction, photoreceptor impairment, and central vision loss. Furthermore, during its progression, AMD is classified by either a dry or wet pathology. Advanced dry (nonexudative) AMD involves RPE atrophy, leading to increasing area of geographic atrophy and loss of support for overlying photoreceptors. Meanwhile, the hallmark of wet (neovascular) AMD (nAMD) is choroidal neovascularization (CNV). Activation of immune and inflammatory pathways causes secretion of proangiogenic cytokines, most notably vascular endothelial growth factor (VEGF).
growth factor. Consequently, aberrant CNV invades and leaks fluid under and into the disrupted photoreceptor layer. Although less common than its dry counterpart, nAMD accounts for almost 90% of AMD-related vision loss.6

Multiple risk factors involved in AMD pathogenesis have been identified, including smoking, age, genetics, and diet.6–9 However, the precise mechanism behind these associations, particularly lifestyle and environmental factors, remains unclear. Considering this, the gut microbiome has emerged as a promising area of study with important implications on mechanistic and therapeutic studies. Consisting of trillions of bacteria, viruses, and fungi, the diverse microbial community is associated with multiple systemic pathologies, including those of cardiovascular, respiratory, and neurologic etiologies.10 Recently, evidence has emerged that supports a mechanistic link in AMD pathogenesis as well. In this review, key and emerging knowledge regarding the gut microbiota’s multifaceted role in AMD pathogenesis is considered, including inflammatory response, immune dysregulation, and gene expression (Figure 1). These findings are elucidated in the context of both human and animal models, highlighting associative and potentially causative relationships between microbial perturbations and disease pathophysiology. With continued exploration and understanding of this evolving new field, the door may eventually open to more accessible and nuanced therapeutic interventions.

Alterations in Gut Microbiota Associated with AMD

Given its dynamic environment and composition, the characterization of healthy microbiota is often broad and imprecise. Nevertheless, specific compositional qualities have been identified in diseased states such as AMD, including reduced diversity and an imbalanced proliferation of more pathogenic microbial strains—findings often termed as gut dysbiosis.11 In a recent case-control study, Lin et al12 showed that subjects with AMD had increased abundances of genera Prevotella, Holdemanella, and Desulfovibrio alongside reductions in genera Dorea, Blautia, and Oscillobia.13 Furthermore, within the same group, metagenomic analysis implicated 72 significant microbial pathways in patients with AMD, showing enrichment in carotenoid biosynthesis, lipid metabolism, fatty acid biosynthesis, and bacterial chemotaxis. Conversely, control patients were associated with microbial pathways tied to bile acid biosynthesis and peroxisome proliferator-activated receptor signaling. Enrichment in microbial pathways involved in lipid metabolism and fatty acid biosynthesis aligns with previous studies showing a large proportion of dysregulated phospholipid metabolites among rats with photoreceptor degeneration, a sequela of late-stage AMD.14 Subclasses of these metabolites included glycerophosphocholines and long-chain polyunsaturated fatty acids, which were significantly increased in dystrophic retinal tissue. Furthermore, peroxisome proliferator-activated receptors have been suggested as a player in AMD pathogenesis and a target of intervention. In addition to inhibiting vascular endothelial growth factor function through mitogen-activated protein kinase, the pathway can moderate oxidative stress linked to AMD.15

Meanwhile, Zinkernagel et al16 detailed a distinct microbial profile in patients with nAMD compared with healthy controls with enrichment in genera Anaerotruncus and Oscillibacter, as well as species Ruminococcus torques and Eubacterium ventriosum. In addition, patients with nAMD had decreased abundance of species Bacteroides eggerthii and an increased Firmicutes/Bacteroidetes ratio. Although this ratio was previously considered a hallmark of obesity-related gut dysbiosis,17,18 more recent studies have presented an inconsistent, contradictory profile.19–21 Although further work needs to be done to delineate these results, the proportional change in microbial profiles remains one of many components to consider when stratifying risk among patients. Among the increased Firmicutes abundance is the class Clostridia, which has been connected to greater AMD risk in other studies.22 Metabolic functions associated with these compositional changes included down-regulation in fatty acid elongation and up-regulation in glutamate degradation, L-alanine fermentation, and arginine biosynthesis in patients with AMD. This aligns with recent metabolomic studies in preclinical animal models; for example, increased intake of n-3 polyunsaturated fatty acids has repeatedly been associated with decreased AMD risk—a finding reaffirmed by the recent population-based cohort study, Alienor.23 Meanwhile, patients with nAMD have shown increased levels of arginine metabolites, supporting the metagenomic profile.14 Although the significance of this role has yet to be elucidated, L-arginine is known to regulate fatty acid metabolism and oxidation, suggesting a potential connection to retinal health.24

Many microbes found to be enriched in patients with AMD by both Zinkernagel and Lin have been associated with promoting systemic pathologic states.13,16 Anaerotruncus is known to increase in abundance with age and is tied to proinflammatory signaling.25,26 Oscillibacter is shown to be involved in pyruvate metabolism as well as increased gut permeability through dysregulated expression of tight junction components.27,28 Meanwhile, it has been established that increased abundance of Prevotella augments mucosal inflammation and type 17 helper T-cell immune response, whereas Desulfovibrio similarly stimulates gut immune response.29,30 In conjunction, preclinical studies further support the association between AMD and perturbations in microbial composition, such as increased abundances of the proinflammatory genera Candidatus Saccharimonas in fecal samples from mice with CNV.31

In a follow-up study, Zyssset-Burri et al32 demonstrated the relationship between altered gut microbiota and AMD using both clinical and preclinical models. Comparing 57 patients with nAMD with 58 healthy controls, they showed that patients with nAMD were enriched in class Negativicutes and...
less abundant in species *Bacteroides*, with *Negativicutes* ranked as a top potential biomarker based on correlation-adjusted t scores. These findings were reaffirmed by studying mice deficient in complement component 3, a complement factor associated with AMD. Similar to human studies, component 3−/− mice showed a markedly increased *Firmicutes/Bacteroidetes* ratio. Beyond taxonomy, metagenomics analysis revealed unique functional microbial profiles among patients with AMD and component 3−/− mice compared with controls. The intestinal microbiomes of these groups were enriched in genes involved in purine degradation and 5-aminoimidazole.

**Figure 1** Effects of gut microbiota on chorioretinal health and their implications in age-related macular degeneration pathophysiology, including altered immune cell regulation, inflammatory response, gene expression, and metabolic signaling. DC, dendritic cell; PAMP, pathogen-associated molecular pattern; RPE, retinal pigment epithelium; Th17, type 17 helper T cell; TNF-α, tumor necrosis factor-α; Treg, regulatory T cell. Created with BioRender.com (Toronto, ON, Canada).
biosynthesis. This is particularly notable given the proposed involvement of purine signaling and metabolism in the progression of AMD and its implication during immune dysregulation.33 Taken together, these results provide evidence that AMD is associated with a distinct microbial signature; however, these findings should be interpreted cautiously, as the gut microbiota changes reported do not overlap to an extent where specific shifts in composition can be confidently linked with AMD pathology. This encourages further studies in evaluating these changes in gut microbiota, as well as how such findings may potentially be harnessed for interventional or screening purposes.

Inflammation and Immunogenic Effects

Dysregulated immune response and inflammation are large driving forces behind AMD pathogenesis and progression. The gut microbiome has been implicated in inflammatory mechanisms, including activation of the innate and adaptive immune systems, inflammasome release during microbial infection, and activation of macrophages and microglia in a para-inflammatory response.12 In particular, the gut microbiome has been linked to macrophage and microglia regulation via a proposed microbiota-microglia axis.35 Bacteria in the gut microbiome are important for microglia maturation during development and adulthood, leading to stark differences in microglial function between germ-free (GF) and specific pathogen-free (SPF) mice.35,36 In a laser-induced model of CNV, GF mice and SPF mice exhibited significant differences in para-inflammatory response to lesion, with GF mice showing reduced lesion size as well as decreased peripheral microglial activation around the lesion when compared with SPF mice.37 These findings substantiate a microbiota-microglia axis in the context of ocular diseases, and are an important first step in supplementing the understanding of how the microbiome affects the para-inflammatory response in AMD.

Complement system activation is also regarded as a key player in AMD progression. Complement factor H (CFH), a regulator of complement activation, has been widely studied as a genetic risk factor for AMD. More important, CFH polymorphisms, specifically CFH Y402H, have been correlated with microbiome bacteria, including Negativicutes, Clostridiales, Bacteroidetes, and Ruminococcus torques.32 Individuals with the CFH Y402H variant show elevated levels of membrane attack complex, the terminating point of the complement cascade.38 Elevated levels of membrane attack complex may represent a trigger for downstream inflammation and AMD progression.39 Thus, if the microbiome environment is altered, it is feasible that complement pathway regulation may also be affected, and therefore AMD disease progression as well.

The adaptive immune system and inflammatory cytokine levels have also gained interest, particularly with regard to the microbiome. Gut homeostasis is critical for maintaining a nonpathologic level of inflammation. When the microbiome composition and ratio of various bacteria are changed, the intestinal epithelial layer integrity is impaired, allowing gut microbial peptides to enter the systemic system.40 Circulating microbial products act as pathogen-associated molecular patterns and activate proinflammatory adaptive immunity.41 This dysbiosis-regulated inflammation has been demonstrated in various inflammatory ocular diseases, such as uveitis, diabetic retinopathy, and AMD.41,42 Epithelial barrier permeability and proinflammatory cytokine levels [including chemokine (C-C motif) ligand 11, Cxcl11, and Il-1β] were reduced significantly when aged mice received young donor microbiota, further highlighting the implication of the gut microbiome in age-related inflammation and AMD.43 Another possible mechanism by which gut dysbiosis yields maladaptive immunodifferentiation in AMD is the T-cell threshold model.44 In the T-cell threshold model, gut dysbiosis may lead to a disproportionate amount of type 17 helper T-cell effector immune cell types relative to regulatory T-cell immunoregulatory cell types. This increase in type 17 helper T-cell type may lead to type 17 helper T-cell migration from the intestinal tract to other environments, triggering an inflammatory response elsewhere, such as in the retina.45

The presence of specific bacteria in the microbiome may also trigger various inflammatory pathways. As previously mentioned, Zinkernagel et al 16 found that patients with AMD showed enrichment of several bacteria, including Anaerotruncus and Eubacterium ventriosum, associated with proinflammatory cytokine activation in mice and elevated IL-6 and IL-8 in humans, respectively. To further support the theory that microbiome composition may have far-reaching effects on inflammation, other studies have demonstrated that dietary intake of specific probiotics reduces inflammatory mechanisms. Administration of heat-killed Lactobacillus paracasei KW3110 decreased retinal inflammation and age-related retinal cell death in aged mice by reducing macrophage-mediated proinflammatory cytokine production.46 Furthermore, aged mice that received fecal transplant from young mice showed reversal of age-associated changes in complement component 3, a marker of retinal inflammation, and Rpe65, a visual cycle protein critical for maintaining photoreceptor function.47 However, Rpe65 may demonstrate non-specific immunoreactivity and may not necessarily demonstrate retinal degeneration. Future studies should further evaluate the microbiome’s impact on inflammation specifically within AMD, as therapeutics that target the microbiota and microbiome composition may have extensive downstream effects.

Effects of the Gut Microbiome on Chorioretinal Gene Expression

To more precisely elucidate the mechanisms by which diet and the microbiome impact chorioretinal biology and AMD pathogenesis, recent work has focused on gene expression
changes that occur in the retina and RPE/choroid when diet and microbiome are perturbed. One recent study showed that GF mice exhibit altered retinal transcriptomes compared with mice with intact microbiomes. A total of 396 differentially expressed genes were identified, suggesting that the microbiome has a significant influence on retinal gene expression. Enriched genes included those involved in obesity/metabolic syndrome, longevity, and signaling pathways, including insulin-like growth factor (Igf), vascular endothelial growth factor (Vegf), hypoxia-inducible factor (Hif)-1, and S' AMP-activated protein kinase (Ampk). One notable gene that was down-regulated in GF mice was peroxisome proliferator-activated receptor-γ coactivator 1-α (Pgc-1α), a transcriptional co-activator of genes involved in mitochondrial biogenesis, oxidative stress, and lysosomal lipid trafficking. Pgc-1α has been shown to regulate normal and pathological angiogenesis in the retina, RPE mitochondrial function, and antioxidant capacity, and is required for preservation of retinal function in the face of detrimental light exposure. Pgc-1α has also been previously suggested to be linked to AMD pathogenesis. Pgc-1α/Nrf-2 double-knockout mice that lack expression of Pgc-1α and Nrf-2, another transcription factor involved in the oxidative stress response, develop a dry AMD-like phenotype. Furthermore, Pgc-1α+/− mice fed high-fat diets (HFDs) develop AMD-like abnormalities in RPE and retinal morphology and function. In wild-type mice, HFD induces changes in choroidal gene expression of Pgc-1α, with close correlation to the phylum Firmicutes.

The microbiome’s impact on gene expression is more prominent in the RPE/choroid compared with the retina—a profile possibly due to differences in ocular immune privilege or unique cell type involvement. When examining RPE/choroidal tissue, 660 differentially expressed genes were identified between GF and SPF mice, with multiple genes involved in inflammatory response, immune pathways, and angiogenesis regulation (Table 1). Those involved included inflammatory mediator Nlrp3 (NLR family pyrin domain containing 3), known to attenuate CNV through IL-18, and Cfh, a previously established genetic risk factor for AMD. Alongside Vegf, the genes for angiopoietin (Angpt1) and Tie1, an angiopoietin receptor, were also differentially expressed in the RPE/choroid of GF mice, indicating a potential relationship between the microbiome and CNV progression. Future work should dissect the microbiome-dependent and microbiome-independent effects of diet on gene expression in the retina and RPE/choroidal transcriptomes in healthy and diseased states, including AMD, as these interactions are still poorly understood.

### Environmental and Lifestyle Risk Factors

AMD pathogenesis is multifactorial, including environmental and lifestyle factors, such as diet and smoking, which contribute to pathologic inflammation and oxidative stress. The Age-Related Eye Disease Study (AREDS) and AREDS2 randomized clinical trials demonstrated that oral supplementation of antioxidant vitamins and minerals—including zinc, lutein/zeaxanthin, and omega-3 fatty acids—could reduce the risk of developing advanced AMD. Although the protective mechanisms of these compounds are not completely understood, their impact on the intestinal microbiome and interactions with specific microbiota may be essential to their bioavailability and beneficial effects. Other trials have examined other supplements, such as oral docosahexaenoic acid, which have been shown to attenuate metabolic disorders by altering the gut microbiome.

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In fact, gut microbiota may serve as a link between the greater body of epidemiologic nutrition research and AMD risk. These studies include dietary patterns, including Western diets, and individual dietary components, such as fat content and glycemic index.58–60 Western or HFDs, which include red meats and high-fat dairy products, are associated with increased odds for AMD progression.61 Yet, despite epidemiologic evidence, the mechanistic understanding between dietary risk factors and AMD has remained largely unknown, but recent research on the gut-retina axis has shed light on how the gut microbiome may mediate the impact of diet in AMD pathogenesis. HFDs have been shown to profoundly alter gut microbiota composition and intestinal permeability.62 HFD-fed mice have lower microbial diversity, increased Bacteroidetes, increased Proteobacteria, and an altered metabolome.63 Andriessen et al64 showed that HFDs induce gut dysbiosis in mice by inverting the Bacteroidetes/Firmicutes ratio, particularly by expanding Clostridia and its members within Clostridiales, Ruminococcaceae, and Lachnospiraceae. In addition, HFDs promoted the presence of Actinobacteria and Spirochaetes.64 Investigators demonstrated that this HFD-induced gut dysbiosis exacerbated laser-induced CNV irrespective of weight, and that these functional changes were accompanied by elevated serum and

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<th>Table 1</th>
<th>Chorioretinal Gene Expression Profile in SPF Versus GF Mice</th>
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<td><strong>Key DEGs</strong></td>
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Key DEGs in the retinal pigment epithelium/choroid between SPF and GF mice. Multiple DEGs are involved in age-related macular degeneration-associated pathways, including angiogenesis regulation, immune function, and inflammatory response.

DEGs, differentially expressed gene; FC, fold change; GF, germ free; SPF, specific pathogen free.
More important, microbiota transplantation from regular diet-fed mice diminished lesion size by 35% in HFD-fed mice. Similarly, others have shown that HFDs worsen laser-induced CNV, especially in genetically susceptible apolipoprotein E-deficient mice that have abnormal lipid homeostasis at baseline. Duration of diet also appears to be an important consideration, as long-term HFD produces a greater degree of gut dysbiosis. Using a HFD that is both high fat and high sucrose, which closely approximates a Western diet, it was shown that long-term HFD (21 weeks) compared with short-term HFD (8 weeks) further decreases protective Bacteroidetes and Bifidobacteriaceae while increasing Clostridiales. Long-term HFD mice also demonstrated greater CNV lesion size and ionized calcium-binding adaptor molecule 1 (Iba1) immunostaining, a marker of microglia activation. Other studies describe comparable findings, where HFDs increase Firmicutes and Verrucomicrobia abundance and decrease Bacteroides. Beyond microbiota composition, HFDs also lead to dysregulation of microbial metabolic pathways, including up-regulation of heparan sulfate biosynthesis, which is implicated in AMD pathogenesis, and down-regulation of biosynthesis of N-glycans and primary/secondary bile acids, which have shown to inhibit AMD-like features in vitro. Together, these studies lend credence that HFDs induce profound changes to the gut microbiome, which can affect chorioretinal health and thereby the susceptibility to developing AMD-like features. Interestingly, GF mice fed a HFD demonstrate altered choroidal and retinal transcriptomic signatures relative to GF mice fed a regular diet. In the retina, a HFD significantly impacted the expression of 53 genes, affecting pathways involved in

Figure 2  Transcriptomic and phenotypic differences using a germ-free (GF) animal model. Summary diagram outlining several experimental setups and results. A: GF mice on a normal diet (GF-ND) and GF mice on a high-fat diet (GF-HFD) underwent retinal and retinal pigment epithelium (RPE)/choroid RNA extraction, followed by high-throughput RNA sequencing and protein-protein association network analyses. A total of 53 differentially expressed genes (DEGs) and 649 DEGs were identified in the retinal and RPE/choroid transcriptomes, respectively. B: Specific pathogen-free (SPF) mice and GF mice underwent retinal and RPE/choroid RNA extraction, followed by high-throughput RNA sequencing and protein-protein association network analyses. A total of 396 DEGs and 660 DEGs were identified in the retinal and RPE/choroid transcriptomes, respectively. C: SPF and GF mice received laser-induced choroidal neovascularization (CNV), followed by immunostaining and microscopy of choroidal filaments. GF mice had reduced CNV lesion size and decreased peripheral microglia activation. The asterisk indicates up-regulation in GF-HFD mice, relative to GF-ND mice. The caret indicates down-regulation in GF compared with SPF mice on a normal diet. n = 4 GF-ND and GF-HFD mice that underwent retinal and RPE/choroid RNA extraction (A); n = 4 SPF mice and GF mice that underwent retinal RNA extraction (B); n = 4 SPF and GF mice that underwent RPE/choroid RNA extraction (B); n = 20 SPF mice the received laser-induced CNV (C); n = 9 GF mice that received laser-induced CNV (C). Created with BioRender.com (Toronto, ON, Canada).
inflammation, angiogenesis, and RPE function.⁷⁰ Such genes included C1qtnf2, a member of the C1q tumor necrosis factor-related proteins, which have been associated with retinal degeneration, and Fat2, a member of the cadherin superfamily that contributes to maintaining the blood-retina barrier. Gene Ontology enrichment analysis revealed that the three most affected biological processes in the HFD group were regulation of blood vessel diameter, inflammatory response, and negative regulation of endopeptidase. Adjacent to the retina, significant changes in gene expression were also noted in the choroid/RPE. A total of 649 differentially expressed genes were identified, with some of the most significantly up-regulated genes pertaining to natural killer T-cell functioning (Cd244a, Cd48, Gzma, Prf1, and Il12b), complement cascade (C1qb, C2, C4b, and Cfh), and angiogenesis (Vegfc, Angpt1, Angpt2, Tie1, Tie2, Pdgfc, and Pdgfd).⁷¹ These findings were supported by Gene Ontology pathway analysis, which demonstrated that angiogenesis, inflammation, and immune response were among the top 10 up-regulated biological processes in the GF-HFD group. Taken together, these findings underline both microbiome-dependent and microbiome-independent effects of HFDs on retinal and choroidal biology. Moreover, their impact may be modified by duration and individual genetics, as well as tissue-specific sensitivity, adding further complexity to these relationships (Figure 2).

Another important dietary consideration is glycemic index, which refers to how readily a consumable increases blood glucose levels after ingestion. High glycemic (HG) index diets are associated with a greater risk for developing early AMD, with up to 77% increased risk reported in some studies.⁷²,⁷³ To investigate the relationships between HG index diets, AMD, and gut microbiota, Rowan et al.⁷⁴ showed that mice fed HG diets developed phenotypic features of dry AMD, including RPE atrophy, lipofuscin accumulation, and loss of photoreceptor cells. The HG index diet altered gut microbiome composition, increasing α diversity and enriching Firmicutes and Clostridia, changes that correlated with more advanced retinal damage, whereas a low glycemic (LG) index diet was associated with retinal protection and increased Bacteroidales and Erysipelotrichi. Notably, an HG-to-LG diet switch could arrest or even reverse several features of dry AMD, with a corresponding change in gut microbiota similar to that of mice fed only an LG diet. Follow-up experiments demonstrated that fecal transplantation from LG diet mice to HG diet mice reduced retinal lesions, and that this retinal protection was paired with increased Akkermanisia.⁷⁴ To further explore the effects of LG and HG diets, Rowan et al.⁷⁴ showed that the two diets conferred different serum and urine metabolic profiles. Seven metabolites, whose levels are modulated by gut microbiota, were negatively correlated with retinal damage: serotonin, hippurate, trimethylamine, 4-hydroxyphenylacetate, 3-indoxylsulfate, tyrosine, and tryptophan. Each of these metabolites were present at higher abundances in the LG group, and thus likely related to the changes in gut microbiota. For instance, 90% to 95% of serotonin is gut derived, and its production is significantly regulated by gut microbiota.⁷⁵ In the context of these findings, small-molecule metabolite signaling may be a key component of the gut-retina axis given the broad impact of gut microbiota on metabolite composition across numerous organ sites.⁷⁶ Furthermore, using functional Kyoto Encyclopedia of Genes and Genomes pathway analysis, the HG diet was associated with fatty acid metabolism and sugar transport, whereas the protective LG diet was associated with carbohydrate metabolism, findings consistent with other studies that underline dysregulation of metabolic pathways in patients with AMD.⁷⁷,⁷⁸

Other factors, such as obesity and body mass index, are also significant risk factors of AMD.⁷⁷ Although the direct relationship between energy balance, gut microbiota, and AMD pathogenesis is not well studied, obesity is associated with alterations in gut microbiota and can promote systemic inflammation through several mechanisms.⁷⁸ Similarly, smoking, another important AMD risk factor, can alter gut microbiome composition, which may contribute to choroidal and retinal damage by increasing oxidative stress, disrupting the gut-epithelial barrier, and promoting inflammation.⁷⁹ Even without specific risk factors, exposure to different microbiota alone can modify age-related retinal damage. This is evident by the fact that Cfh<sup>−/−</sup> mice raised in conventional SPF environments show decreased retinal inflammation and macrophage infiltration, and maintain a greater number of photoreceptors compared with mice raised in open, non-SPF environments.⁸⁰

A large body of research has established associations with AMD and environmental and lifestyle factors. These studies shed insight into how such elements can alter the gut microbiome, and themselves are affected by gut microbiota, thereby imparting profound functional and physiological changes that can modify the risk of developing AMD and exacerbating this disease.

**GF and AMD Animal Models**

As research relating gut microbiota and AMD continues to proliferate, so too will the necessity for reliable preclinical models. GF animal models are the gold standard for in vivo preclinical studies involving the microbiome, revealing systemic changes in response to its perturbation.⁸¹ The absence of microbiota decouples the confounding effects associated with wild-type commensal bacteria and avoids the disadvantages of using broad-spectrum antibiotics to deplete microbiota, including off-target drug effects, inconsistent microbial depletion, and promotion of antibiotic-resistant strains alongside fungal outgrowth.⁸²–⁸⁴

Given this, and growing evidence of gut involvement in AMD pathogenesis, efforts have been made to produce a similarly high-fidelity GF animal model for AMD. One of the most widely used animal models for nAMD is the CNV model, produced by laser-induced lesions through the Bruch
membrane complex. Its advantages, including short time course, precise intervention, and recapitulation of pathologic choroidal neoangiogenesis, have made it a foundational model for understanding choroidal angiogenesis pathobiology and therapeutic developments for nAMD. Recently, the first protocol for the GF and gnotobiotic operation of CNV models was developed, demonstrating a sustainable GF laser-induced mouse model. The nature of CNV treatment, including mouse positioning and management of the laser apparatus, necessitates direct maneuvers that would normally interfere with sterility in traditional GF protocols. Thus, by addressing these challenges, this study is the first to generate a reproducible, sterile protocol for eye-related procedures. The ability to use the gold standard GF model when studying AMD is critical for future studies and opens the door for many exciting directions. For instance, causal relationships between the microbiota and AMD can be assessed independently of dietary effects. This can further be studied in the context of genetic predispositions by incorporating mice of different genetic backgrounds, or in the context of targeted colonization with specific microbiota populations. Finally, just as important, this protocol can be adapted to allow for similar studies in other laser-induced or GF ocular disease models, such as glaucoma, optic neuropathy, and retinal vein occlusion. Many advantages of the GF model compared with other methods of microbiota manipulation have been discussed. However, some important considerations remain, especially regarding development. As GF mice are raised in a sterile environment from birth, they demonstrate altered immune system development and metabolism, which can affect angiogenesis and the blood-brain barrier. Only a few studies have examined the development of CNV in GF mice in the context of AMD. Although both GF and SPF mice will reliably mount CNV in response to laser induction, the CNV lesion size and degree of microglial infiltration around the lesion are diminished, which is consistent with the current understanding of GF status on immune development and angiogenesis. Additional studies are required to characterize the underlying mechanisms of CNV development in GF mice and how they may differ compared with mice raised conventionally. Nevertheless, this model, alongside others, will allow for greater sustainability and standardization of technique when investigating the gut-retina axis in the context of AMD and other retinal pathologies.

Conclusion

As the literature continues to grow, the connection between the microbiome and AMD pathophysiology becomes clearer and further reaffirmed. Most important, the multifactorial nature of this relationship has gained recognition. Both clinical and preclinical models have revealed unique microbial signatures seen in AMD cohorts, the local and systemic effects of these perturbations, and the potential for pathologic rescue. Meanwhile, other studies have identified associations between gut dysbiosis and the hallmark inflammatory and immunologic changes seen in AMD. Finally, the rapid development of next-generation sequencing technologies has resulted in the identification of numerous differentially expressed genes in chorioretinal tissues secondary to changes in gut homeostasis. However, despite these recent advances, further multi-omics studies need to be done to more precisely characterize the significance of identified associations and exact pathways of proposed mechanisms. Doing so will pave the way for not only more efficacious therapeutic strategies but also more robust primary prevention strategies and screening techniques.

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