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Implication of gut microbiome in age-related macular degeneration

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Age-related macular degeneration (AMD) is the most common cause of blindness in the United States in adults over 55 years of age and is one of the leading global causes of blindness: at least 196 million of the worldwide population have AMD, and prevalence is projected to rise to 288 million by 2040 (Lin et al., 2021). As cases and disease burden increase, improvements in the characterization of AMD pathobiology and exploration of potential therapeutic solutions are necessary first steps in addressing this global health concern.

AMD can be subdivided into dry and wet (neovascular) AMD, and while the latter is the less common form, wet AMD is the cause for 90% of AMD-related vision loss. The etiology of wet AMD has been linked to oxidative damage and changes in choroidal vascularization caused by chronic inflammation in response to debris and drusen accumulation in the retinal Bruch's membrane (Lin et al., 2021). To elucidate disease pathogenesis, attention has recently turned to the dynamic ecosystem of the gut microbiome. The gut microbiome is integral to maintaining homeostasis, as it plays a major role in digestion, vitamin synthesis, and immune system regulation. When gut dysbiosis, an unhealthy imbalance of gut microbiota, occurs, inflammatory mechanisms trigger downstream systemic effects like colitis, type 1 diabetes mellitus, rheumatoid arthritis, and multiple sclerosis (Lin et al., 2021). Gut microbial composition is heavily influenced by diet and lifestyle factors, and diets high in saturated fats (closely representing Western diets) have been shown to cause dysbiosis, perturb immune homeostasis, and prompt inflammatory immune responses in mice (Devkota et al., 2012). The exact mechanism of how the gut microbiota impacts AMD progression remains unknown, but the disease pathogenesis may be related to increased intestinal permeability due to microbial composition changes, allowing more systemic circulation of pathogen-associated molecular patterns and binding of pathogen-associated molecular patterns to pattern recognition receptors on microglia or retinal pigment epithelial (RPE) cells (Andriessen et al., 2016). These interactions may induce neovascularization and inflammation, precipitating AMD (**Figure 1**). In this perspective, we will highlight key findings pointing to a novel gut-retina axis in AMD from a preclinical and clinical context and discuss future work that needs to be explored.

To study microbiome interactions *in vivo*, the germ-free (GF) mouse model has been considered the gold standard. Comparisons can be made between GF mice (without any microbes) and specific pathogen-free (SPF) mice (with conventional microbiome) to study the effects of the microbial organ overall. GF models also allow the use of gnotobiotic mouse models in which GF mice are colonized with specific microbial populations to study the causal relationship of host-microbe interactions and underlying mechanisms. Leveraging the GF/gnotobiotic model in the context of AMD has proven difficult: simply applying the traditional laser-induced choroidal neovascularization (CNV) model, the most commonly used mouse model of wet AMD, is impossible because the standard laser positioning is not compatible with the sterility of the GF mice. To circumvent this problem, our lab developed the first GF/gnotobiotic protocol for sterile laser-induced CNV in GF mice (Movahedan et al., 2021). All steps were carried out in a sterile laminar flow hood, all materials used were sterilized, mouse handlers used sterile gloves and gowns, and the laser slit lamp was modified such that the laser beam could reach the mice while under sterile laminar air flow. We confirmed successful laser delivery with lesion formation and maintained sterility, and these results have been reproduced in several studies (Zhang et al., 2022).

By using GF animal models, our group has further corroborated the role of the microbial organ in gut-retina/RPE choroidal axis and CNV development. RNA sequencing of RPE/choroidal tissue revealed 660 differentially expressed genes (DEGs) between GF and SPF mice (Zhang et al., 2022). In GF mice, the absence of microbiota resulted in the downregulation of several notable genes, including *CXCR3* (CXC motif chemokine receptor 3) and *TIE1* (tyrosine kinase with immunoglobulin-like and EGF-like domains 1), and *TNF* (tumor necrosis factor) and *CFH* (complement factor H), involved in the regulation of angiogenesis and inflammation respectively. Of note, *CFH* has been previously established as a genetic risk factor for AMD. In a separate study investigating the whole retinal transcriptome, our group identified 396 DEGs between GF and SPF mice (Nadeem et al., 2020). Genes of interest included *HIF-1* (hypoxia-inducible factor), *IGF* (insulin-like growth factor), *VEGF* (vascular endothelial growth factor), *AMPK* (5'AMP-activated protein kinase), and *PGC1α* (PPARG coactivator 1 alpha). Metabolic dysfunction, induced by changes in diet for example, has been shown to affect several of these aforementioned pathways. Zhang et al. (2022) showed that, in response to laser-induced CNV, GF mice had smaller CNV lesion area and reduced IBA-1 macrophage/microglia infiltration around the CNV lesion (a correlate for inflammatory response) compared to SPF mice. Taken together, these findings highlight the presence of a gut-retina axis and distinct gut-RPE/choroidal axis as well as its role in choroidal angiogenesis, the hallmark lesion of neovascular AMD.

While Zhang et al. (2022) and Nadeem et al. (2020) showed how microbial organ affects RPE/choroidal transcriptome at baseline status, other studies from our group examined the effects of diet on retina and RPE/choroid transcriptome in the absence of microbiota. Dao et al. (2021) compared the retinal transcriptome from GF mice on either a normal diet or high-fat diet (HFD), and 53 DEGs using high-throughput RNA-sequencing were identified, several of which are key genes involved in retinal inflammation, angiogenesis, and RPE function. These genes included *C1qtnf2*, part of the TNF-related-protein family and associated with retinal degeneration, and *Fat2* (FAT-like cadherin 2), which may have a role in maintaining the blood-retinal barrier. Similarly, Xiao et al. (2022) demonstrated RPE/choroidal transcriptomic changes between GF-normal diet and GF-HFD mice. A total of 649 DEGs were identified in the RPE/choroid transcriptome, and all but 33 genes were upregulated in the GF-HFD cohort. Inflammatory markers, including tumor necrosis factor receptor superfamily member 13B (*Tnfrsf13b*) and prostaglandin-endoperoxide synthase 2 (*Ptgs2*), as well as mediators of angiogenesis, like Vegfc and angiopoietin genes (*Angpt1, Angpt2, Angptl2*), were upregulated in GF-HFD mice, suggesting underlying molecular pathways of the complex diet-gut-retina interactions in AMD pathobiology.

These transcriptomic differences support prior hallmark studies that indicate a functional interaction between diet, gut microbiome, and AMD (Andriessen et al., 2016; Rowan et al., 2017). Andriessen and colleagues demonstrated that administration of an HFD both increased CNV and induced gut dysbiosis in mice by enriching *Firmicutes* via expansion of *Clostridia* and *Proteobacteria*. Mice on HFD had threefold higher intestinal permeability and higher levels of serum inflammatory cytokines interleukin (IL)-1β, IL-6, TNF-α, IL-17A, and interferon-γ, along with anti-inflammatory IL-10. Moreover, a microbial transplant from regular diet mice to HFD-fed mice suppressed CNV, validating that HFD's effects on CNV are regulated by gut microbiota. Rowan et al. (2017) showed that mice on a high-glycemia (HG) diet developed AMD-associated phenotypes, including subretinal deposits, RPE hypopigmentation and atrophy, lipofuscin granule accumulation, and photoreceptor disorganization. HG mice also had altered gut microbiome compositions, showing *Clostridia* enrichment, which correlated with advanced retinal damage. Conversely, mice on a low-glycemia diet were protected from developing AMD features and had enriched *Bacteroidales*. Further, mice that switched from an HG to a low-glycemia diet arrested or reversed AMD features, alongside changes in the gut microbiome that resembled those of a microbiota from the low-glycemia diet. HG mice were also shown to have higher plasma levels of lipids and advanced glycation end products, such as glucosepane and Nε(1-carboxyethyl)-lysine, which are quantitatively associated with retinal damage (Rowan et al., 2017). Their data also showed the accumulation of lipid peroxidation-related products, 2-ω-carboxyethyl pyrrole and 4-hydroxy-2-nonenal, in HG RPE tissue. Additional metabolomics analysis indicated that C40:6 phosphatidylcholine, C3 carnitine, and C22:6 lysophosphatidylethanolamine plasma metabolites were correlated with retinal damage (Rowan et al., 2017). Similar to dietary effects, aging has been understood to alter bacterial diversity, change the proportions of gut flora, and weaken the gut-mucosal barrier (Parker et al., 2022). Microbiome composition as a function of age and its effect on retinal inflammation was demonstrated through a recent notable donor experiment, in which fecal microbiota transfer of aged donor gut microbiome into young mice accelerated retinal inflammation, measured by microglial activation, and loss of key functional proteins in the eye (Parker et al., 2022). Conversely, the transfer of young donor gut microbiome into aged mice reversed the aforementioned negative effects.

As clinical studies involving the gut microbiome and AMD continue to be explored, many important associations have already been established. A clinical case-control study comparing the microbiomes of advanced AMD patients to control patients, showed an abundance of *Prevotella*, *Holdemanella*, and *Desulfovibrio* in AMD patients and several of these species have been associated with other inflammatory disease states (Lin et al., 2021). Zinkernagel et al. (2017) sequenced the gut metagenomes of nAMD patients and of controls and demonstrated an increase in *Anaerotruncus*, *Oscillibacter*, *Eubacterium ventriosum*, and *Ruminococcus torques* and a decrease in *Bacteroides eggerthii* in nAMD patients. nAMD patients also showed an enrichment of genes involved in the amino acid breakdown and a decrease in genes of the fatty acid elongation pathway. In a different study, Zysset-Burri et al. (2020) also sequenced gut metagenomes of nAMD patients and of controls to evaluate both compositional and functional changes. Consistent with previous studies, patients with AMD showed enrichment of the class *Negativicutes*, which was selected as a top potential biomarker for AMD. Metagenomics analysis also revealed that gut microbiota in AMD patients were enriched in genes involved in the purine ribonucleosides degradation pathway, which has been implicated in immune dysregulation. These pilot clinical studies suggest that AMD patients have a distinct microbial landscape, both compositionally and functionally, that may be associated with the disease state.

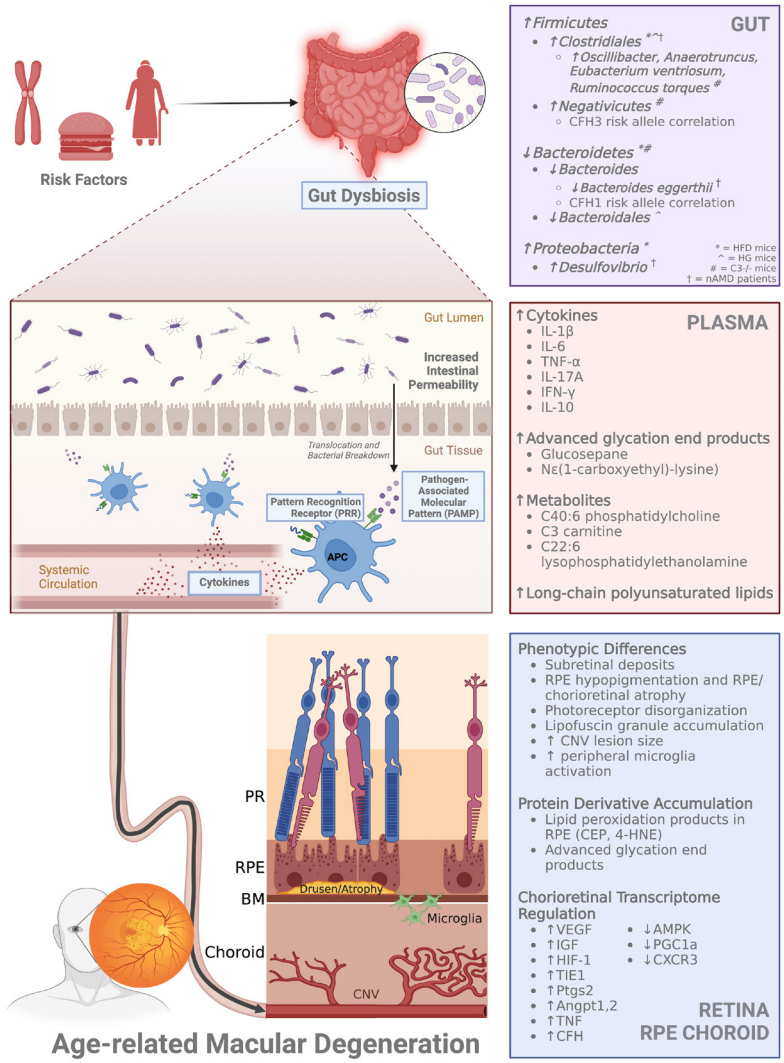

Figure 1 | **Potential interactions between gut microbiome and age-related macular degeneration (AMD).** This illustrative figure summarizes key findings on a gut microbiome, circulatory, and chorioretinal level. Several risk factors, including genetics, diet/lifestyle, and aging, may lead to gut dysbiosis. The purple box describes the microbial composition from several hallmark studies, where * = High Fat Diet (HFD) mice, ^ = High Glycemic (HG) mice, # = C3-deficient ($C3^{-/-}$) mice, and † = neovascular AMD (nAMD) patients. The red box shows the plasma levels of cytokines, advanced glycation end products, metabolites, and long-chain polyunsaturated lipids, along with a schematic representation of cell-signaling in gut tissue. Finally, the blue box highlights phenotypic differences, protein accumulation, and transcriptomic changes on the chorioretinal level. There is also an illustrative representation of disruption in the retina/RPE choroid. APC: Antigen presenting cell; BM: Bruch's membrane; CNV: choroidal neovascularization, a classic lesion of AMD; PR: photoreceptor cells; RPE: retinal pigment epithelium. Created with BioRender.com.

Results from clinical studies utilizing genetic approaches have also better characterized the relationship between genetics, the gut microbiota, and human AMD pathogenesis (Lin et al., 2021). Variations in several genes involved in complement activation and inflammatory pathways, such as complement factor H (*CFH*) and age-related maculopathy susceptibility 2 (*ARMS2*), were found to be highly associated with AMD. Furthermore, Lin et al. (2021) found that genetic risk scores from AMD-related risk alleles, including *ARMS2* and *CFH*, are inversely correlated with alpha diversity of the gut flora in AMD patients. Findings from work led by Zysset-Burri further highlight the associations between genetics, gut microbiome, and AMD (Zysset-Burri et al., 2020). They showed that complement C3-deficient mice had an abundance of *Firmicutes* relative to *Bacteroides* compared to wildtype mice, reflective of the ratio typically observed in nAMD. Moreover, *CFH3*, a single nucleotide polymorphism in *CFH*, was associated with patients with nAMD, and was positively correlated with increased *Negativicutes* (Zysset-Burri et al., 2020). Individuals with *CFH3* polymorphism were also positively correlated with *Clostridiales*, while those with *CFH1* and *CFH2* variants showed a negative correlation. These findings corroborate the interaction of genetics and the gut environment on AMD development, as genetic predispositions to gut dysbiosis may trigger changes in complement cascade activation (and ultimately AMD pathogenesis). Strengthening our understanding of this complex interplay will be crucial towards developing therapeutics that account for individual variation.

Taken together, advancements in preclinical and clinical areas continue to bolster and deepen our understanding of the complex, multifactorial relationship between the gut microbiome and AMD disease mechanisms. With the advent of a novel animal model to study host-microbial interactions in ocular diseases, further preclinical studies should continue to manipulate other genetic or lifestyle factors to better characterize the gut-retina axis in AMD. Clinical studies for AMD should continue to elucidate the synergistic relationship between genetics, environment, and age on disease development, as this may pave the way for identifying important risk factors for AMD onset and progression. Much progress is needed in the realm of identifying therapeutic solutions – as more therapeutic targets are identified, future studies need to account for the feasibility of the application of microbiome-based therapeutics. There is much to look forward to as we further delve into the exploration and elucidation of the gut microbiome contribution to AMD pathogenesis and identify novel therapeutic strategies.

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