Review Article

A systematic review of gut microbiome and ocular inflammatory diseases: Are they associated?

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The primary focus of this review was to establish the possible association of dysbiotic changes in the gut bacterial microbiomes with both intestinal and extra-intestinal diseases with emphasis on ocular diseases such as bacterial keratitis, fungal keratitis, uveitis, age-related macular degeneration, and ocular mucosal diseases. For this particular purpose, a systematic search was conducted using PubMed and Google Scholar for publications related to gut microbiome and human health (using the keywords: gut microbiome, ocular disease, dysbiosis, keratitis, uveitis, and AMD). The predictions are that microbiome studies would help to unravel dysbiotic changes in the gut bacterial microbiome at the taxonomic and functional level and thus form the basis to mitigate inflammatory diseases of the eye by using nutritional supplements or fecal microbiota transplantation.

Key words: Age-related macular degeneration, dysbiosis, gut microbiome, keratitis, ocular disease, uveitis

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Every inch of the human body harbors microorganisms (bacteria, fungi, and viruses) and they together constitute the human microbiome. Microbiomes vary depending on the particular niche they occupy on the human body. The most enormous is the gut microbiome which has a preponderance of bacteria whose numbers (3.8×10^{13}) are similar to the total number of cells in the human body (3.0×10^{13}).[1] With an estimated 3.3 million genes, the gut bacterial microbiome has 150 fold greater numbers of genes compared to the 23,000 genes in the human body.[2] Thus, it is logical to assume that the vast number of bacteria and their associated genes are likely to have functions that impact human health.

Gut Microbiome Functions

The canonical role of the gut bacterial microbiome is to aid in digestion, to protect against pathogenic bacteria, to aid in the development of the host immune system, to aid in production of vitamins and synthesis of short-chain fatty acids (such acetate, propionate, and butyrate). In addition, the microbiome helps to preserve homeostasis of several T-cell populations in the gut, comprising regulatory T cells (Treg), T helper 1 (Th1), and 17 (Th17) cells which are vital in hosting an immune response against pathogens.[3] Studies have also indicated that commensal bacteria that are native to the human gut, that is, the autochthonous or indigenous gut microbiota are diverse between individuals and may thus be responsible for the variations observed between individuals at the physiological level.[4] Thus unravelling the gut bacterial microbiome is important and needs to be understood in totality.

Core Gut Microbiome and 'Dysbiosis'

Gut microbiome is primarily composed of the phyla Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria, and Verrucomicrobia. Of these, the phyla Firmicutes and Bacteroidetes are the most predominant and represent 70–90% of the gut microbiota.[5] The above four phyla together constitute the "core microbiome" and have been consistently detected in the gut microbiome of all normal individuals. In a healthy human being, the gut bacterial microbiome maintains a delicate balance between the 'good or beneficial' (probiotic and anti-inflammatory) and "bad or harmful" (pro-inflammatory and pathogenic) bacteria. But under certain conditions, such as high-fat diet, excessive of sugar intake, sedentary lifestyle, excess uptake of antibiotics, and under diseased conditions the balance in the microbiome tilts from 'beneficial' to 'harmful' bacteria. This imbalance or alteration in the gut microbiome recognized by the increase or decrease in diversity, abundance, and function of the gut microbes as compared to that in the

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healthy human gut is referred to as "dysbiosis". Over the last decade, dysbiosis in the gut microbiome has become a hallmark of disease [Fig. 1].

Evidence that Bacteria in the Gut Microbiome are Associated with Disease

That bacteria in the gut microbiome cause the disease during dysbiosis was obvious when it was elegantly demonstrated that lean mice following fecal transplantation with the gut bacteria from fat mice were transformed into obese mice.[6] The converse was observed when skinny germ-free mice plumped up on receiving a fecal transplant from a human obese donor.[6,7] It was also demonstrated that fecal transplants supplemented with *Christensenella minuta* rendered the recipient mice thinner, indicating that *C. minuta* controls obesity.[8] In the gut microbiome, there are other beneficial bacteria such as *Akkermansia muciniphila*, which when present in abundance reversed obesity and decreased insulin resistance probably mediated by endocannabinoids secreted by *A. muciniphila*[9] and *Faecalibacterium prausnitzii*, which protects against intestinal inflammation.[9] In addition, the gut microbiome may also be associated, with bacteria which exert deleterious effects like *Klebsiella pneumoniae* and *Proteus mirabilis*, which have been implicated in colitis in mice.[10] Thus, just as a pathogen could cause a disease, a "good" microbe could prevent a disease? An important aspect that has emerged, over the years, is that the gut microbiome is prone to changes depending on host factors (such as age, gender , region of origin, genetics and intrinsic factors of the gut such as pH, bile acids, transit time and mucus), environmental factors (e.g., nutrients and medication) and microbial factors (e.g., adhesion capability, bacterial enzymes, metabolic strategies, bacteriophages).[11] These confounding factors need to be accounted for when comparing microbiomes between healthy and diseased individuals.

Gut Microbiome Dysbiosis and Human Health

Establishing an association between dysbiosis and disease is the first step in appreciating the important role of the gut microbiota in disease. But the formidable challenge is to connect the property/function of a microbe or microbes to a disease so as to be able to manipulate the microbe for the benefit of mankind. Establishing this connection between the gut microbe and the disease is a mammoth task considering that the numbers and species of microbes that inhabit a niche are mindboggling and thus singling out one or a few bacteria may not always be possible.

Dysbiosis in the gut bacterial microbiome has been associated with several intestinal diseases like obesity,[12] Crohn's disease,[13] Type 1 Diabetes Mellitus, Type 2 DM,[14,15] colorectal cancer[16] and gastric cancer.[17] Dysbiois has also been associated with several extra-intestinal diseases such as cancers, muscular dystrophy, vaginosis, neuro-developmental, and neuro-degenerative diseases.[18-24] Overall the above studies indicated that the connection between gut microbiome dysbiosis and diseases may be based on the functional attributes of the dysbiotic taxa in the gut microbiome.[25] But, it may not always be possible to interpret the dysbiotic changes vis a vis the disease.[26] It should also be dealt with caution that much of the interpretation is based on inferred functions of the bacteria and most of the time the extrapolations are from the genus to the species level.

Gut Microbiome Dysbiosis and Ocular Diseases

Several systemic diseases also manifest in patients as ocular diseases. For instance approximately 10% of individuals with inflammatory bowel disease manifest as episcleritis, uveitis, and conjunctivitis,[27,28] which are inflammatory diseases of eye. Some of these diseases also occur due to non-infectious conditions like idiopathic or auto-immune uveitis or Steven Johnson syndrome–induced keratitis. It is likely that under non-infectious conditions, these diseases are influenced by dysbiosis in the gut microbiome. More recently, dysbiosis has been implicated in ocular diseases like bacterial[29] and fungal Keratitis,[30] Uveitis,[31-33] Ocular mucosal disease[34] and Age-related macular degeneration[35] which implied a possible connect between the gut microbiome dysbiosis and ocular diseases.[36,37] It was also demonstrated that the ocular fungal microbiome changes under conditions of fungal Keratitis.[38,39] Thus maintaining a healthy gut microbiome or organ microbiome is crucial and sacrosanct to human health and the challenge is to be able to identify and establish a connect between the microbe and the disease.

(a) Gut microbiome dysbiosis and Uveitis

Two studies on Indian and Chinese Uveitis patients demonstrated that uveitis and healthy control (HC) microbiomes are distinctly different and an overall decrease was observed in the diversity and abundance of the bacterial communities in the gut microbiomes of uveitis patients compared to HC[31,32] [Fig. 2]. Several bacteria like *Lachnospira, Dialister, Dorea, Blautia, Clostridium, Coprococcus, Odoribacter, Faecalibacterium prausnitzii, Akkermansia muciniphila, Mitsuokella, Magasphaera and Roseburia* which are known butyrate producers and contribute to anti-inflammatory response were decreased in abundance. In addition *Ruminococcus, Bacteroides, Bifidobacterium adolescentis, Oscillospira and Veillonella dispar* which are known to exhibit probiotic properties were also decreased several folds in uveitis microbiomes compared to HC [Table 1]. Thus, it may be concluded that in uveitis subjects, the decrease in gut bacteria with anti-inflammatory and probiotic properties may contribute or exacerbate the inflammatory reaction. Several other taxa were also significantly enriched in HC and substantially reduced in uveitis patients but the physiological relevance of these enrichments in HC vs. uveitis patients is not known. It was also observed[40] that a few short chain fatty acid producing bacteria viz. *Faecalibacterium* and *Roseburia* were present in the guts of diseased individuals, but their abundances were less than half when compared to healthy controls implying that abundance is important. One of the major challenges is to establish a connection between dysbiosis and the ocular disease. In a recent review Horai and Caspi[41] provided evidence that gut commensal microbes impact not only intestinal diseases but also extra-intestinal diseases like the diseases of the eye. They used mice models of experimental autoimmune Uveitis (EAU) and the spontaneously uveitic R161H mice to address the issue as to whether commensal gut microbiota could trigger the development of Uveitis.[33,42] EAU was induced by active immunization of B10.RIII mice with interphotoreceptor retinoid binding protein (IRBP), a retinal

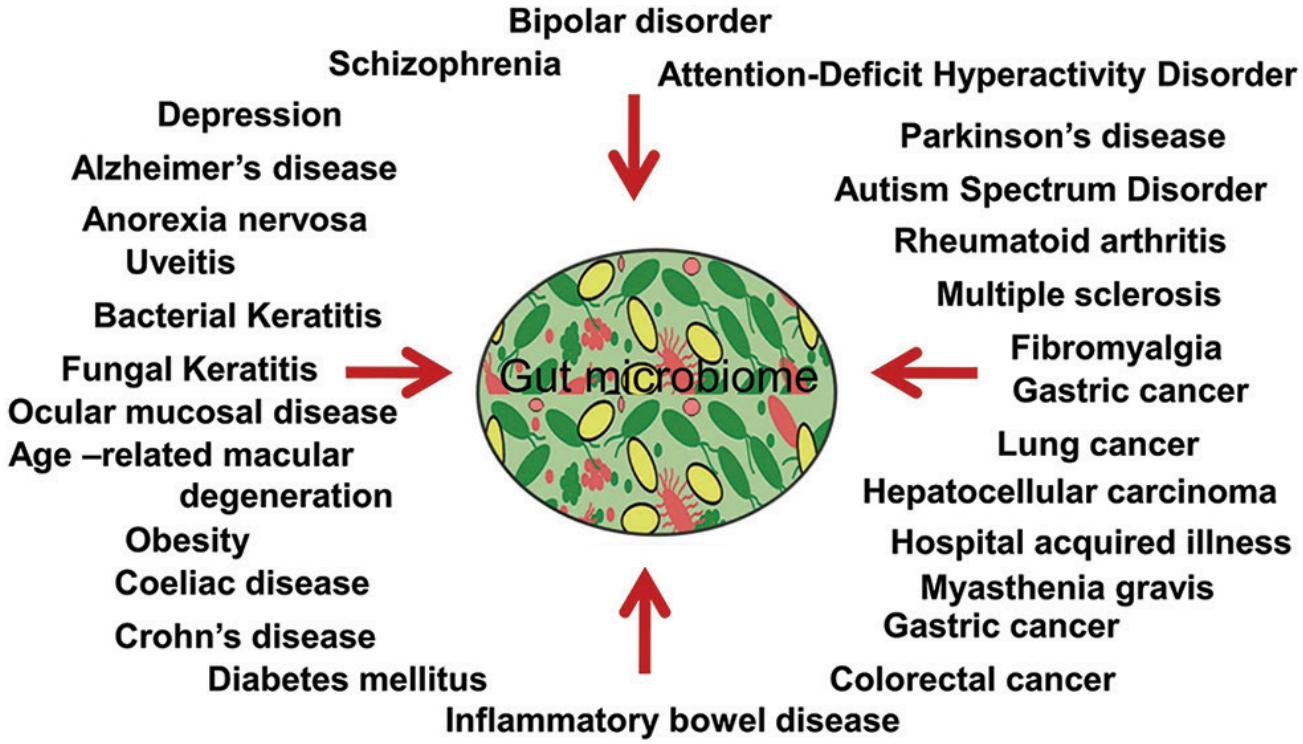

Figure 1: Gut bacterial microbiome dysbiosis and human diseases

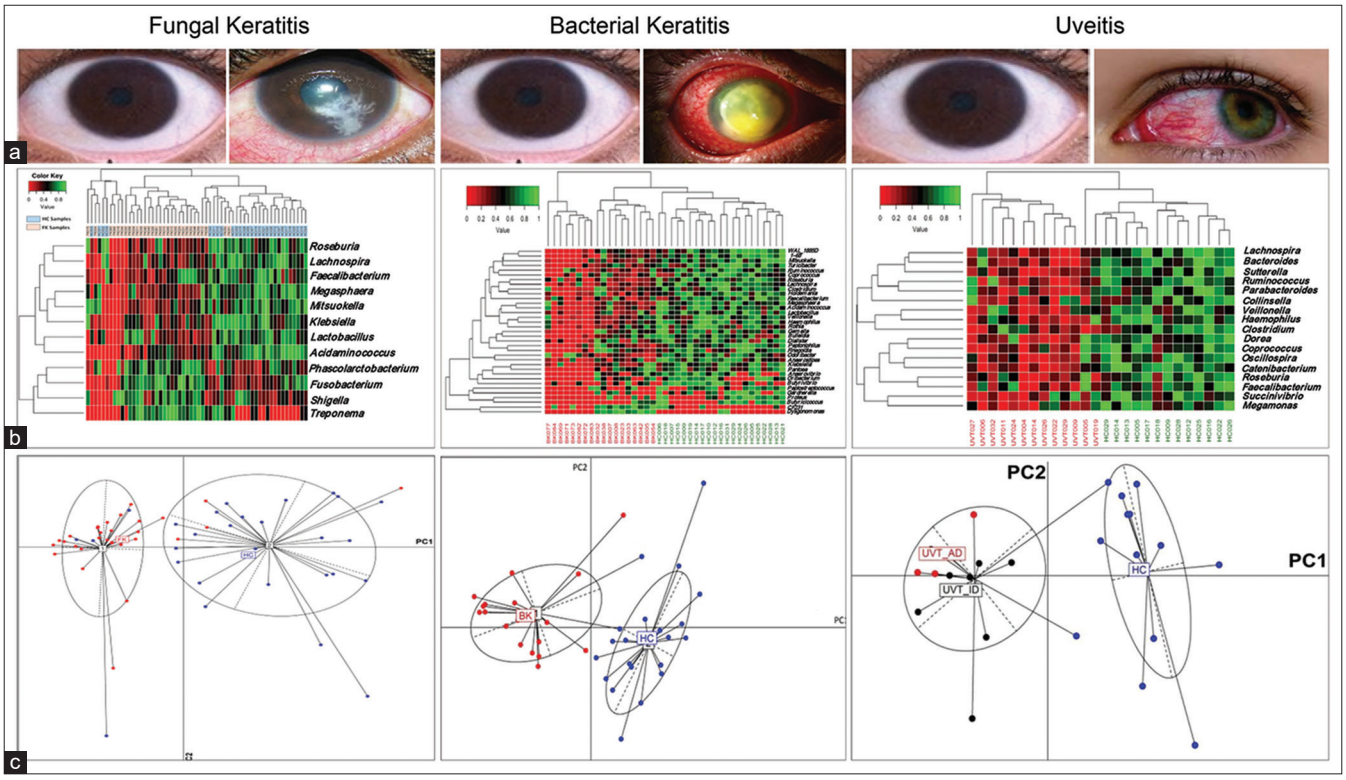

Figure 2: Human gut bacterial microbiome dysbiosis associated with individuals with bacterial and fungal keratitis and Uveitis. (a) depicts the healthy and the diseased eyes. (b) (heat map) (c) (principal component analysis) depict a comparison of the abundance of the bacterial genera in the gut microbiomes of the healthy individuals and individuals with fungal Keratitis , bacterial Keratitis and and Uveitis. Figure based on data from references 29-31

Table 1: Candidate bacterial genera associated with the gut microbiomes of individuals with ocular diseases

Disease	Bacterium*	Increase/Decrease in disease condition	Function and reference
Bacterial Keratitis	*Acidaminococcus* (Ai), *Bacteroides caccae* (Ai), *Bifidobacterium adolescentis* (Ai), *Blautia* (Ai), *Clostridium* (Ai), *Coprococcus eutactus* (Ai), *Dialister* (Ai), *Dorea* (Ai), *Faecalibacterium prausnitzii* (Ai) *Lachnospira* (Ai), *Lactobacillus* (Ai/Pr), *Megamonas* (Ai), *Megasphaera* (Ai), *Mitsuokella multacida* (Ai) *Odoribacter* (Ai), *Parabacteroides* (Ai), *Phascolarctobacterium* (Ai), *Roseburia* (Ai) *Ruminococcus* (Ai), *Streptococcus* (Ai), *Veillonella dispar* (Ai)	Decreased	Anti-inflammatory.[29]
Bacterial Keratitis	*Prevotella copri* (Pi), *Bilophila* (Pi)	Increased	Pro-inflammatory.[29]
Bacterial Keratitis	*Bacteroides* (Pr), *Bifidobacterium adolescentis* (Pr), *Blautia, Dialister* (Pr), *Lactobacillus mucosae (Pr), Lactobacillus ruminis* (Pr), *Megamonas* (Pr), *Oscillospira* (Pr), *Phascolarctobacterium* (Pr), *Ruminococcus* (Pr), *Veillonella* (Pr), *Streptococcus* (Pr), *Turicibacter* (Pr),	Decreased	Probiotic.[29]
Bacterial Keratitis	*Bacteroides* (Ab), *Lactobacillus ruminis* (Ab), *Bifidobacterium adolescentis* (Ab), *Blautia* (Ab)	Decreased	Antibacterial[29]
Fungal Keratitis	*Megasphaera* (Ai), *Ruminococcus* (Ai), *Roseburia* (Ai), *Lachnospira* (Ai), *Acidaminococcus* (Ai), *Clostridium* (Ai), *Dialister* (Ai), *Dorea* (Ai) *Mitsuokella multacida* (Ai), *Faecalibacterium prausnitzii* (Ai), *Lactobacillus* (Pr), *Bacteroides plebeius* (Pr), *Bifidobacterium adolescentis* (Pr), *Klebsiella* (Pi), *Sutterella* (Pi)	Decreased	Anti- and pro-inflammatory and probiotic.[30]
Fungal Keratitis	*Bacteroides fragilis* (Ab), *Dorea* (Ai), *Shigella* (Pi), *Treponema* (Pa)	Increased	Anti-bacterial, anti-inflammatory, pro-inflammatory and pathogenic.[30]
Uveitis	*Lachnospira (Ai), Ruminococcus (Pr), Bacteroides (Pr), Dialister (Ai), Dorea (Ai), Blautia (Ai), Clostridium Ai), Coprococcus (Ai), Bifidobacterium adolescentis (Pr), Oscillospira (Pr), Odoribacter (Ai), Veillonella dispar (Pr), Faecalibacterium prausnitzii (Ai), Akkermansia muciniphila (Ai), Mitsuokella (Ai), Magasphaera (Ai), Roseburia (Ai)*	Decreased	Anti-inflammatory and probiotic.[31-33]
Uveitis	*Bifidobacterium adolescentis (Pr), Bifidobacterium longum (Pr)*	Decreased	Probiotic.[31]
Ocular mucosal disease	*Pseudobutyrivibrio* (Ai) *Escherichia* (Pa)/*Shigella* (Pi), *Blautia* (Ai), *Streptococcus* (Ai)	Increased	Anti- and pro-inflammatory and pathogenic.[34]
Ocular mucosal disease	*Bacteroides* (Pr), *Parabacteroides* (Ai), *Faecalibacterium* (Ai), *Prevotella* (Pi)	Decreased	Probiotic, anti- and pro-inflammatory.[34]
Age-related macular degeneration	*Anaerotruncus* (Ai), *Oscillibacter* (Pi), *Ruminococcus torques* (Pr, Ai), *Eubacterium ventriosum* (Ai)	Increased	Probiotic, anti- and pro-inflammatory.[35]
Age-related macular degeneration	*Bacteroides eggerthii* (Co)	Decreased	Commensal.[35]

*Pi, Pro-inflammatory; Ai, Anti-inflammatory; Pa, Pathogenic; Pr, Probiotic; Co, Commensal

protein, which was coadministered with a killed mycobacterial antigen adjuvant, to induce ocular inflammation. In these EAU mice, the severity of uveitis was associated with increased abundance of *Coprococcus, Dorea, Adlecreutzia and Desulfovibrio* genera in the uveitic state compared with normal healthy controls.[43] Further, it was observed that altering the intestinal microbiota of uveitic mice with a cocktail of four broad-spectrum oral antibiotics (ampicillin, metronidazole, neomycin and vancomycin) substantially reduced the severity of uveitis. Individual antibiotics, ampicillin, and neomycin, had no effect on uveitis severity whereas oral metronidazole or vancomycin alone significantly reduced uveitis severity.[43] It was also observed that systemically administered antibiotics did not have an effect on uveitis severity arguing against any direct anti-inflammatory effects of the antibiotics.[43] Yet another study confirmed that altering the microbiome with either germ-free rearing of animals or treatment with oral metronidazole and ciprofloxacin resulted in markedly reduced uveitis severity.[44] The possible reason for the amelioration of uveitis following antibiotic treatment could be attributed to the increased regulatory T cells (Tregs) both in lymphoid tissues and in the eye of EAU mice which acted as the trigger.[43]

In addition to the use of EAU model, the spontaneous uveitis model in R161H mice also confirmed that the

gut microbiotas are involved in triggering autoimmune uveitis.[33,42] It was observed that R161H mice reared under the germ-free (GF) conditions or following depletion of commensal microbiota by a cocktail of oral broad-spectrum antibiotics (ampicillin, metronidazole, neomycin, and vancomycin) showed attenuation of spontaneous uveitis.[41] Further, disease development in the spontaneously uveitic R161H mice was associated with the gut microbiome activating Uveitis-relevant cells, the TH17 cells in the intestine even before the onset of clinical Uveitis.[41] These studies supported a causative role of microbiota in triggering uveitis, but the direct proof that auto-reactive T cells in the gut migrate and reach the eye to cause uveitis is still lacking.[41,43] From the foregoing information, it was hypothesised that metabolites of gut microbiota could possibly modulate or attenuate uveitis either by enhancing Tregs in the colon and cervical lymph nodes and (or) by reducing the trafficking of effector T cells between the intestines and the spleen during uveitis.[45,46] Accordingly, it was demonstrated that exogenous administration of short chain fatty acids, which are normally produced by gut microbiota, could reduce the severity of uveitis by the above two mechanisms.[45,46] Thus an effective method to treat uveitis could be to alter intestinal bacteria diversity so as to enhance beneficial metabolites.

Molecular Basis of Gut Microbiota-Induced Uveitis

The molecular basis of the gut microbiota induced uveitis is yet not clearly understood. A few studies demonstrate that in the EAU mice model dysbiosis in intestinal, pharyngeal, oral, and ocular microbiomes could lead to epigenetic reprogramming and inflammation making the host more susceptible to ocular diseases such as autoimmune uveitis, AMD and open-angle glaucoma.[47] Evidence for this mechanism is multifold and includes several important observations. Foremost is the discovery of the transcription factors Tbx21 and Rorc whose methylation changes were associated with the production of the Th1/Th17 cells associated with uveitis. Hypomethylation of these transcription factors due to reduction in the expression of DNA methyltransferase (DNMT1) was discovered in the retinas and RPE choroidal tissues of EAU mice and was associated with increased production of Th1/Th17 specific cytokines (IFNγ and IL-17).[48] But whether a similar mechanism operates in human uveitis patients is not known. It was also observed that miRNA-223 which is associated with microbiome dysbiosis and which promotes inflammation was upregulated in the EAU rat model.[49] In addition, a uveitis associated miRNA cluster of six miRNAs, which is linked to inflammatory signalling cascades, was detected in serum miRNA profiles of patients [49]. Yet we do not understand how changes in the gut cause inflammation in the eye, which is normally immunologically privileged.

(b) Gut microbiome dysbiosis and Bacterial Keratitis (BK)

Dysbiotic changes in the bacterial gut microbiome were observed in individuals with BK compared to HC individuals[29] [Fig. 2]. Functionally the bacteria in BK patients which showed significant differences in abundance compared to the gut microbiome of healthy controls could be categorised as anti-inflammatory (21 nos.), pro-inflammatory (2 nos.), anti-bacterial (4 nos.) and probiotic (12 nos.) [Table 1]. The pro-inflammatory bacteria increased whereas the anti-inflammatory, probiotic and anti-bacterial decreased in abundance in BK patients. It was concluded that this combination of a decrease in anti-inflammatory and probiotic bacteria and increase in pro-inflammatory bacteria would support BK, an inflammatory condition.[29] These observations also confirmed earlier studies that *Prevotella copri* and *Bilophila*, which are pro-inflammatory are increased in BK patients.[50,51] It was also observed that known pathogens like *Enterococcus*, *Bacteroides fragilis*, genera CF231, and *Dysgonomonas*[50,51] which cause gastroenteritis[52] were also enriched in the gut microbiomes of BK patients. It is worth mentioning that in both HC and BK microbiomes *Prevotella copri* which has a pro-inflammatory function and associated with rheumatoid arthritis is enriched but its abundance is greater in BK patients.[29] Thus decrease in anti-inflammatory and probiotic bacteria may be contributing to the inflammatory reaction in BK patients.

(c) Gut microbiome dysbiosis and fungal Keratitis (FK)

Fungal keratitis (FK) is estimated to affect over a million cases annually and significantly contributes to corneal blindness in tropical countries. Common causative organisms include *Aspergillus* spp., *Fusarium* spp., *Candida* spp., *Curvularia* spp., *Penicillium* spp., *Rhizopus* spp., and *Mucor* spp. In a recent study, in an Indian cohort, it was demonstrated that gut bacterial richness and diversity in FK patients was significantly decreased demonstrating dysbiosis in the gut bacterial microbiomes compared to healthy controls[30] [Fig. 2]. In FK subjects several anti-inflammatory bacteria (11 numbers), which are involved in promoting several health benefits like those affiliated to Lachnospiraceae and Ruminococcaceae and to the *genera Megasphaera, Ruminococcus, Roseburia, Lachnospira, Acidaminococcus, Clostridium, Dialister, Dorea* and the species *Mitsuokella multacida and Faecalibacterium prausnitzii* were decreased in abundance compared to the HC individuals [Table 1]. This prominent decrease in anti-inflammatory bacteria along with the decrease in probiotic bacteria like *Lactobacillus, Bacteroides plebeius* and *Bifidobacterium adolescentis* would support the inflammatory condition in FK patients. Further increase in pro-inflammatory *Shigella* and a single pathogen *Treponema* would also support FK.[30] Thus, in FK subjects, the decrease in gut bacteria with anti-inflammatory and probiotic properties exacerbate the inflammatory reaction in Keratitis.

(d) Gut microbiome dysbiosis and ocular mucosal disease

Sjögren syndrome (SS) is a common mucosal autoimmune disease and primarily affects the secretory glands and mucosal tissues of the eye and mouth. In the eye, it causes severe dry eyes. Investigations on the ocular, oral, and stool microbiomes of patients with SS revealed significantly altered diversity in the oral and intestinal microbiome in SS patients [Table 1].[34] Thus SS associated dry eye disease patients showed dysbiosis in the gut microbiome but the trend was not very clear. For instance anti-inflammatory (*Pseudobutyrivibrio, Blautia,* and *Streptococcus*), pro-inflammatory (*Shigella*) and pathogenic (*Escherichia*) bacteria were increased in abundance under dry eye condition and in contradiction anti-inflammatory (*Parabacteroides, Fecalibacterium),* proinflammatory *(Prevotella)* and probiotic *(Bacteroides)* were also significantly reduced in stool samples from SS individuals.[34] Thus dysbiotic changes in the gut microbiomes of patients with dry eye is clear but the possible involvement is obscure.

(e) Gut microbiome dysbiosis and age-related macular degeneration (AMD)

AMD is the most frequent cause of blindness in the elderly. Factors such as nutrition, inflammation, and genetic risk factors have been implicated in the development of AMD. In fact, it was demonstrated using a mouse model that high-fat diet[53] alters the gut microbiota and exacerbates choroidal neovascularization, a feature of AMD. Just about the same time, it was demonstrated that wild-type mice fed a high-glycemic-index diet had an altered gut microbiota and the mice developed AMD like in the diseased state. Further when the mice were treated with a low-glycemic-index diet, the development of AMD was reverted. Recent studies suggest that dysbiosis in the gut microbiome is also associated with AMD in human beings[35] [Table 1]. *Anaerotruncus, Oscillibacter, Ruminococcus torques, and Eubacterium ventriosum* were relatively enriched in patients with AMD, whereas *Bacteroides eggerthii* was decreased in AMD patients [Table 1].[35] In individuals with advanced AMD the abundance of *Prevotella* increased whereas the abundance in Ruminococcaceae and Rikenellaceae bacteria were decreased compared to healthy controls.[45] It was also observed that the microbiomes of AMD patients were enriched in genes of the L-alanine fermentation pathway, glutamate degradation pathway and arginine biosynthesis pathways. Simultaneously decrease in genes of the fatty acid elongation pathway and the carotenoid biosynthetic pathways were observed thus implicating these pathways in the pathogenesis of AMD.[35,45] Taking cue from these observations a study titled "Age related eye disease study 2" (AREDS2) was undertaken to ascertain whether nutritional supplements could prevent or slow down AMD. The AREDS2 formulations tested contained antioxidants and carotenoids (vitamin C, Vitamin E, cupric oxide, Lutein, Zeaxanthin and Zinc) and so far it is the only nutritional intervention that slowed the progression of AMD.[54] In all likelihood, these oral supplements altered the gut microbiota, and this is yet to be demonstrated.[54] But, gut microbiota were altered by supplementation with AREDS which unlike AREDS2 had all the above constituents but lacked Lutein and Zeaxanthin. The most predominant change was an increase in *Peptoniphilius,* in AMD individuals taking AREDS. It is also known that variations in or near the complement genes (*CFH* , *CFI* , *CFB*, and *C3)* and a polymorphism (rs10490924) in *ARMS2* showed the highest association with AMD.[55] But the majority of intestinal bacterial changes could not be associated with the presence of ARMS2 rs10490924 or variations in CFH (complement factor H).[54,56]

Molecular Basis of Gut Microbiota Induced AMD

The foregoing studies indicate that gut microbiome dysbiosis is associated with AMD. But, as yet, a possible molecular basis of dysbiotic gut microbiota influencing AMD is not clear. A few epigenetic changes like DNA methylation and histone acetylation have been observed in the retina of AMD patients. Hypermethylation of glutathione S-transferase P1 (GSTP1) promoter is known to repress mRNA expression of the two isoforms of glutathione S-transferase (GSTM1 and GSTM5) thus leading to a decrease in scavenging of reactive oxidative species which is detrimental to retina.[57-59] Further, hypomethylation of interleukin 17 receptor C (IL17RC) promoter leads to increased expression of the receptor which is known to promote pro-inflammatory cascades.[57-59] Finally, histone deacetylation has been shown to limit the accumulation of clusterin, a protein produced by the retinal pigment epithelium. The environmental trigger for these epigenetic changes has not been defined but it is possible that the microbiome and its byproducts may influence such modifications.[57-59]

(f) Ocular microbiome dysbiosis and fungal Keratitis

Recent ocular surface studies indicated that Proteobacteria, Firmicutes, and Actinobacteria constituted the core phyla[60,61] and the Corynebacterium genus was the most abundant on the ocular surface.[62-64] Compared to the bacterial microbiome, little is known about the ocular surface fungal microbiome. In a recent study, NGS detected 65 distinct fungal genera with *Aspergillus*, *Setosphaeria, Malassezia*, and *Haematonectria* present in all the 25 eyes in which fungi were detected. Alpha diversity in the two eyes was similar and sex had no effect, but Chao1 and Simpson indices were altered by age.[39] In a subsequent study, it was demonstrated based on Alpha diversity indices, phylum and genera level diversity and abundance differences and heat map analysis that the fungal microbiomes of individuals with fungal keratitis exhibited dysbiosis compared to the ocular surface microbiome of the healthy control individuals.[38] Based on the diversity and abundance it was suggested that as compared to the conjunctiva from healthy controls, the conjunctiva and corneal scraping of fungal keratitis individuals had a greater abundance of opportunistic pathogens or pathogens which could be related to ocular disease.[38] This was the first report implicating dysbiosis in the fungal microbiome of conjunctival swabs and corneal scrapings in individuals with fungal keratitis. Such studies on ocular surface bacteria are lacking.

Modulation of the Gut Microbiome as a Therapy

The realization that we are what we eat and the fact that the microbes are not passive partners in the gut but could positively influence human health (under conditions of dysbiosis) has opened up several avenues for effective treatment. The most obvious approach was to try and reverse the dysbiotic changes and restore normalcy by the use of antibiotics, prebiotics, and probiotics. But success was not forthcoming with the use of antibiotics and prebiotics. A distinct ray of hope was apparent when probiotics were used to reverse dysbiosis. For instance in animal models of rheumatoid arthritis, the beneficial effects of probiotics were obvious but probiotic use has not been unequivocally replicated in clinical settings. In Rheumatoid arthritis patients who received *Lactobacillus rhamnosus* alone or in combination with *Lactobacillus reuteri* or *Bacillus coagulans* the outcomes were not consistent and varied from improved subjective well-being to a reduction in inflammatory markers and cytokine levels.[65] This lack of consistent outcome could be attributed to the observation that the stool microbiota compositions before and after probiotic courses of *Lactobacillus rhamnosus* or *Bifidobacterium* were retrieved in >90% of the subjects' stools and were similar to those of the placebo group.[66] Despite these attempts to modulate the gut bacterial microbiome to overcome diseases, it has been demonstrated that 1-month treatment with probiotic eye-drops of *Lactobacillus acidophilus* improved signs and symptoms in patients with Vernal keratoconjunctivitis.[67] Another method which has positive clinical outcomes is fecal microbial transplantation (FMT), involving transfer of fecal microbiota of a normal healthy

individual to a diseased person. FMT has proved successful in the treatment of *Clostridium difficile* diarrhea and IBD.[68,69] At the moment in the field of ocular biology, this approach is far from being considered due to various social and ethical reasons.

What Needs to be Done

Gut microbiome studies have proved an association between the gut microbiome and ocular diseases[36,37] and the future of microbiome studies would however be cause or effect. But, the importance of the microbiome vis a vis ocular health would become even more appreciated if the following are addressed:

(i) Establish a connection between the gut and ocular microbiomes
(ii) Carry out longitudinal studies to define the dynamics of the ocular microbiome vis a vis the severity of the disease
(iii) Undertake studies in close relatives to confirm the changes
(iv) Understand the mechanism by which gut microbiome influences a disease through metabolites, inflammatory molecules, and cytokines thus opening up a co-ordinated effort between microbiomes and metabolomics
(v) Use animal models like mouse and zebrafish to study the molecular mechanism underlying the disease.[69]

Microbiome research offers hope by way of a new therapy for ocular diseases involving gut microbes and their metabolites.

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Conflicts of interest

There are no conflicts of interest.

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