Inherited retinal degenerations (IRDs) represent a genetically and clinically heterogeneous group of progressive and visually debilitating disorders that can lead to irreversible visual loss. Our understanding of IRD pathogenesis at both the genetic and cellular levels has increased tremendously over the past two decades, but the exact pathogenic mechanisms remain incompletely understood. Enhanced understanding of the pathophysiology of these diseases can result in new treatment targets. Alterations in the human gut microbiome play a key role in the pathogenesis of many ocular and nonocular diseases, such as age-related macular degeneration, neurologic and metabolic disorders, and autoimmune conditions. The gut microbiome regulates the susceptibility of mice to develop experimental autoimmune uveitis, a model for autoimmune disease of the posterior portion of the eye elicited by the systemic response to retinal antigens. Because of the mounting evidence in favor of a role for local and systemic inflammatory and autoimmune-mediated components to IRD pathogenesis, this review presents the current knowledge of gut microbiome in IRDs and discusses the association between possible changes in gut microbiome and pathogenesis of these diseases, with special attention to their possible contribution to the inflammatory underpinnings of IRDs. (Am J Pathol 2023, 193: 1669–1674; https://doi.org/10.1016/j.ajpath.2023.03.005)
disease (NIH Human Microbiome Project, https://www.hmpdacc.org/hmp, last accessed October 14, 2022). Alterations in gut microbiota and immune and blood metabolomes have been reported in several diseases, including multiple sclerosis, obesity, and diabetes. Studies have also demonstrated that alterations in the gut microbiota are linked to several ocular diseases, such as age-related macular degeneration, diabetic retinopathy glaucoma, and uveitis, suggesting that it may play a significant role in the pathogenesis and/or progression of the disease. The gut microbiome regulates the susceptibility of mice to develop experimental autoimmune uveitis, a model for autoimmune disease of the posterior portion of the eye elicited by the systemic response to retinal antigens. Potential therapeutic interventions include intermittent fasting, fecal microbial transplant, prebiotics, probiotics, as well as antibiotics. However, because most of the studies are conducted in animal models, translatability and applicability to the human diseases are limited, and further studies are required to elucidate the role of gut dysbiosis in the pathogenesis and the progression of ocular diseases.

Notwithstanding these important advances in our understanding of the role of gut microbiome in both human and experimental preclinical disease, the literature regarding gut microbiota composition in IRDs is sparse. To the best of our knowledge, to date, the gut microbiome and the gut-retina axis have not yet been studied at the clinical level in human populations with IRD. However, the investigation of this field in IRDs is likely to yield important new insights in the complex pathogenesis of IRDs. Particularly, there is mounting evidence in favor of a role for local and systemic inflammatory and autoimmune-mediated components to IRD pathogenesis, which builds on prior knowledge in the field. This review presents the current knowledge of the role of gut microbiome in animal models of IRDs and discusses the association between possible changes in gut microbiome and pathogenesis of these diseases, with special attention to their possible contribution to the inflammatory underpinnings of IRDs. It presents the current knowledge of the potential role of the microbiome dysbiosis in IRD expression and progression and summarizes a potential framework within which the gut microbiome may modulate the autoreactivities against retinal and optic nerve antigens that many patients with IRD appear to develop in the course of their disease.

Retinitis Pigmentosa

Retinitis pigmentosa (RP) comprises a group of rare genetically determined disorders. It is characterized by progressive rod and cone degeneration and presents clinically with symptoms of night blindness, peripheral visual field loss, and, ultimately, often central vision loss. Although considered a rare, orphan disease estimated to affect 1:3000 to 1:4000 people, with no sex predilection, RP is the most common IRD and can be transmitted primarily as an autosomal dominant, autosomal recessive, or X-linked trait. More rarely, RP can also result from mitochondrial genome mutations. Several subtypes of RP are also characterized by the association with systemic findings, such as hearing loss, obesity, and neurologic findings, identifying specific syndromic entities.

Only a small number of studies have investigated the gut microbiome in animal models of RP. Kutsyr et al studied the gut microbiome composition in the retinal degeneration 10 (rd10) mouse model of RP, which has a missense mutation in the phosphodiesterase 6B (Pde6b) gene, and compared it with C57BL/6J wild-type control mice. They noted that both rd10 and control mice share similar general microbial features, with predominance of Bacteroidoida and Firmicutes phyla regarding taxonomic features and Lactobacillus and Muribaculaceae species. However, significant differences in α (microbiome diversity within a sample) and β diversity (similarity or dissimilarity between samples) of the gut bacteria were found between these two groups, with four species that commonly present in healthy gut microbiome being absent in rd10 mice (Rikenella, Muribaculaceae, Prevotellaceae UCG-011, and Bacilli species), whereas Bacteroides caecimuris was overrepresented in the gut of these mice but was absent in the controls.

Kutsyr et al also evaluated the effect of high-fat diet (HFD) and regular chow diet on rd10 mice and compared it with that in wild-type control C57BL/6J mice. The study revealed that HFD was associated with further gut microbiome dysbiosis with significant alterations in α and β diversity of the gut bacteria. In HFD mice, Bilophila, Alistipes, and Mucispirillum schaedleri species, which are positively correlated to inflammatory processes of the gut, were most abundant. On the other hand, Muribaculaceae bacteria, which are involved in the gut homeostasis, were remarkably decreased. Short-term feeding of HFD for 2 to 3 weeks had a detrimental impact on several molecular, histologic, and functional aspects of the disease, which led to significant worsening of the retinal degeneration in rd10 mice. Overall retinal function, as assessed with a- and b-wave dark-adapted full-field flash electroretinography, was significantly decreased in amplitude in rd10 mice fed the HFD versus standard chow diet at virtually all tested intensity reductions. The adverse effects of HFD were similar after 2 and 3 weeks of HFD. Visual acuity measured by optomotor response was also significantly lower in rd10 mice on HFD compared with rd10 mice on chow diet (and controls). Furthermore, the mean number of photoreceptor rows calculated from temporal to nasal retina through the optic nerve head was significantly higher in rd10 mice on chow diet compared with those on HFD (2.4 ± 0.3 versus 3.6 ± 0.3 rows of photoreceptor nuclei in the outer nuclear layer, respectively). The cones of mice on chow diet had also better morphologic features compared with those on HFD, in which the cones were smaller in size and with diminished inner and outer segments.
The adverse impact of HFD on the rd10 phenotype was similar to what Ryals et al \(^{44}\) had observed in a prior study, which also included evaluation of a ketogenic (high-fat and low carbohydrate) and a ketogenic plus low-protein diet on the progression of retinal degeneration in the rd10 compared with C57BL/6J control wild-type mice and a standard chow from postnatal day 23 to postnatal day 50. Photoreceptor outer nuclear layer thickness, full-field electroretinography amplitudes, and optokinetic tracking to estimate psychophysical visual performance were assessed. The earlier study had demonstrated that ketogenic plus low-protein diet in rd10 mice decelerated retinal degeneration, with significant improvement of photoreceptor thickness, full-field electroretinography amplitudes, and optokinetic thresholds. On the basis of the above findings, the authors proposed that this type of diet may promote retinal neuroprotection. \(^{44}\)

Although the gut microbiota compositions of these mice were not investigated in the study, the change in the severity of the retinal degeneration in rd10 mice based on distinct dietary regimens further emphasizes indirectly the impact that dietary changes can have on the retinal phenotype. One can envision how this protective diet could well be associated with anti-inflammatory diet-associated gut microbiome favorable changes. It will be definitely of interest to observe the outcome of future studies that may ultimately allow us to make potential dietary recommendations to patients with RP that may modulate favorably the severity of their primary disease.

### Batten Disease

Batten disease, also known as neuronal ceroid lipofuscinoses, is an autosomal recessive inherited neurodegenerative disease that is characterized by progressive loss of vision, loss of language and motor skills, as well as seizures, behavioral changes, and cognitive decline, which can ultimately result in death. \(^{15}\) More than 12 genes and 430 mutations have been identified thus far, which encode lysosomal enzymes, lysosomal and transmembrane proteins, or other proteins involved in secretory and/or endolysosomal pathway; the most common subtypes being CLN1, CLN2, and CLN3. \(^{45}\) In retina and other ocular tissues, accumulation of storage material can lead to optic nerve atrophy, pigmentary retinopathy, vascular attenuation, and bull’s eye maculopathy. \(^{46}\) In 2017, enzyme replacement therapy with cerliponase \(\alpha\) was approved by the US Food and Drug Administration as an effective treatment for the CLN2-linked Batten subtype. In addition, several novel treatments for either decelerating or halting disease progression are under investigation. \(^{45}\)

Although there are no studies investigating the gut microbiota composition and its effect on the visual function or the retinal phenotype in Batten disease in either humans or animal models, the research team of Kovács and co-workers has published three interesting studies on the three most common subtypes of Batten disease and the changes of gut microbiota compared with controls in mice along with neurologic changes. \(^{38}-^{50}\)

A comparative analysis of the gut microbiota composition in mouse models for the infantile CLN1-linked and late infantile CLN2-linked forms of Batten disease (\(\text{Cln1}^{R151X}\) and \(\text{Cln2}^{R207X}\), respectively) and that of three wild-type mouse strains (129S6/SvEv, mixed 129S6/SvEv x C57 BL/6J, and C57BL/6J) was conducted. \(^{50}\) Two different genetic backgrounds were used for \(\text{Cln1}^{R151X}\) mice (mixed 129S6/SvEv x C57BL/6J and pure C57BL/6J), and in both groups increased numbers of bacterial species of the family of Erysipelotrichaceae were reported. Studies have shown that Erysipelotrichaceae is associated with inflammatory diseases of gastrointestinal tract, such as Crohn disease-like ileitis and obesity. \(^{51,52}\) \(\text{Cln1}^{R151X}\) mice on C57BL/6J background had greater proportion of Streptococcaceae family bacteria but less Bacteroides genus bacteria. In the \(\text{Cln2}^{R207X}\) mice, abundance of Bacteroidetes phylum, Bacteroidaceae family, Bacteroides genus, and Alistipes genus was observed. Another interesting finding of this study was the relative decrease in lactic acid—producing bacteria (Lactococcus genus) in all \(\text{Cln1}^{R151X}\) and \(\text{Cln2}^{R207X}\) mice, except the \(\text{Cln1}^{R151X}\) mice on C57BL/6J background. The anti-inflammatory and cytoprotective properties of these bacteria have been well documented. \(^{53}\) Thus, it was postulated that this difference may play a crucial role in the development and progression of the neurologic symptoms commonly seen in these disorders. \(^{50}\)

In \(\text{Cln3}^{-/-}\) mice (originally backcrossed with 129S6/SvEv mice for 12 generations and maintained as a homozygous \(\text{Cln3}^{-/-}\) colony), the model for CLN3-associated Batten disease, acidified water (pH 2.5 to 2.9) was administered from postnatal day 21, and its effects on gut microbiota along with motor behavior and neuropathologic changes were assessed at 3 and 6 months. \(^{38}\) The 129S6/SvEv mouse model was used as a control for the same background as the \(\text{Cln3}^{-/-}\) mice. The study demonstrated that acidified drinking water attenuated motor deficits at 3 months and microglial activation in the thalamus, motor cortex, and striatum in \(\text{Cln3}^{-/-}\) mice at 6 months but had no effect on the lysosomal storage material in the same areas. A relative reduction in the abundance of Lactobacillus was also reported in the \(\text{Cln3}^{-/-}\) mice on nonacidified water. On the basis of the ensemble of the above-mentioned findings, the authors postulated that similar differences may contribute to the neurologic deficits seen in these patients. \(^{58}\)

Last, a similar method to the previous study \(^{48}\) was used to assess the therapeutic effect and the gut microbiota alterations of acidified water in \(\text{Cln1}^{R151X}\) mouse model of the CLN1-linked subtype of Batten disease, where the neurologic phenotypes and the neuropathologic changes in male mice were examined. \(^{39}\) Significant changes in the gut microbiota of \(\text{Cln1}^{R151X}\) (129S6/SvEv x C57BL/6J) were identified and were altered following administration of acidified drinking water. Similar to what was observed in
Cln3−/− mice, acidified water also resulted in attenuation of microglia activation in the hypothalamus and the somatosensory barrel field cortex. On the contrary, the improvement in motor skills was maintained at both 3 and 6 months of age in Cln1R151X mice. At 3 months, α diversity in Cln1R151X mice on nonacidified water was higher compared with controls. Although this was reduced in the diseased mice on acidified water at 3 months, this change was no longer significant at 6 months. The β diversity was substantially altered among the diseased animals on either types of drinking water, and the most pronounced differences were found in Cln1R151X mice on acidified water at 3 and 6 months, with a significant reduction in the proinflammatory Deferrribacteres at 3 months and Verrucomicrobia at 3 and 6 months. By 6 months of age, bacteria of the anti-inflammatory Bifidobacterium genus also dramatically increased in the Cln1R151X mice drinking acidified water. Therefore, amelioration of the underlying neurologic deficits in hypothalamic, somatosensory, and motor cortices was in part attributed to the anti-inflammatory properties of the observed changes in the bacteria of the gut microbiome induced by the acidified water.

Discussion

To our knowledge, there are no human studies thus far on the gut microbiome composition in patients with IRD with or without syndromic, extraretinal manifestations. Some studies on mouse models of IRDs have been conducted, highlighting changes in the gut flora composition that are associated with retinal and, in the case of Batten disease models, systemic neurologic phenotype differences. It has also been shown how modifications of the gut microbiome induced via diet or drinking water changes that lead to more anti-inflammatory characteristics of the gut flora were associated with reduced severity of the retinal and/or neurologic manifestations of the genetically driven conditions, with these changes impacting especially the inflammation-driven features of RP or Batten disease.

Consistent with early studies pointing to an important role of inflammation and the immune system in IRDs, a more recent reappraisal and further corroboration of an important role of inflammation in modulating the overall severity of IRDs has been corroborated not only clinically but also in animal models of IRDs, whose disease severity has been successfully mitigated by either direct interference with the immune effectors of inflammation-driven damage or prevention of accumulation of proinflammatory, immunogenic intraretinal deposits. The presence of clinically detectable and treatable inflammatory complications in patients with IRD has been retrospectively evaluated in recent years as well. A key unanswered question is why do certain genetic subtypes of IRDs appear to be more prone than others to these inflammatory complications. It is not presently known if this may represent only a stronger locally proinflammatory effect of specific changes induced at the retinal level by mutations in these particular genes, leading first to activation of the resident immunocompetent retinal cells (mainly the microglial cells) followed by secondary activation of the immune system at the systemic level, as proposed already by Adamus. This possibility is supported by antiretinal—and anti–optic nerve—autoantibodies developed by patients with IRD affected with these complications that correlate well with the observed inflammatory phenotypes and features thereof. However, the likelihood that these complex inflammatory events may be happening in a vacuum is highly unlikely, as the interplay of retinal degeneration with inflammation is certainly multifaceted and far reaching.

The preclinical animal studies reviewed herein, albeit limited in number, and details with regard to specific ocular phenotype manifestations, are strongly suggestive of an interplay between IRDs, inflammatory complications, and changes in the gut microbiome. The favorable impact of dietary modifications in mice on the phenotypes expressed by these IRD models also suggests that there may well be untapped opportunities from this vantage point to modulate favorably IRD phenotypes and mitigate their severity. To this end, it is valuable to characterize the gut microbiome in patients with IRD, investigate differences in disease severity and/or rates of progression between. For example, obese patients with IRD versus lean patients with IRD or between patients on different diets, and potentially test prospectively interventions based on these differences. Clinical differences that support this possibility have been anecdotal observed. For example, morbidly obese patients with metabolic syndrome appear to be commonly refractory to treatments for a common inflammatory complication of IRDs, cystoid macular edema, that are typically readily effective instead in other patients, including other affected family members sharing the same genetic cause of the disease, who are leaner or on healthier diets. Extreme variability was observed in IRD severity expression within families in fraternal twins who differed radically in their diets and body mass index. For example, the case of a woman with severe macular dystrophy expression (macular atrophy and 20/200 acuity in the best seeing eye) and body mass index >50 kg/m² despite undergoing gastric bypass surgery and her twin brother, who shares the same genetic changes but is fit and into anti-inflammatory diets and nutritional supplements. He was subjectively asymptomatic at baseline with 20/20 visual acuity. However, once examined, he was clearly affected by imaging criteria, although only at the subclinical level. After 4 years of follow-up, the overt imaging changes seen in this patient had not progressed, and he retained 20/20 acuity. Furthermore, several anecdotal cases have been documented of patients with autoimmune retinal manifestations who experienced a visible and clinically measurable reduction in their inflammatory manifestations following spontaneously enacted
dietary changes, such as switching to gluten- or dairy-free diets. Lastly, it is also possible that the various past trials of RP that have shown benefits toward rates of disease progression from several dietary supplements may have themselves impacted favorably also the gut microbiome in ways that remain to date, unknown.

Conclusion

Although the relationship between the gut microbiome and the manifestations of IRDs is not at all well characterized yet, the existing data are highly suggestive of a role of the gut microbiome in IRD onset, severity, and progression, especially as it may pertain to their inflammatory manifestations. Although it is not possible to draw precise conclusion from the anecdotal clinical observations briefly summarized above, studying the role of diet and the gut microbiome in IRDs is highly likely to reveal information about disease pathogenesis that could be of significant prognostic and, potentially, therapeutic relevance. We propose that investigating the gut microbiome at the clinical level in IRDs may offer an important opportunity to better understand the pathophysiology of these conditions and to mitigate, at least in some cases, the severity of these visually debilitating disorders.

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