

# Evidence Suggesting the Role of Gut Dysbiosis in Diabetic Retinopathy

Pratima Singh Thakur,<sup>1</sup> David Aggarwal,<sup>1</sup> Brijesh Takkar<sup>ID</sup>,<sup>1,2</sup> Sisinthy Shivaji<sup>ID</sup>,<sup>3</sup> and Taraprasad Das<sup>ID</sup><sup>1</sup>

<sup>1</sup>Anant Bajaj Retina Institute–Srimati Kanuri Santhamma Centre for Vitreoretinal Diseases, L V Prasad Eye Institute, Hyderabad, India

<sup>2</sup>Indian Health Outcomes, Public Health, and Economics Research (IHOPE) Centre, L V Prasad Eye Institute, Hyderabad, India

<sup>3</sup>Prof. Brien Holden Eye Research Centre, L V Prasad Eye Institute, Hyderabad, India

Correspondence: Brijesh Takkar, LV Prasad Eye Institute, Banjara Hills, Hyderabad 500034, India; [britak.aiims@gmail.com](mailto:britak.aiims@gmail.com), [brijeshthakkar@lvpei.org](mailto:brijeshthakkar@lvpei.org).

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**PURPOSE.** Gut dysbiosis has been identified and tested in human trials for its role in diabetes mellitus (DM). The gut–retina axis could be a potential target for retardation of diabetic retinopathy (DR), a known complication of DM. This study reviews the evidence suggesting gut dysbiosis in DR.

**METHODS.** The published literature in the past 5 years was reviewed using predetermined keywords and articles. The review intended to determine changes in gut microbiome in DR, the hypothesized mechanisms linking to the gut–retina axis, its predictive potential for progression of DR, and the possible therapeutic targets.

**RESULTS.** The gut microbiota of people with DM differ from those without it, and the gut microbiota of people with DR differ from those without it. The difference is more significant in the former (DM versus no DM) and less significant in the latter (DM without DR versus DM with DR). Early research has suggested mechanisms of the gut–retina axis, but these are not different from known changes in the gut microbiome of people with DM. The current evidence on the predictive value of the gut microbiome in the occurrence and progression of DR is low. Therapeutic avenues targeting the gut–retina axis include lifestyle changes, pharmacologic inhibitors, probiotics, and fecal microbiota transplantation.

**CONCLUSIONS.** Investigating the therapeutic utility of the gut ecosystem for DM and its complications like DR is an emerging area of research. The gut–retina axis could be a target for retardation of DR but needs longitudinal regional studies adjusting for dietary habits.

Keywords: diabetes mellitus, diabetic retinopathy, gut microbiome, gut–retina axis, prevention of blindness

Diabetes mellitus (DM) is estimated to affect about 463 million people worldwide. It is likely to increase by nearly 50% in the coming quarter of the century.<sup>1</sup> Diabetic retinopathy (DR) is the most common microvascular complication of DM. As point prevalence, about 35% of people globally with DM have DR, a third of which may be vision threatening.<sup>2</sup> DR has emerged as a leading cause of visual impairment in working-age people in many regions of the world. In the past three decades, the prevalence of disability secondary to DM has increased by 146% (higher than other chronic diseases).<sup>3</sup> Also, by the Global Burden of Disease estimate, the crude prevalence of DR-related blindness is increasing while blindness due to all other causes has decreased due to international efforts.<sup>4</sup> DR has a very long latent period for development in type 2 DM (T2DM), typically exceeding 5 to 10 years, and progression to vision-threatening DR (VTDR) is even longer.<sup>5</sup> Given the very high incidence of VTDR, often despite intensive control of DM,

its prevention in incipient stages is prudent.<sup>5</sup> Gut dysbiosis is a potential target of such prevention.

The human gut microbiota consists of at least 1500 different microbial species. The gut bacterial ecosystem is a dynamic community of inhabitant bacteria, and dysbiosis (changes in abundance, diversity, and function) of this gut microbiome has been associated with DM and several autoimmune and inflammatory diseases. Its association with ophthalmic disorders such as Sjögren syndrome, uveitis, age-related macular degeneration, and infective keratitis has been established.<sup>6–14</sup> Previous studies have indicated that the onset of type 1 DM (T1DM) is preceded by an increase in inflammation-associated microorganisms in the gut.<sup>15–26</sup> Tetz et al.<sup>27</sup> reported activation of pathways leading to a prediabetic state in children susceptible to T1DM due to *Escherichia coli* that produce amyloid. Zhao et al.<sup>28</sup> indicated that viromal changes in the intestine precede autoimmune changes in the gut of children at risk for T1DM.

Such studies have indicated the role of gut microbiota in causing a permeable gut barrier, altered inflammatory cascades, altered glucose metabolism, and insulin resistance that impacts retinal neurons.<sup>18–20</sup> Despite this gross overlap with mechanisms that cause DR, the evidence connecting gut dysbiosis to DR is inconclusive.

In 2018, murine model experiments revealed that behavioral interventions could restructure the gut microbiome.<sup>29</sup> In continuation, the retinal targets and neural pathways were successfully tested for their links between the gut and the retina, thus confirming the existence of the gut–retina axis in DR.<sup>26,30–36</sup> Furthermore, clinical studies by our author group have documented variations in the gut microbiome and mycobomes of healthy individuals and people with DM and those with and without DR.<sup>30,31</sup> These changes were later confirmed by other studies too.<sup>26,32,33</sup> This review summarizes the evidence generated thus far supporting the role of gut dysbiosis in DR. We discuss the research gaps and suggest further courses necessary before human trials can be initiated to evaluate the manipulation of the gut–retina axis for the prevention of DR.

## GUT MICROBIOME IN DM

Several microorganisms have been identified in the gut of people with DM, some of these in incremental proportion and some of these associated negatively. The abundance of the bacteria has been an outcome measure in most studies. In a systemic review of 42 publications, Gurung et al.<sup>34</sup> found *Bacteroides* and *Bifidobacterium* were more often identified across these studies, but the  $\alpha$  and  $\beta$  diversity were not associated with T2DM. The  $\alpha$  diversity, representative of intraindividual microbiome diversity, was defined using the Chao1, Shannon, and Simpson indices by the authors, and  $\beta$  diversity represented interindividual differences in the microbiome. In many studies, the ratio of *Bacteroides* to Firmicutes is counted; some authors did not find any preferential equation, positive or negative, in their review.<sup>34,37,38</sup> Bhute et al.<sup>39</sup> have reported different microbiomes in newly diagnosed and “known” people with DM. Pandolfi et al.<sup>40</sup> have shown the link between Firmicutes, obesity, and diabetes. Many inconsistencies in the abundance of some of the associated microbes are probably related to the impact of oral hypoglycemic agents and variable immunity.<sup>30,41</sup> This is further compounded in the presence of hyper-abundant bacteria like *Lactobacillus* in the gut with many species and stains, each possibly impacting differently. Bacterial dysbiosis of the gut has also been a target of therapy for DM. Oral administration of *Bifidobacterium* to diabetic mice led to an increase in the expression of proteins involved in the insulin signaling pathway and reduced serum glucose level in diabetic mice.<sup>42</sup> This has been shown in several other preclinical studies.<sup>43–46</sup> Apart from bacteria, some authors established an association of pathogenic viral infection (particularly enterovirus, rotavirus, cytomegalovirus, and norovirus) with T1DM.<sup>47–53</sup>

At least four mechanisms have been suggested to explain the relationship between DM and the gut. First, inflammation is a significant pathophysiologic phenomenon. End products of microbial metabolism such as lipopolysaccharides (LPSs) cause endotoxemia and promote inflammation, whereas the production of other molecules may improve insulin resistance.<sup>54</sup> *Lactobacillus*, *Bacteroides*, *Roseburia*, and *Faecalibacterium* are known to downregulate proinflammatory cytokines in the gut.<sup>34</sup> Butyrate production by the latter two

is responsible for reducing nuclear factor- $\kappa$ B, a well-known transcription factor involved in multiple inflammatory cascades manifesting in clinical signs of DR.<sup>55</sup> *Bacteroides* and *Akkermansia* can upregulate the tight junction of the intestinal epithelium, resulting in lesser endotoxaemia.<sup>55</sup> Second, various species of *Bacteroides* and *Lactobacillus* can increase glucose uptake; these microbes possess bile salt hydrolases and produce secondary bile acids (BAs) that may be neuroprotective. Third, *Lactobacillus* and *Akkermansia* can decrease carbohydrate metabolism, resulting in lesser hyperglycaemia.<sup>34,55</sup> Fourth, many probiotic bacteria can induce fatty acid metabolism, reducing obesity.

These investigations on the gut microbiome in DM have led to research into the interactions between antidiabetic drugs and the gut microbiome. Furthermore, there are many challenges in humanizing this therapy as the local culture, dietary habits, individual immunity, and health status influence the gut microbiome. Evidence has increased, and randomized trials have shown that standardized fecal transplants can diminish insulin resistance.<sup>56,57</sup>

## DR AND THE GUT–RETINA AXIS

Gut dysbiosis is less explored in DR than in DM. In an experimental study of diabetic mice, Beli et al.<sup>29</sup> reported restructuring of the mice gut microbiome with an increase in Firmicutes after intermittent fasting. This resulted in an increase in tauroursodeoxycholate (TUDCA), known to stimulate retinal ganglion cells and act as a neuroprotective agent. In another arm of the study, the authors reported stimulation of the TUDCA receptor in retinal cells by another molecule (INT-767), known to be protective against DR.<sup>29</sup> Our author group studied gut dysbiosis in DM-induced rats. We found that the microbiome in the control rats was different from that of the diabetic rats, and there were overlaps between the microbiomes of the diabetic rats with or without retinopathy (Table 1).<sup>2</sup>

In our clinical study of gut bacteria in people with DM and DR, we observed dysbiosis in the gut at phylum and genus levels, in T2DM and DR compared to the healthy human controls.<sup>30</sup> Four phyla (Firmicutes, Actinobacteria, Bacteroidetes, Proteobacteria) were the predominant microbiomes. The  $\alpha$  diversity demonstrated a significant difference in the gut microbiome of healthy controls and people with T2DM but did not significantly differ between people with T2DM with and without DR. On evaluating  $\beta$  diversity, two major phyla (*Actinobacteria* and *Bacteroidetes*) were significantly less in DR than in controls.<sup>30</sup> Thus, gut microbiome of the DR cohort differed from that of the controls and T2DM at the phyla level. Compared to the controls, there was a significant reduction of 10 genera in T2DM and 20 genera in DR (Table 2, Fig. 1). We linked increased inflammation in DR to a reduced abundance of anti-inflammatory bacteria. An increase in abundance of only a single proinflammatory bacterium (*Shigella*) was demonstrated in our study in DR.<sup>30</sup> The study also demonstrated a decrease in the relative abundance of two probiotic bacteria (*Bifidobacterium* and *Lactobacillus*). Hence, we concluded that DM and DR changes could be attributed to an altered balance between proinflammatory, anti-inflammatory, and pathogenic gut bacteria. We did not identify any effect of the duration of DM on gut dysbiosis.

Our study of gut mycobome in people with DM with and without DR<sup>31</sup> showed a significant reduction of Mucoromycota in DR compared to the controls. While there were

TABLE 1. Studies Evaluating Gut Microbiome in Diabetic Retinopathy

Study No.	Author (Publication Year), Study Type, and Place	Methods	Major Results	Conclusions
1.	Beli et al. <sup>29</sup> (2018) Preclinical study Sample size—not specified	Two groups of mice, homogeneous (diabetic) and heterogeneous (control), were obtained. Diabetic mice were subjected to intermittent fasting and another set was on ad libitum diet. Microbiome analysis: Fecal samples of mice were collected every 4 hours during the time span of 48 hours. Retinal evaluation: Morphometric analysis, acellular capillary analysis, peptidoglycan estimation from blood plasma, BA analysis, RT-PCR of mouse retinal tissue, and immunofluorescence staining of retina cross section were done.	(a) No significant change in glycated hemoglobin was noted. (b) Significant longevity and reduction in DR changes were observed in <i>db/db</i> mice on IF. (c) Microbial analysis demonstrated increased abundance of Firmicutes and decreased abundance of Bacteroidetes and Verrucomicrobia. (d) Colon morphometric analysis revealed increased gut mucin, number of goblet cells, and an increase in villi length in <i>db/db</i> mice on fasting as compared to <i>db/db</i> mice on ad libitum feeding. (e) Plasma peptidoglycan was reduced in the IF group. (f) Significant increase in TUDCA (neuroprotective agent) was noted in <i>db/db</i> mice on IF. (g) Reduction in TNF $\alpha$ mRNA, which serves as downstream target of TGR5, was noted. INT-767 was noted to prevent DR by pharmacologic activation of TGR5 in a second model of diabetic mice.	IF plays a significant role in prevention of DR as it restructures gut microbiota in a fashion that increases species producing TUDCA and therefore subsequent neuroprotective effect is exerted by increased TGR5 activation. IF can act as a promising pathway for increased neuroprotective TUDCA production.
2.	Jayasudha et al. <sup>31</sup> (2020) Clinical study sample size—75 South India	Fecal samples (300 mg) were collected from participants of all three cohorts (total 75 individuals). Healthy control (30), T2DM (21), and DR (24) individuals were recruited.	(a) Dysbiotic changes were significantly noted in T2DM and DR in comparison of HC. (b) The $\alpha$ diversity demonstrated significant reduction in DR Mycobiomes. (c) Abundance of <i>Candida</i> was noted in T1DM and T2DM. Six genera decreased exclusively in DR ( <i>Aspergillus</i> , <i>Diutina</i> , <i>Pseudogymnoascus</i> , <i>Cladorrhinum</i> , <i>Kazachstania</i> , and <i>Oliveonia</i> ). (d) None of the genera demonstrated increased abundance in DR. (e) Increase/decrease in pathogen along with decrease in commensal was noted in T2DM. However, only decrease in pathogen was observed in DR.	First study to demonstrate differences in gut mycobiome at phylum and genera level in DM and DR
3.	Das et al. <sup>30</sup> (2021) Clinical study sample size—83 South India	Fecal samples were collected from participants of all three cohorts. Total of 83 individuals (HC, 30; T2DM, 25; DR, 28) were recruited.	The $\alpha$ diversity demonstrated a significant difference in gut microbiome of HC and T2DM. No significant difference in $\alpha$ diversity was noted in T2DM and DR. $\beta$ diversity: Two major phyla (Actinobacteria and Bacteroidetes) were significantly less in DR in comparison with HC. <u>Abundance</u> : Several genera were significantly decreased in T2DM (10 genera) and DR (20 genera) in comparison with HC.	Dysbiosis was confirmed in T2DR as compared to HC. No significant difference at genera level was noted in DM and DR. Gut microbiome of DR patients shows reduction in anti-inflammatory, pathogenic, and probiotic bacteria.
4.	Huang et al. <sup>32</sup> (2021) Clinical study Sample size—75 China	Clinical information and fecal samples were collected from 75 participants (HC, 25; DM, 25; DR, 25).	(a) Both $\alpha$ and $\beta$ diversity were significantly reduced in DM and DR groups as compared to HC group. (b) Most abundant genus observed was <i>Blautia</i> (especially in T2DM). (c) Decrease in probiotic bacteria (mainly two, <i>Lactobacillus</i> and <i>Bifidobacterium</i> ) was also noted in DR. (d) Significant increase in level of <i>Bifidobacterium</i> and <i>Lactobacillus</i> and decrease in abundance of <i>Faecalibacterium</i> , <i>Escherichia</i> , <i>Sbigella</i> , <i>Eubacterium</i> , and <i>Clostridium</i> were noted in DM and DR groups as compared to HC group. (e) Twenty-five bacterial families were identified as a biomarker set for distinguishing DR from DM and HC. Pasteurellaceae was identified as an independent predictive biomarker to differentiate DM from DR.	Gut microbiota data can be used as a noninvasive biomarker for diagnosing cases of DM and DR in the future.

TABLE 1. Continued

Author (Publication Year), Study No.	Study Type, and Place	Methods	Major Results	Conclusions
5. Khan et al. <sup>33</sup> (2021)	Clinical study Sample size—58 South India	An association was studied between sight-threatening DR and gut microbial abundance in T2DM. Sample size—58 Cases—37 were diagnosed with sight-threatening DR (CSME/PDR) Controls—21 with history of diabetes for past 10 years and no diagnosis of DR Fecal sample was collected using fecal swabs.	(a) No significant difference was noted in the microbiome abundance of two groups at the phylum level. (b) Overall, most common phyla in two groups: Bacteroidetes and Firmicutes. Least common: Proteobacteria and Actinobacteria (c) B/F ratio was increased in cases as compared to control in univariate analysis. Optimal cutoff value determined for B/F ratio was 1.05.	B/F ratio serves as a significant biomarker in the differentiation of patients with and without sight-threatening DR.
6. Padakandla et al. <sup>26</sup> (2021)	Preclinical study sample size—48	Gut bacterial microbiome of the Sprague Dawley rats in which diabetes was induced. Total of 48 rats were recruited, which included 24 in the control arm and 12 each in DM and DR cohorts. Histology and immunochemistry of retinal section were done to note the progression of DR changes.	(a) $\alpha$ diversity: Differentially abundant genera forming separate cluster of microbiomes were noted in all healthy diabetic and mice with retinal changes. (b) $\beta$ diversity analysis differentiated microbiome of control rats from DM and rats with DR changes. However, an overlap was noted in the microbiome of DM rats and rats with DR changes. (c) Diabetic rats: Most abundant phyla—Firmicutes and Bacteroidetes; ratio: increased in comparison of DM1 to control rats (d) Rats with retinal changes showed decrease in 12 and increase in 4 genera in DM1 and decrease in 8 and increase in 5 genera in DM2 group compared to control rats.	Decreasing trend was observed in anti-inflammatory bacteria and increased trend was noted in pathogenic and proinflammatory bacteria.

CSME, clinically significant macular edema; HC, healthy control; IF, intermittent fasting; PDR, proliferative diabetic retinopathy; DM1 refers to rats sacrificed at 1 month, DM2 refers to rats sacrificed at 2 months of DM induction.

differences between DM and controls, the gut mycobiome changes were overlapping in individuals with or without DR. An increase in four additional genera was noted in T2DM (*Cladosporium*, *Kodamaea*, *Meyerozyma*, and *Mortierella*). The  $\alpha$  diversity was significantly reduced in the DR group. Eighteen genera showed a significant reduction in DR compared to controls with an overlap of 12 genera in T2DM; 6 genera decreased exclusively in DR (*Aspergillus*, *Diutina*, *Pseudogymnoascus*, *Cladorrhinum*, *Kazachstania*, and *Oliveonia*). None of the genera demonstrated increased abundance in DR. Mycobiomes were thus significantly discriminated in between three cohorts. Lesser pathogenic fungi (human/plant) were present in the controls than in the other two groups. Based on our observations of a decrease in anti-inflammatory commensal gut bacteria and fungi, we inferred that inflammation probably incites DR.

Gut dysbiosis can be “personal” and vary from individual to individual. In 2021, Huang et al.<sup>32</sup> reported a decrease in individual-level  $\alpha$  and  $\beta$  diversity in people with DM and DR than in the controls, but the difference between people with DM (without DR) and people with DR was less obvious. The abundance of bacteria differed in DM and DR. The most abundant genus was *Blautia* in people with T2DM. A significant increase in the level of *Bifidobacterium* and *Lactobacillus* and a decrease in abundance of *Faecalibacterium*, *Escherichia*, *Shigella*, *Eubacterium*, and *Clostridium* were noted in DM and DR groups than in the control group. These changes indicated higher pathologic and complex diversity

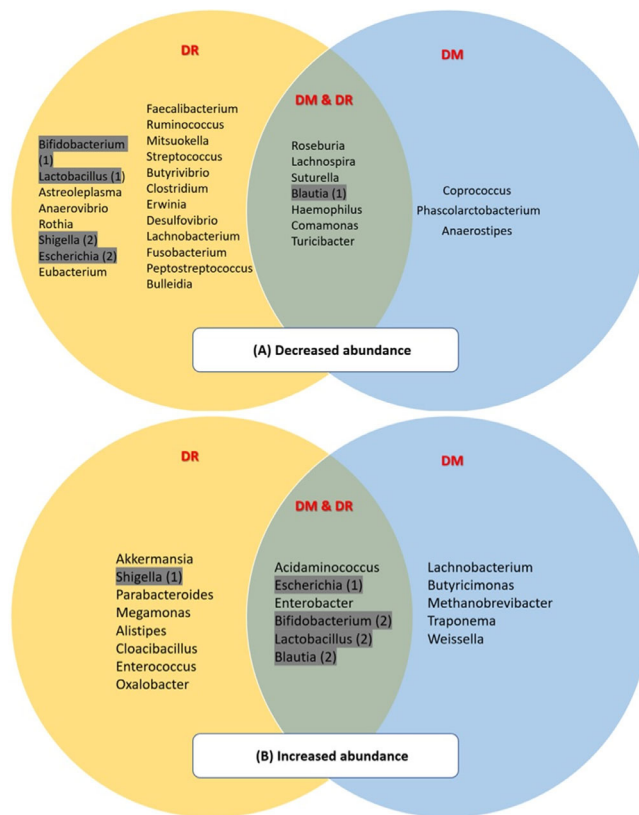
in people with diabetes. We have also noted similar changes in our studies. But another study from South India that examined the gut microbiome of people with at least 10 years of T2DM did not find any of these changes. These authors employed fecal swabs for sample collection and reported a lack of difference in the relative abundance of different phyla in patients with or without VTDR (clinically significant macular edema and proliferative diabetic retinopathy).<sup>33</sup> In this study, Bacteroidetes and Firmicutes were the most common microbes; Proteobacteria and Actinobacteria were the least common microbes.

The six studies we have cited in this review<sup>26,29–33</sup> have indicated a significant difference in the gut micro-ecosystem between the controls and people with DM, but the difference is less compelling between DM and DR. We have summarized the chief findings in Tables 1 and 2. Beli et al.<sup>29</sup> have indicated neurodegeneration as a possible link in the gut–retina axis; hence, evaluating the gut dysbiosis in the context of diabetic retinal degeneration in the absence of clinical DR is an important question. There is a variation in the distribution of severity of DR in the clinical studies we discussed. While our studies included fewer patients with early DR (10.8% in the microbiome study and 8% in the mycobiome study), the study by Huang et al.<sup>32</sup> included 33% and the study by Khan et al.<sup>33</sup> included 36% of patients with early DR. The diagnosis of DR was based on histopathology in our preclinical study on diabetic mice. One should also evaluate the changes in the neural stages of DR and early clinical DR

TABLE 2. Comparison Between Healthy Controls (HCs) and Patients With DR for Relative Abundance in Gut Microbiome in the Published Human Studies

Study No.	Bacteria	Studies Showing Increased Prevalence in DR Compared to HCs		Studies Showing Decreased Prevalence in DR Compared to HCs		Remarks and Comparison With DM Individuals Without DR
		DR Compared to HCs	DR Compared to HCs	DR Compared to HCs	DR	
	<b>PHYLUM</b>					
1	Bacteroidetes	Khan et al.	Huang et al.	Das et al.		B/F ratio important biomarker for sight-threatening DR
2	Firmicutes	Khan et al.		Huang et al.		
3	Proteobacteria	Khan et al.				
4	Actinobacteria	Khan et al.		Das et al.		
	<b>FAMILY</b>					
	Verrucomicrobia	Das et al.				
	<b>PASTEURRELLACEAE</b>					
1	Pasteurellaceae			Huang et al.		Increased in DM and decreased in DR
	<b>GENUS</b>					
1.	<i>Faecalibacterium</i>			Huang et al.	Das et al.	
2.	<i>Bifidobacterium</i>	Huang et al.		Huang et al.	Das et al.	Decrease in DR as compared to DM also (Das et al.)
3.	<i>Lactobacillus</i>	Huang et al.		Das et al.		Unique hub in DR (Das et al.)
4.	<i>Escherichia</i>	Das et al.		Huang et al.		
5	<i>Eubacterium</i>			Huang et al.		Highest relative abundance compared to DM and HC
6	<i>Clostridium</i>			Huang et al.	Das et al.	
7	<i>Peptostreptococcus</i>			Huang et al.		
8	<i>Roseburia</i>			Das et al.		
9	<i>Lachnospira</i>			Das et al.		
10	<i>Mitsuobella</i>			Das et al.		Decrease in DR as compared to DM also (Das et al.)
11	<i>Streptococcus</i>			Das et al.		Decrease in DR as compared to DM also (Das et al.)
12	<i>Sutterella</i>			Das et al.		Unique hub in DR (Das et al.)
13	<i>Haemophilus</i>			Das et al.		Decrease in DR as compared to DM also (Das et al.)
14	<i>Blautia</i>			Das et al.		
15	<i>Ervinia</i>			Das et al.		Decrease in DR as compared to DM also (Das et al.)
16	<i>Desulfovibrio</i>			Das et al.		Decrease in DR as compared to DM also (Das et al.)
17	<i>Bulleidia</i>			Das et al.		
18	<i>Butyrivibrio</i>			Das et al.		
19	<i>Aseroleplasma</i>			Das et al.		Decrease in DR as compared to DM also (Das et al.)
20	<i>Anaerovibrio</i>			Das et al.		Decrease in DR as compared to DM also (Das et al.)
21	<i>Comamonas</i>			Das et al.		
22	<i>Rothia</i>			Das et al.		
23	<i>Turicibacter</i>			Das et al.		Increase in DR as compared to DM also (Das et al.)
24	<i>Abkermansia</i>					
25	<i>Parabacteroides</i>	Das et al.				
26	<i>Megamonas</i>	Das et al.				
27	<i>Acidaminococcus</i>	Das et al.				
28	<i>Escherichia</i>	Das et al.				
29	<i>Alistipes</i>	Das et al.		Huang et al.		Increase in DR as compared to DM also (Das et al.)
30	<i>Enterobacter</i>	Das et al.				
31	<i>Cloacibacillus</i>	Das et al.				Increase in DR as compared to DM also (Das et al.)
32	<i>Enterococcus</i>	Das et al.				Increase in DR as compared to DM also (Das et al.)
33	<i>Oxalobacter</i>	Das et al.				
34	<i>Shigella</i>	Das et al.		Huang et al.		Increase in DR as compared to DM also (Das et al.)
35	<i>Klebsiella</i>	—		—		Decrease in DR as compared to DM (Das et al.)
36	<i>Lachnobacterium</i>	Das et al.		Das et al.		Decrease in DR as compared to DM also (Das et al.)
37	<i>Wissella</i>	Das et al.		Das et al.		Decrease in DR as compared to DM (Das et al.)
38	<i>Phascolarctobacterium</i>					Increase in DR as compared to DM also (Das et al.)
39	<i>Ruminococcus</i>	Das et al.		Das et al.		

Das et al.: reference number 30; Huang et al.: reference number 32; Khan et al.: reference number 3.



**FIGURE 1.** Venn diagrams depicting differences in abundance of gut microbiome at genera levels in DM and DR in comparison to healthy human controls. **(A)** Reduced abundance. **(B)** Increased abundance. The highlighted organisms indicate differences in findings of studies, where one study showed increased while the other showed decreased. Citation (1) refers to a study by Das et al.<sup>30</sup> and (2) refers to a study by Huang et al.<sup>32</sup>

and compare them to VTDR to make a definite conclusion. This will widen the perspective on the role of gut dysbiosis in DR. Furthermore, a cohort model study evaluating people with DM without “investigative/neural DR” is equally desirable.

## MECHANISMS LINKING THE GUT–RETINA AXIS IN DR

The following mechanisms could explain our current knowledge of the gut–retina axis in DR (Fig. 2). Many of these mechanisms overlap between the causation of DM and DR.

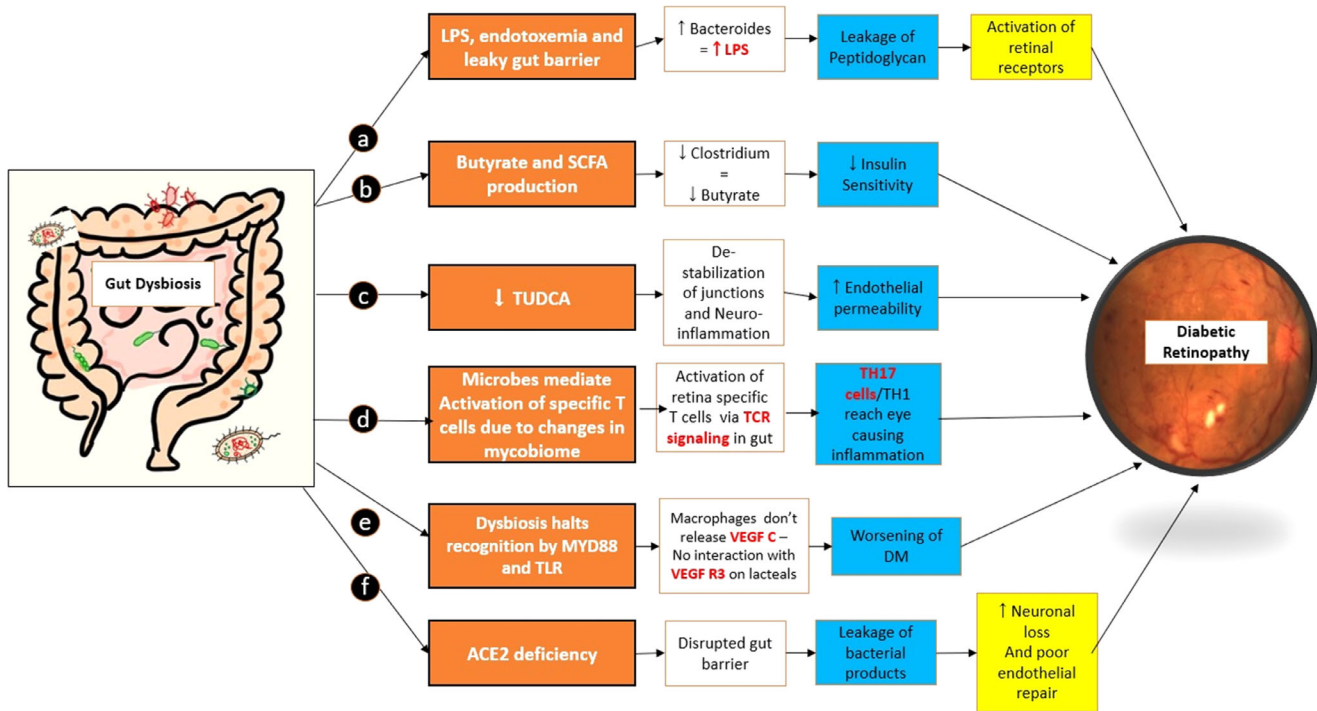
1. **LPS, endotoxemia, and leaky gut barrier:** An increase of Bacteroides is common in the guts of people with DM and DR.<sup>30,33,58</sup> The cell wall of this bacteria is rich in LPS. It has been shown that repeated exposure to systemic LPS in hyperglycemic mice leads to a 3.5-fold increase in endothelial cell injury.<sup>54</sup> Optical coherence tomography of these mice showed progressive retinal thinning.<sup>54</sup> It is thus hypothesized that leakage of peptidoglycan through a permeable gut barrier in DM activates the receptors in the retina later, leading to leakage in the retina. Furthermore, Huang et al.<sup>32</sup> reported higher Desulfovibrio in people with DR than people with DM and ascribed its butyrate-lysis activity as possibly linked to the LPS release, thus

hypothesizing it as the initial point of LPS release in the leaky diabetic gut.

2. **Butyrate and short-chain fatty acid production:** Huang et al.<sup>32</sup> also hypothesize that reduction in *Clostridium* and other bacteria reduces butyrate production. Butyrate is produced by lysis and fermentation of carbohydrates. Butyrate has a regulatory role in insulin sensitivity too, and thus its reduction can be implicated to increase DM and DR as a later consequence as well because insulin resistance is a known risk factor for DR.<sup>30,55</sup>
3. **TUDCA:** *Lactobacillus* increases TUDCA in the gut in DM and impacts the dendritic receptors.<sup>59</sup> Increased endothelial permeability is a known consequence of this pathway. Twin studies by Beli et al.<sup>29</sup> have shown the role of TUDCA and its interaction with the TGR5 (G-protein coupled bile acid) receptor in retinal ganglion cells to be protective for DR. They have even evaluated and proposed a pharmacologic targeting of this receptor in the retina as an endpoint on the gut–retina axis.
4. **Uveitis-like inflammation:** Nakamura et al.<sup>60</sup> stated a microbial difference in antibiotic-treated mice, which protected them from uveitis, suggesting a role of the gut biome in uveitis. T-helper 1 (Th1) and Th17 lymphocytes are important subsets of immune cells that contribute to inflammatory ophthalmic conditions. It is postulated that intestinal dysbiosis affects DR like the gut microbiome and uveitis.<sup>31</sup> The precise component of uveitis-related possible gut microbiome to retina axis is not very clear yet with regard to DR.
5. **Modulation of VEGF:** Dysbiosis may play a role in DR by modulation of vascular endothelial growth factor (VEGF). Suh et al.<sup>61</sup> have stated that intestinal villus macrophages secrete VEGF-C upon recognition of microbes. Hence, gut microbiota plays an important role by regulating villus macrophages in the small intestine to produce VEGF locally in the gut of individuals with DM. The correlation of circulating serum VEGF with DR is well known, and VEGF is also central to its therapy, thus completing another possible loop of the gut–retina axis in DR.
6. **Angiotensin-converting enzyme (ACE2) deficiency:** In type 1 diabetic mice, ACE2 deficiency promotes disruption of gut barrier integrity and results in the leakage of bacterial products into the circulation. ACE2-deficient diabetic mice within the gut parenchyma displayed reduced myeloid angiogenic cells (MACs) without a concomitant increase in inflammatory monocytes and hence the lack of gut barrier repair mechanisms, thus supporting the pathogenic role of ACE2 deficiency locally in the diabetic gut. Exogenous administration of MACs restored gut epithelial and vascular barriers and beneficially altered the microbiome by decreasing the genes associated with peptidoglycan biosynthesis.<sup>62,63</sup> Thus, there seems to be a link between the local ACE2 down-regulation and gut microbiome in DM, which can be an important component of the gut–retina axis.

## GUT MARKERS OF DR TOWARD PREDICTION POTENTIAL

The results of two clinical studies<sup>32,33</sup> have suggested the utility of gut microbiota as a noninvasive marker of DR.



**FIGURE 2.** The links between gut–retina axis and its components in DR. The image describes the various possible mechanisms linking the gut to retina in DR, as well as the components of each hypothetical gut–retina axis. The six axes revolve around LPS and leaky gut barrier, short-chain fatty acid production, modulation of VEGF, gut inflammatory changes, TUDCA, and ACE2 deficiency.

Huang et al.<sup>32</sup> have identified 25 families of bacteria that can be potentially employed for differentiating people with and without DR. Using a random forest model, the area under curve (AUC), and the receiver operator curve, they reported that AUC was nearly 0.7 for differentiating DR from DM and about 0.8 for differentiating DR from the controls. In three families of bacteria that could distinguish between these categories, Pasteurellaceae had the highest AUC for discriminating between DR and DM (nearly 0.75).

Khan et al.<sup>33</sup> used Bacteroidetes to Firmicutes (B/F) ratio to predict the development of VTDR. Utilizing the Bray Curtis coordinates analysis, they identified a positive correlation between elevated B/F ratio and VTDR. In their study, the VTDR had a higher B/F ratio at a cutoff of 1.05, with a sensitivity and specificity of nearly 60%.<sup>33</sup> Thus, there is a predictive potential for utilizing the gut flora changes as a predictor of DR and VTDR. What remains to be seen is whether these changes parallel the development of DR or occur independently. These predictive models will also be prone to regional dietary and microbial flora variations.<sup>64</sup>

### HYPOTHETICAL THERAPEUTIC STRATEGIES

On the basis of the early results discussed by us, it cannot be affirmed whether the gut dysbiosis is directly causative of DR; rather, a possible role of gut dysbiosis in the progression of DR may be concluded. While further clinical studies, especially longitudinal ones, will focus to answer these questions with higher precision, certain hypothetical strategies may be considered that could reduce the occurrence of DR or its progression. Lifestyle changes like intermittent fasting restructured the gut microbiome in the experimental murine model by Beli et al.<sup>29</sup> In the gut, Firmicutes is increased

in abundance with intermittent fasting, which metabolizes primary bile acids to produce secondary BAs and results in an increased production of TUDCA.<sup>29</sup> TUDCA can relieve oxidative stress and improve phagocytosis.<sup>65</sup> Lawson et al.<sup>66</sup> report that injections of TUDCA can prevent damage to retinal function and its architecture. This is particularly true for preventing damage to photoreceptors in the detached retina that can occur in a subset of people with diabetic macular edema (DME).<sup>67</sup> TUDCA also improves channelization (differentiation) and homing (mobility) of hematogenic stem cells for endothelial repair.<sup>68</sup> It is also hypothesized that TUDCA acting on the TGR5 receptor of retinal ganglion cells can be an important link in the gut–retina axis, resulting in neurodegeneration. In addition, pharmacologic activation of TGR5 is shown to prevent DR in mice.<sup>29</sup> For these reasons, TUDCA and its pathways appear to be a possible pharmacologic target for controlling DR through the gut–retina axis.

### Prebiotics, Probiotics, Antibiotics, and Fecal Transplants

Considering the changes in the gut microbiome, we noted in our three studies and those reported by Huang et al.<sup>32</sup> that there is evidence supporting gut flora as a possible target for manipulation and control of DR.<sup>26,30–33</sup> This hypothesis stems from the early success of experiments with objectives to controlling DM through the gut microbiota. For example, the transmissibility of obesity or adiposity has been demonstrated in murine models through fecal transplantation.<sup>69</sup> In a randomized trial, Vrieze et al.<sup>70</sup> reported small intestinal infusions of allogenic (lean donors) gut bacteria to result in higher levels of butyrate-producing bacteria and

improved insulin resistance compared to the subjects who had received autologous infusions. Similarly, other authors have reported fecal microbial transplantation to preserve insulin production, linking several bacteria and consequent metabolites to this beneficial action.<sup>71,72</sup> While we lack such human studies for DR, the murine model studies by Beli et al.,<sup>29</sup> later summarized by Floyd and Grant,<sup>73</sup> indicate the promising potential for human trials.

## SUMMARY

The gut–retina axis has immense potential and must be exploited for retardation of DR. Arguably, there is an overlap between DM and DR for this benefit, and the roots of the protection may lie in improvement in DM itself. This is highlighted by the commonalities we have identified earlier in this review. Others and we have documented differences in the gut microbiome of controls and people with DM, as well as DM individuals with and without DR, although the differences are less in the latter groups. A longitudinal model study evaluating gut biomes of DM individuals without DR would prove helpful.

Gut microflora may support a predictive model for the development of DR or VTDR. Such models may be based on clusters of families of individual flora or their relative proportions. However, currently, only moderate validity exists with such tests. Therapy for DR based on the gut–retina axis has not been evaluated yet in humans, but some hypothetical strategies based on lifestyle or pharmacotherapeutics may have a role, given the early results and experience with DM itself.

## CHALLENGES AND THE WAY FORWARD

The local gut–environmental components, including nonspecific and specific host factors, influence the status of the gut microbiome and impact the local gut immunity. Other influencing factors include antibiotic usage, antidiabetic medication, diet, season, geography, ethnicity, and age of individuals.<sup>74</sup> Similarly, DM and DR are complex diseases that depend on a large number of variables and factors including genomics and lifestyle factors such as diet, smoking, and physical activity. Other comorbidities also modulate the disease progression, which also varies in different stages of the disease.<sup>75</sup> All these factors must be weighed in future evaluations of the gut microbiome vis-à-vis DM and DR.

The available evidence is either from a murine model or extrapolated from cross-sectional studies on people with DM. Furthermore, the human studies come from a limited geographic area of the world. The studies by our group<sup>30</sup> and Khan et al.<sup>33</sup> involved South Indian participants, while Huang et al.<sup>32</sup> included Chinese subjects. Thus, although the approach is promising, worldwide data are needed as the microbiome is different the world over, confounded by geographic, ethnic, genomic, lifestyle, and dietary factors. Global differences in the microbiome may be responsible for the global differences in presentations and outcomes in DR too. Long-term studies with longitudinal follow-up for DR and gut dysbiosis are needed in all regions of the world, as further therapeutic strategies would need such a region-specific database for a region-specific therapy. Personalized medication adjusted for an individual specific therapy can be the other way to develop strategy.

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