**Gut Microbiome and Kidney Disease**

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<td>Shankaranarayanan, Divya; The George Washington University School of Medicine and Health Sciences, Division of Kidney Diseases and Hypertension  Raj, Dominic; The George Washington University School of Medicine and Health Sciences, Division of Kidney Diseases and Hypertension</td>
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Gut Microbiome and Kidney Disease:
Reconciling Optimism and Skepticism

Divya Shankaranarayanan MD and Dominic S. Raj MD
Division of Kidney Diseases and Hypertension
George Washington University School of Medicine
Washington, DC 20037

Address for communication
Dominic S Raj MD
Professor of Medicine and Chief
George Washington University School of Medicine
Washington DC 200037
Email: draj@mfa.gwu.edu
Phone: 202 741 2283
Fax: 202 741 2285
The Microbiome Hype

Integrative analyses of metagenomics, metatranscriptomics, metaproteomics and metabolomics have provided an unprecedented insight into physiological role of the human microbiome in health and disease. Studies employing genome-scale metabolic networks and Metagenome-Assembled Genomes reveal that several metabolic pathways in humans are the result of the combined activities of the human genome and microbiome. A recent study showed that gut microbiota associate with 38 self-reported common diseases and 51 medications. Changes in the gut microbiota could promote CKD progression through alterations in immune response, blood pressure regulation and metabolic changes. In this perspective we focus on the recent advances in the field of microbiome that is relevant to kidney disease.

Dysbiosis in CKD

The concepts of ‘niche partitioning’ and ‘functional redundancy’ are highly relevant to the shaping of microbiome in CKD. The former refers to the process by which competing species use the environment differently, permitting them to co-exist. Functional redundancy is a mechanism by which many phylogenetically unrelated taxa carry similar genes and perform similar functions. It is possible that CKD milieu results in loss of ‘key taxa’, shifting the community structure. The resulting dysbiosis drives CKD progression through generation of multitude of uremic toxins. Impaired protein digestion in CKD results in delivery of undigested protein to the colon fosters preferential proliferation of bacteria with urease and uricase enzymes and taxa involved in indole and phenol metabolism. Concomitant reduction in saccharolytic bacteria leads to reduced generation of short chain fatty acids, which are involved in energy homeostasis, maintaining gut barrier, blood pressure control and immune regulation.

Gut and Blood Pressure
Hypertension is an important risk factor for CKD progression. Disturbed gut microbiota and hypertension could be causally related. Experimental studies suggest T cell subsets such as T helper and Treg cells are involved in the regulation of blood pressure. High-salt diet caused depletion of *Lactobacillus murinus* in mice.\(^3\) Treatment of mice with *L. murinus* reduced TH17 cell numbers and prevented salt-sensitive hypertension.\(^3\) Reduced potassium consumption and low urinary potassium excretion are associated with increased risk for developing hypertension. A recent study showed that microbiome and host co-metabolism are altered by potassium.\(^4\)

Renin release from the afferent arteriole mediated by Olfr78 activated by short chain fatty acids is counteracted by the vasodilatory action of GPR43 expressed in major blood vessels. Interestingly, gut microbiota encodes several enzymes that influences the metabolism of xenobiotics that might impact the excretion, transport, and bioavailability of antihypertensive medications.

**Dysmetabolism in CKD**

Protein catabolism by gut microbiota is generally viewed as detrimental since it results in production of toxins such as ammonia, amines, phenols, indoles and sulfurous compounds, which accumulate in CKD. Gut microbiota converts tryptophan to indole and indole derivatives, such as indoxyl sulfate. Bacterial fermentation of aromatic amino acids tyrosine and phenylalanine generates phenolic compounds, such as p-cresol sulfate.

Dietary choline can be metabolized to trimethylamine by the microbiota, which is oxidized in the liver to trimethylamine N-oxide. Several studies have shown that trimethylamine N-oxide alters cholesterol transport, promotes formation of foam cells and exacerbates atherosclerosis. Members of *Lactobacilli, Bifidobacteria, and Clostridia* genera can deconjugate bile acids and convert them to secondary bile acids, including deoxycholic acid (DCA) and lithocholic acid.
DCA is elevated in CKD and is directly toxic to vascular smooth muscle cells. Mechanistic studies have shown that microbiome-derived indoles, phenols and amines could mediate CKD progression through glomerular and interstitial fibrosis.

It is becoming evident that elevated plasma levels of microbiome-derived uremic retention solutes in CKD cannot be fully explained by differences in bacterial generation rates alone.\(^5\) Retention of these solutes due to decreased tubular secretion and to a smaller extent reduced glomerular filtration contribute to accumulation of these molecules in CKD.\(^5\)

**Microbiome Therapeutics**

With the expanding knowledge of microbiome, recent efforts have sought to harness the power of microbiome for health benefit. (Figure 1) These therapeutics could be broadly classified as (a) supplementing the host microbiota with fecal transplantation, specific strains of microbiota or a consortium of natural or engineered microorganisms; (b) elimination of specific deleterious members of the microbiota using nonspecific or targeted antimicrobials such as bacteriocins and bacteriophages; (c) modulation of host microbiota by administration of agents such as prebiotics and (d) postbiotics that target downstream signaling pathways of microbiome.

Advances in orthogonal niche engineering in which uncommon/unused nutrients are employed to enable engraftment of therapeutic bacteria.

For a probiotic to be effective, the bacteria should be able to colonize, proliferate and be metabolically active in that environment. Furthermore, microbes are interdependent on each other for nutrients and signaling molecules, so the effective probiotic need supportive microbiome as well. These phenomena explain the mixed results seen with prebiotic and probiotic based interventions in patients with CKD.
Cardiovascular disease contributes to CKD progression and remains the leading cause of death in CKD patients. Researchers have explored several avenues to reduce trimethylamine N-oxide and stall the atherosclerotic process. Methanogenic archaea can use methylated amines such as TMA as growth substrates. Colonization of Apoe\(^{-/-}\) mice with \textit{M. smithii}, resulted in a sustained reduction in plasma trimethylamine N-oxide concentrations and a tendency for reduction in atherosclerosis.\(^6\) 3,3-dimethyl-1-butanol (DMB) is a structural analog of choline that inhibits trimethylamine lyases.\(^7\) DMB inhibits choline-induced endogenous macrophage foam cell formation and atherosclerotic lesion development in apoE\(^{-/-}\) mice.\(^7\) FMO3 is the rate limiting enzyme in the conversion of trimethylamine to trimethylamine N-oxide. Knockdown of FMO3 mice has been shown to attenuate atherosclerosis. Iodomethylcholine is a suicide substrate inhibitor, which selectively accumulate within gut microbes, reducing production of trimethylamine by inhibiting trimethylamine-lyase. In animal models, iodomethylcholine reduces kidney fibrosis and preserves kidney function.\(^8\)

Knowledge about the biochemical pathways in disease and microbiome has led to novel therapies. In the intestine, bacterial urease converts host-derived urea to ammonia and carbon dioxide, contributing to hyperammonemia. A consortium of 8 bacteria, with minimal urease gene content resulted in sustained reduction in ammonia production in antibiotic treated mice.\(^9\) Tryptophanase involved in conversion of tryptophan to indole is expressed by gut commensal \textit{Bacteroides}. Delvin et al showed that indole production could be inhibited by deleting the tryptophanases or eliminating the bacteria carrying the enzyme.\(^10\) Bacterial catabolism of the sulfur-containing amino acids produces hydrogen sulfide, which could function as an endogenous signaling molecule and a substrate for mitochondrial energization. High sulfur amino acid-containing diet resulted in post-translationally modified microbial tryptophanase activity and preservation of kidney function in a mouse model of CKD.\(^11\)
Advances in DNA technologies for manipulation of microbial genome has permitted scientist to engineer smart bacteria that could deliver therapeutic molecules and reprogram host cells by delivering transcription factors. However, safety and biocontainment remain major concerns that have not yet been fully addressed yet. In our quest for microbiome therapeutics, we need to be mindful of undesired propagation of genetically modified bacteria or genetic material into the ecosystem.

Concluding Remarks

The lack of large scale metagenomic data has greatly impeded progress in our understanding the role of microbiome in CKD. Existing evidence indicate that dysbiosis drives the production of many uremic retention solutes. The field of microbiome therapeutics is transitioning from prebiotic and probiotic to postbiotics. Employing bacteria as engineered therapeutics is a rapidly evolving field that is poised to transform the management of many chronic diseases, including CKD. As we strive to decipher the language of microbiome, a healthy skepticism is good, but we should be open to embrace true scientific discoveries and be prepared for future microbiome-based therapies.
Disclosures

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Author Contributions

Dominic S. Raj: Conceptualization, Funding acquisition, Visualization, Writing – original draft, Writing – review & editing
Divya Shankaranarayanan: Conceptualization, Visualization, Writing – original draft, Writing – review & editing
References


Figure 1 Legend

Microbiome-based therapeutics and their site of action. Fecal transplants could be least specific with large-scale changes in microbial community, while engineered bacteria are specific. Recent advances in our understanding of the molecular basis for disease and have enabled us to alter the function rather than changing the microbiome profile. TMA= Trimethylamine, TMAO= Trimethylamine N-oxide, SCFA= Short chain fatty acid, FM03=Flavin-Containing Monooxygenase 3, FM03 KO= FMO3 knock out, DMB= 3,3-dimethyl-1-butanol