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Role of the Gut Microbiome in Uremia: A Potential Therapeutic Target

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Abstract

Also known as the “second human genome,” the gut microbiome plays important roles in both the maintenance of health and the pathogenesis of disease. The symbiotic relationship between host and microbiome is disturbed due to proliferation of dysbiotic bacteria in patients with chronic kidney disease (CKD). Fermentation of protein and amino acids by gut bacteria generate excess amounts of potentially toxic compounds such as ammonia, amines, thiols, phenols, and indoles, but generation of short chain fatty acids is reduced. Impaired intestinal barrier function in CKD permits translocation of gut-derived uremic toxins into the systemic circulation, contributing to progression of CKD, cardiovascular disease, insulin resistance, and protein energy wasting. The field of microbiome research is still nascent, but evolving rapidly. Establishing symbiosis to treat uremic syndrome is a novel concept, but if proven effective will have significant impact on the management of patients with CKD.

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Index words

gut microbiome; uremic toxin; microbial metabolite; metabolome; ammonia; urea; amine; thiol; phenol; indole; p-cresol sulfate (PCS); uremic syndrome; chronic kidney disease (CKD); end-stage renal disease (ESRD); review

Keywords

Gut microbiome; chronic kidney disease; uremic toxins

BACKGROUND

Findings from the Human Microbiome Project (HMP) and the Metagenomics of the Human Intestinal Tract (Meta-HIT) project have shown that the human intestine is home to an extraordinarily complex and dynamic consortium of bacteria that play a pivotal role in human health and disease.^{1, 2} Bacteria have co-evolved with humans, and this symbiotic relationship has expanded our capabilities beyond what is coded in our own genome.³ Indeed, genetically, we are vastly outnumbered by our own *microbiome*, the microbial genome. As the Nobel Laureate Joshua Lederberg has asserted, “We should think of each host and its parasites as a superorganism with the respective genomes yoked into a chimera of sorts.”⁴ The central role of gut in human health has been long recognized, dating back to 400 B.C. when Hippocrates stated, “death sits in the bowels.”⁵ This review will provide an overview of the bidirectional relationship between chronic kidney disease (CKD) and the gut microbiome, discuss the consequence of gut dysbiosis in the pathogenesis of systemic inflammation and uremic toxicity, and highlight the recent advances in targeting gut microbiome for therapeutic purposes.

CASE VIGNETTE

A 65-year-old man with CKD stage G4 presented with lethargy and chronic constipation to the emergency room. Clinical examination was unremarkable except for generalized muscle weakness and distended abdomen with sluggish bowel sounds. Laboratory investigation showed a sodium level of 138 meq/L; potassium, 6.3 meq/L; chloride, 115 meq/L; bicarbonate, 16 meq/L; anion gap, 14; serum urea nitrogen, 60 mg/dl; serum creatinine, 3.8 mg/dl (corresponding to an estimated GFR of 16 ml/min/1.73 using the *** equation); glucose, 100 mg/dL; calcium, 8.1 mg/dL; phosphate, 7.1 mg/dL; albumin, 3.6 g/dL; WBC count, $8.1 \times 10^9/L$; and hemoglobin, 10.1 g/dL. Computed tomography (CT) of the brain was normal except for mild cortical atrophy. Abdominal CT showed abundant fecal matter in a dilated rectum and sigmoid colon. After disimpaction with enemas and laxatives, the patient felt better. He was discharged with recommendation to take laxatives on a regular basis.

He was seen in the outpatient clinic two months later. He appeared energetic and said that he was taking a prebiotic (*p*-inulin) and continuing the laxative when needed. Repeat laboratory evaluation showed a sodium level of 139 meq/L; potassium, 4.0 meq/L; chloride, 110 meq/L; bicarbonate, 20 meq/L; anion gap, 11; serum urea nitrogen, 51 mg/dl; serum

creatinine, 3.4 mg/dL (corresponding to an eGFR of 18 ml/min/1.73 m²); glucose, 82 mg/dL; calcium, 8.3 mg/dL; phosphate, 6.2 mg/dL; and albumin, 3.8 g/dL.

In the case presented, the patient's clinical symptoms and biochemistry improved with relief of constipation, possibly through decreased generation and increased elimination of uremic toxins. This highlights the importance of colon health in patients with CKD.

PATHOGENESIS

Gut microbiome in health

The human gut harbors ~10¹⁴ bacteria with an enormous metabolic potential.^{6–8} Under physiologic conditions, the microbiota provide complementary functions by participating in metabolic activities that are not fully evolved in the human host, such as digestion of complex polysaccharides,⁹ endogenous synthesis of certain vitamins and amino acids,¹⁰ metabolism of bile acids,¹¹ degradation of dietary oxalates,¹² and maturation of the immune system.¹³

On average, an individual's gut microbiota is composed of 500–1,000 bacterial species.¹⁴ Findings from the HMP suggest that each individual has a unique microbiome, each niche features one or a few signature taxa, and the gut microbiome is characterized by the greatest diversity with little variation over time.^{15, 16} The predominant bacterial groups in the human gastrointestinal tract are Bacteroidetes, Firmicutes, and Actinobacteria.^{17, 18} The phylogenetic composition of gut microbiota tend to be similar between individuals living in the same region, belonging to the same family, and having a similar diet.¹⁹ Muegge et al. studied gut microbiome profile in 33 mammalian species, including 18 humans, and reported that the difference in microbiome profile stems from differing metabolic functions required to utilize the diet.²⁰ Thus, the gut microbiome appears to change adaptively to the needs of the host organism.

Gut Microbiome in Kidney Disease

The term “dysbiosis” was first coined in early twentieth century by the Russian Nobel Laureate Elie Metchnikoff.²¹ Dysbiosis is defined as an imbalanced intestinal microbial community with quantitative and qualitative alterations in the composition and in metabolic activities of the gut microbiota. Preliminary evidence indicates that microbiome profile might be altered in patients with chronic kidney failure and earlier stages of CKD.²² (Table 1) Vaziri et al. found that 190 microbial operational taxonomic units (OTUs) differed significantly in abundance between ESRD patients and apparently healthy controls.²³ Hida et al. reported that the number of aerobic bacteria including *Enterobacteria* and *Enterococci* is higher in patients treated with maintenance hemodialysis than in controls.²⁴ Among anaerobic bacteria, Hida et al observed that hemodialysis patients have significantly lower number of *Bifidobacteria* and higher organism counts for *Clostridium perfringens*.²⁴

The main contributing factors to gut microbiome dysbiosis in patients with kidney disease include slow intestinal transit time,²⁵ impaired protein assimilation,²⁶ decreased consumption of dietary fiber,²⁷ iron therapy,²⁸ and frequent use of antibiotics.^{29, 30} Antibiotic treatment decreases the diversity and alters the relative abundances of members of

the bacterial community, with some patients exhibiting incomplete recovery post-treatment.³¹

Gut-Derived Uremic Toxins and Microbial Metabolites

In 1965, Einheber and Carter showed that germfree anephric mice survived longer than anephric mice with intact gut microbiome.³² Aronov et al. showed that a number of uremic retention solutes are present only in hemodialysis patients with intact colon.³³ Recently, using untargeted metabolomic mass spectrometry, Wikoff et al. reported that the presence of several protein-bound uremic toxins, such as indoxyl sulfate (IS), hippuric acid, and phenylacetic acid are dependent on the presence of gut microflora.³⁴

Impaired protein assimilation in uremia leads to large influx of undigested proteins into the distal intestine, which favors the proliferation of proteolytic bacteria.³⁵ (Fig 1) Increased protein fermentation results in generation of potentially toxic metabolites, such as ammonia, phenols, amines, indoles, and thiols.³⁶ The clinical manifestations of these uremic toxins are rather nonspecific and may include neurologic disorders, protein energy wasting, cardiovascular disease (CVD), and progression of CKD. The potential pathways linking the accumulation of some of the major toxic metabolites to patho-physiological consequences in patients with CKD are shown schematically in Fig 2. Increased levels of these toxins in CKD may be related to increased generation from dysbiotic microbiome or decreased elimination from reduced kidney function. In this review we will focus on the role of gut microbiome in generation of uremic toxins. (Table 2)

Ammonia and Urea—Inter-dependency between humans and microbes in the metabolic process is exemplified by the urea nitrogen salvage pathway. The end-product of mammalian protein catabolism is ammonia, which is toxic to cells in higher concentrations and thus converted to urea through the ornithine–urea cycle. Mammals cannot break down urea, but gut bacteria expressing urease cleave urea into ammonia and carbon dioxide.³⁷ (Fig 3) Some of the ammonia can be utilized for microbial synthesis of amino acids or can enter the host circulation and serve as a substrate for synthetic processes.³⁸ Ammonia in the large intestine could also be generated by microbial fermentation of glutamine, serine, threonine, and glycine. In a series of studies using CKD rats and human colonocytes, Vaziri et al. demonstrated that exposure to ammonia and ammonium hydroxide damages the intestinal epithelial tight junction and impairs its barrier function.^{39–41}

Creatinine, Guanidine and Uric Acid—Jones and Burnett estimated that about 16 to 60% of orally administered C¹⁴ labeled creatinine is either metabolized or excreted by routes other than the urine in subjects with kidney failure.⁴² Creatinine concentration in ileal effluent is similar to plasma, but it is undetectable in stool, suggesting that it is possibly degraded by colonic bacteria.⁴³ Furthermore, 1-methyl guanidine is produced by the metabolism of creatinine by *Pseudomonas stutzeri*.⁴³ Guanidine compounds accumulate in CKD, and some of them are considered uremic toxins.⁴⁴ Administration of methyl guanidine to rats with kidney failure results in a dose-dependent increase in mortality.⁴⁵

Urate (uric acid) is the end product of purine metabolism. Through excretion, the kidneys play an important role in maintaining serum urate levels, and sustained hyperuricemia in CKD is associated with gout, hypertension, and CVD.^{46, 47}

Although the kidney is the primary route for the excretion of urate, there is a minimal rise in its serum concentration in advanced CKD due to CKD-induced adaptive secretion of uric acid by the colon.⁴⁸ Consequently, bacterial families possessing urease, uricase, and enzymes capable of forming indole and *p*-cresol are expanded in ESRD patients.⁴⁹

Indoles—Indoles are an aromatic group of compounds containing a pyrrole ring. Metabolism of tryptophan by bacterial tryptophanase generates more than 600 indoles in the gut, which are absorbed and sulfate conjugated in the liver.⁵⁰

Indoxyl Sulfate (IS): The concentration of IS in the serum is negatively correlated with the level of kidney function,⁵¹ and can be used as a predictor of CKD progression.⁵² IS is normally cleared by the proximal tubules of the kidneys, but accumulates in CKD patients. Cellular transport of IS is mediated by the organic anion transporter (OAT) 1 and OAT3,⁵³ expression of which has been shown to be reduced in experimental models of kidney failure.⁵⁴ Accumulation of IS in renal proximal tubular cells induces nephrotoxicity by activating nuclear factor (NF)- κ B and plasminogen activator inhibitor type 1 expression.^{54, 55} Following its administration to uremic rats, IS increases the expression of genes such as tissue inhibitor of metalloproteinases and transforming growth factor (TGF) β 1, which are known mediators of tubulointerstitial fibrosis.⁵⁶

Elevated level of IS is associated with aortic calcification, vascular stiffness, and increased risk of overall and cardiovascular mortality in patients with CKD.⁵⁷⁻⁵⁹ Experimental studies show that IS enhances oxidative stress in endothelial cells,⁶⁰ increases shedding of endothelial microparticles,⁶¹ impairs endothelial cell repair mechanism,⁶² and induces vascular smooth muscle cell proliferation.⁵⁹ Interestingly, this indole may decrease erythropoietin production by interfering with oxygen sensing in erythropoietin producing cells.⁶³ Furthermore, IS is taken up by osteoblasts, where it augments oxidative stress and down-regulates parathyroid hormone receptor expression, leading to low-turnover bone disease.⁶⁴ IS also has an inhibitory effect on osteoclast function.⁶⁵ In non-dialysis-dependent CKD patients, IS has been found to be positively associated with bone formation rate.⁶⁶ IS is a ligand of the aryl-hydrocarbon receptor (AhR), a transcriptional regulator that has been shown to cause podocyte injury.⁶⁷ Similar to *p*-cresol sulfate, IS is also highly protein bound, preventing it from being effectively removed by hemodialysis.^{68, 69}

Indole Acetic Acid (IAA): A protein-bound uremic solute, IAA is generated from tryptophan by both intestinal bacteria and normal cells. Plasma concentrations of IAA are elevated in CKD and the compound is only partly removed during hemodialysis.⁷⁰ IAA has been shown to induce glomerular sclerosis and interstitial fibrosis in subtotal nephrectomized rats, thus contributing to progression of CKD.⁷¹ IAA is also a significant predictor of mortality and cardiovascular events in patients with CKD.⁷² IAA activates nongenomic AHR pathway, resulting in induction of the proinflammatory enzyme cyclooxygenase-2 and oxidative stress.⁷²

Phenols—Phenols are aromatic compounds with one or more hydroxyl groups attached to a benzene ring. Partial breakdown of tyrosine and phenylalanine by several intestinal bacteria genera including *Bacterioides*, *Bifidobacterium*, *Lactobacillus*, *Enterobacter*, and *Clostridium* generate phenols and *p*-cresol.⁷³ Most of the phenols produced in the colon are quickly absorbed and modified by sulfate, acetate, and—more rarely—glucuronide conjugation, especially in the liver or colonic mucosa, making them less toxic and facilitate their excretion by organic ion transport systems.⁷⁴

***p*-cresol sulfate (PCS):** PCS is a 188-Da uremic retention solute and a uremic toxin.^{73, 75} Urinary excretion depends on tubular secretion through specific transporters,⁷⁶ leading to progressive accumulation in patients with CKD.⁷⁷ In experimental studies, IS and PCS activate the intrarenal renin-angiotensin system, TGF/Smad pathway, and possibly epithelial mesenchymal transformation leading to fibrosis of the kidney.⁷⁸ Watanabe et al. showed that administration of PCS causes significant renal tubular damage in 5/6-nephrectomized rats by increasing oxidative stress and inflammatory cytokines.⁷⁹ Elevated plasma PCS level is associated with all-cause mortality and CVD in patients with chronic kidney failure and earlier stages of CKD.^{80–82} Koppe et al.⁸³ demonstrated the contribution of PCS to CKD-associated insulin resistance and cachexia by its action on adipose tissue and increase of lipolysis. Mice treated with PCS have been shown to display altered insulin signaling in skeletal muscle through activation of ERK1/2.

Phenylacetylglutamine (PAG): PAG is a major microbe-derived nitrogenous metabolite that accumulates in uremia.^{84, 85} Most PGA is derived from β -phenylethylamine formed in the large intestine by decarboxylation of phenylalanine released by bacterial proteolysis of unabsorbed protein.⁸⁶ PGA is also produced in the liver by metabolism of phenylacetic acid, derived from phenylalanine.⁸⁷ The precursor phenylacetic acid itself is toxic and induces nausea, vomiting, diarrhea, and convulsion.⁸⁸ The latter is associated with impaired immunoregulation,⁸⁹ increased oxidative stress,⁹⁰ and osteoblast dysfunction.⁹¹ Autopsy studies in dogs have shown that exposure to phenylacetic acid causes renal tubular damage, and thus may contribute to progression of kidney disease.⁸⁸

Hippurate—Gut microbial metabolism generates benzoate from dietary aromatic compounds, and the subsequent hepatorenal conjugation of benzoate with glycine, forms hippurate.^{92, 93} Hippurate is generally believed to be non-toxic, except for contributing to the anion gap acidosis.^{94, 95} There is some evidence that hippurate may cause glucose intolerance and interfere with erythropoiesis and platelet cyclo-oxygenase activity.⁹⁶

Amines

Polyamines: Generated by the gut microbiota from precursor amino acids, polyamines include putrescine, agmatine, cadaverine, tyramine, and histamine. Igarashi et al. observed a decrease in spermine and an increase in putrescine, as well as acrolein (a major toxic compound deriving from spermine), in the plasma of patients with CKD.⁹⁷ Cellular downregulation by polyamines is proposed to play a role in the lack of tissue response to hormones in uremia.⁹⁸ Polyamine also inhibit the activity of erythropoietin and may hence play a role in the anemia of CKD.⁹⁹

D-amino Acids: Among other potential uremic toxins are some of the D-amino acids. It has been shown that plasma levels of certain D-amino acids increase as GFR declines.¹⁰⁰ D-lactic acid originates from endogenous production by the methylglyoxylase pathway, or as a byproduct of bacterial metabolism in the intestine. Oh et al. first described D-lactic acidosis, which has been associated with neurotoxicity.¹⁰¹

RECENT ADVANCES

Trimethylamine N Oxide (TMAO)

In a landmark study, Wang et al. performed metabolomics studies and screened more than 2,000 compounds in 75 patients with CVD, and identified TMAO, choline, and betaine as being associated with heart disease.¹⁰² In a study involving 4,007 patients undergoing elective coronary angiography, elevated TMAO level was found to predict an increased risk of major adverse cardiovascular events after adjustment for traditional risk factors.⁸ Choline is catabolized by the intestinal microbiota to form trimethylamine gas, which is subsequently metabolized by the liver into TMAO. Dietary carnitine, which is found in red meat, is another substrate for gut flora to produce TMAO.¹⁰³

Microbial taxa belonging to the Clostridiaceae and Peptostreptococcaceae families are positively associated with blood levels of TMAO in humans.¹⁰³ The way in which TMAO promotes atherosclerosis remains speculative, but it has been shown to cause alteration of cholesterol and sterol metabolism, promote foam cell formation by increasing expression of scavenger receptors on macrophages, and lead to alterations in bile acid metabolism and sterol transporters in the liver and intestine.^{102, 103} High TMAO level is a predictor of increased long-term mortality in patients with heart failure, which is independent of traditional risk factors.¹⁰⁴

Plasma level of TMAO is elevated in patients with CKD and is associated with increased risk of death.¹⁰⁵ In animal models, feeding with TMAO and choline leads to tubulointerstitial fibrosis and collagen deposition, which is accompanied by a significant increase in the phosphorylation of Smad3.¹⁰⁵

Hydrogen sulfide (H₂S)

Sulfate-reducing bacteria in the human colon can use H₂ or organic compounds as electron donors for reduction of sulfate or other oxidized sulfur compounds to generate H₂S. This toxic gas belongs to the family of gasotransmitters and inhibits mitochondrial respiration through blockade of cytochrome c oxidase,¹⁰⁶ with genotoxic, cytotoxic, and inflammatory effects.¹⁰⁷ Despite these reports of toxicity, findings from animal models of ischemia/reperfusion injury and heart failure suggest that H₂S could be cardioprotective.¹⁰⁸ In animal models of CKD plasma, H₂S production by the kidney and liver is reduced due to down-regulation of the H₂S-producing enzymes.¹⁰⁷ Preliminary evidence indicates that plasma H₂S level is reduced in hemodialysis patients compared to controls.¹⁰⁹ In *in vitro* studies, sodium hydrogen sulfide, a hydrogen sulfide donor, decreases inflammation and inhibited renal fibrosis, possibly through inhibiting the TGF-β1/Smad and mitogen-activated protein

kinase signaling pathways.¹¹⁰ The potential beneficial effect of H₂S in CKD and whether it retains its toxicity in higher concentration needs further study.

Endotoxin

Endotoxin is a phospholipid that forms the outer membrane of most Gram-negative bacteria. Circulating endotoxin binds lipopolysaccharide binding protein (LBP), forming a complex that interacts with the MD-2 part of the Toll-like receptor 4, anchored by CD14.¹¹¹ This binding stimulates, via activation of NF- κ B, the translation and production of inflammatory cytokines.¹¹² Endotoxin translocation from the gut has been suggested as one of the causes of inflammation in CKD.^{103, 113} Furthermore, endotoxin is known to play an important role in initiation and progression of atherosclerosis by mediating endothelial cell injury, boosting recruitment of monocytes, transforming macrophages to foam cells, and activating coagulant activity.^{114, 115} We have demonstrated that soluble CD14 is associated with progression of CKD, CVD, and mortality in patients with kidney disease.^{116–118} Furthermore, endotoxin has been identified as an inflammatory trigger of insulin resistance, obesity, and diabetes in mice.¹¹⁹

Gut Microbe-Derived Mediators of Immune Regulation

It is now evident that gut microbes play a key role in shaping of the human immune system.¹²⁰ Polysaccharide A produced by *Bacteroides fragilis* induces accumulation of Foxp3-positive regulatory T cells and production of interleukin (IL)-10.¹²¹ Another molecule that is derived from gut microbiota and can modulate peripheral immune function is peptidoglycan, an essential component of the cell wall of virtually all bacteria. Upon entry to blood, peptidoglycan systemically primes the innate immune system.¹²² Peptidoglycan has been shown to signal via the pattern-recognition receptor Nod1.¹²² The role of these and other microbe-derived molecules in mediating dysregulated immune response in patients with CKD warrants further exploration.

Short-Chain Fatty Acids (SCFA)

An altered dysbiotic gut microbiome will not only produce an array of harmful metabolites and uremic toxins, but can also potentially cease to produce the otherwise beneficial metabolites such as SCFA. These one- to six-carbon aliphatic organic acids are the products of anaerobic bacterial fermentation of dietary polysaccharides, and include acetate, propionate, and butyrate. SCFA enter the systemic circulation through colonocytes by passive diffusion as well as active transport mechanisms. They affect a range of host functions, including energy metabolism, immune regulation and gut motility, and blood pressure regulation through activation of G protein-coupled receptors such as GPR41 and GPR43.¹²³

Butyrate is the primary source of energy for colonocytes and is thus associated with maintenance of the epithelium. More recently, researchers have expanded the role of SCFA to explain the gut-kidney connection in ischemia reperfusion injury (IRI) showing that treatment with SCFA reduces kidney injury of this type in a germ-free mouse model system.^{124, 125} The key mechanism protecting against acute kidney injury was suggested to

be reduction in inflammation mediated by an epigenetic mechanism. If SCFA are important in AKI, it might be of interest to also define their role in progression of CKD.

Interestingly, SCFA may also influence blood pressure through activation of GPR41 and GPR43, which are expressed in adipocytes, neutrophils, and sympathetic ganglia.¹²⁶ Olfactory receptor 78 (Olf78) expressed in the kidney responds to SCFA where it mediates renin secretion and increases blood pressure³ this increase is counteracted by GPR43, which induces vasodilatation.¹²⁶

Advances in Technology and Discovery of Uremic Toxins

In 2003, the European Uremic Toxin Work Group catalogued 90 different uremic retention solutes,¹²⁷ and this list has since grown in number to more than 150.¹²⁸ The discovery of the newer solutes is primarily driven by the advent and implementation of “omic” technologies such as metagenomics, transcriptomics, proteomics, and metabolomics.¹²⁹

It is apparent that uncultured microorganisms represent about 99% of the gut microbiota. Fortunately, since 16S ribosomal RNA sequences are highly conserved within organisms of the same genus and species, they can be used to determine phylogeny. Metagenomics is culture-independent analysis of microbial genomes, which allows understanding the dynamics and diversity of microbial community and its interaction with host.¹³⁰

In the search for uremic toxins, use of proteomics and metabolomics could be complementary. Proteomics focuses on study of peptides and proteins, whereas metabolomics is useful in identification of small metabolites (<1000 Daltons) such as amino acids, alcohols, vitamins, polyols, organic acids, as well as nucleotides. Since metabolites are downstream of both transcription and translation, they may be more reflective of disturbed metabolism than proteins, messenger RNA, and genes, but each technique has a unique role in the discovery of uremic retention solutes.

Metabolite profiling of plasma samples from 1,434 Framingham study participants demonstrated that 9 metabolites predict the development of CKD.¹³¹ Interestingly, choline was one of the three markers that remained significant after adjustment for estimated glomerular filtration rate, age, sex, diabetes mellitus, hypertension, and proteinuria at baseline.¹³¹ A metabolomic study in stage 3–4 CKD patients revealed that 14 metabolites were elevated in uremic plasma.¹³² In addition to confirming the retention of several previously identified uremic toxins, including PCS, this study detected two novel uremic retention solutes, dimethyl sulphone and 2-hydroxyisobutyric acid.¹³² It is essential to emphasize the importance of investigating the underlying biochemical mechanisms of any newly discovered uremic solute in order to determine its pathophysiologic importance.¹²⁹

The Microbiome as a Therapeutic Target

Unlike the human genome, the gut microbiome has a dynamic composition that is susceptible to manipulation and selective “farming” of desired microbial populations for the benefit of the human host. As we unravel the details of interactions between the host and the microbiota, as well as those within the microbiota itself, new classes of therapeutics will emerge to harness the vast therapeutic potential of this extraordinary natural resource.

Among the seemingly limitless potential applications of human gut microbiome, its use as disease biomarker, alteration of the microbiome composition to treat disease, and genetic engineering of the microbes to gain new functions or deliver small therapeutic molecules, have been proposed.

Gut microbiome-based biomarkers—Gut microbiome composition has been considered as a potential tool for diagnosing, monitoring and prognostication of diseases. Microbiome profile is not only altered in CKD, it also varies by underlying etiology.^{133–135} Whether the dysbiotic microbiome is a cause and/or consequence of CKD, is not quite clear.¹³⁶ The potential utility of gut microbiome as a biomarker for screening individuals with susceptibility to develop CKD remains to be investigated.

Manipulation of the microbiome composition—An improved understanding of the gut microbiome's physiologic functions and the pathologic effects of dysbiosis have triggered interest in various ways of reestablishing symbiosis.²² The generation of uremic toxins could be reduced by selectively increasing saccharolytic bacteria and reducing proteolytic bacteria in the colon. A number of therapeutic interventions have been explored to modulate gut microbiota, or adsorption of uremic toxin end products of microbial fermentation (Table 3).

Probiotics are defined as “live microorganisms” that when administered in adequate quantities bestow a health benefit on the host. However, administration of enteric capsule preparation of *Bifidobacterium longum* to patients with CKD was reported to have only minimal effect on CKD progression.¹³⁷ In a randomized, double-blind, placebo-controlled crossover study involving 22 hemodialysis patients, Renadyl (a specific probiotic formulation) was not observed to have an effect on microbe-derived uremic toxins.¹³⁸

Despite these negative findings and the absence of large-scale studies, a recent randomized, double-blind, placebo-controlled trial involving 39 peritoneal dialysis patients reported significant reduction in the serum levels of endotoxin and pro-inflammatory cytokines, increase in the serum levels of anti-inflammatory cytokine (IL-10), and preservation of residual kidney function following a 6 month probiotic therapy.¹³⁹ In another double-blind, randomized placebo-controlled trial with 30 CKD patients (stages 3–4), the symbiotic Probinul neutro® was shown to significantly lower total plasma *p*-cresol concentrations after 2–4 weeks of treatment.¹⁴⁰ One of the main limitations to probiotic therapy is that no study has yet demonstrated sustained survival of probiotics in the colon of dysbiotic CKD patients. Furthermore, care should be taken when choosing probiotics since the contribution of bacteria with the ability to hydrolyze urea may be more harmful.

Prebiotics are nondigestible food ingredients whose positive effects are due to stimulating the growth or activity of one or a limited number of bacteria in the colon. Meijers et al. showed that oligofructose inulin significantly reduces PCS generation rates and serum concentrations in hemodialysis patients, but has no effect on IS.¹⁴¹ In contrast, a randomized controlled trial showed that resistant starch decreased IS level in patients treated with hemodialysis.¹⁴² In rats, a high amylose-resistant starch diet was observed to retard CKD progression and attenuated oxidative stress and inflammation.¹⁴³ A small study in

hemodialysis patients showed that a synbiotic (combining *Lactobacillus casei*, *Bifidobacterium breve* and galacto-oligosaccharides) decreased *p*-cresol but not IS.¹⁴⁴

Genetic engineering of the gut microbes—Genetic engineering of bacteria for delivery of drugs and small therapeutic molecules holds a tremendous potential as a future approach to treat a wide range of disease and promote health. Prakash and Chang used microencapsulated *E. coli* cells genetically engineered to contain a *K. aerogenes* gene, and showed that these modified bacteria were able to effectively remove urea and ammonia *in vitro*¹⁴⁵ and lower plasma creatinine in rats when given orally on a daily basis.¹⁴⁶ At present, safety concerns prevent the release of genetically engineered cells into the body, and the field anxiously welcomes further pioneering research to improve our knowledge on the gut microbial ecology so that it can make more educated attempts to manipulate the gut microbial community for therapeutic purposes.

Another method for decreasing gut-derived uremic toxins is to use oral sorbents. The oral sorbent AST-120 was found to decrease plasma IS in a dose-dependent manner.¹⁴⁷ The primary effect of AST-120 appears to be mediated through its adsorption of urea-derived ammonia and interruption of enterohepatic urea recycling.¹⁴⁸ Although small randomized controlled animal studies have suggested a renoprotective effect for AST-120, a subsequent large randomized controlled trial could not confirm it.¹⁴⁹ The larger study has some methodological limitations, but it also raises the possibility that targeting specific microbiome-derived uremic toxin may not be sufficient, since the microbiomes generate a myriad of yet unidentified toxins.

Thus, the quality of evidence emerging from the small, uncontrolled mostly single-center studies targeting the microbiome needs careful scrutiny and these results need replication in well-designed large scale multicenter trials. Among the currently ongoing studies, some are worth mentioning. In a double-blind, placebo-controlled, randomized cross-over trial, Rossi et al., is recruiting 37 CKD patients for a 6 week-long synbiotic therapy (or placebo) to determine the efficacy of synbiotic therapy for lowering the serum IS and PCS.¹⁵⁰ The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) is proposing to conduct two highly intensive phase II randomized, double-blind, placebo-controlled, multicenter studies examining the effect of prebiotic therapy on microbiome and the associated metabolomics profile in CKD patients not yet on dialysis and maintenance hemodialysis patients; these studies are expected to be launched shortly.

SUMMARY

It is becoming evident that interaction of the human host with our resident microbes at the gene, protein, and metabolite levels could have significant impact on health and disease. The features and composition of the gut flora reflects selective forces acting on both the microbial community and the host. Whether an adaptive change in microbiome in response to the uremic state becomes maladaptive, leading to increased generation of uremic toxins and associated complications, needs to be rigorously examined using modern techniques in clinical and experimental settings. It is also possible that the presence of specific microbiome or absence of protective microbiome increases the susceptibility to kidney

disease when exposed to injury. (Fig 4) Supported by preliminary evidence and enticed by novelty of the concept, a number of interventions have been proposed to reduce uremic toxicity targeting the gut microbiome and tested with mixed results. The findings from these studies should be viewed cautiously and further examined in well-designed large scale studies prior to implementation. Needless to say, if proven to be effective, gut-microbiome targeted interventions will have a significant impact on the management of patients with chronic kidney failure and earlier stages of CKD.

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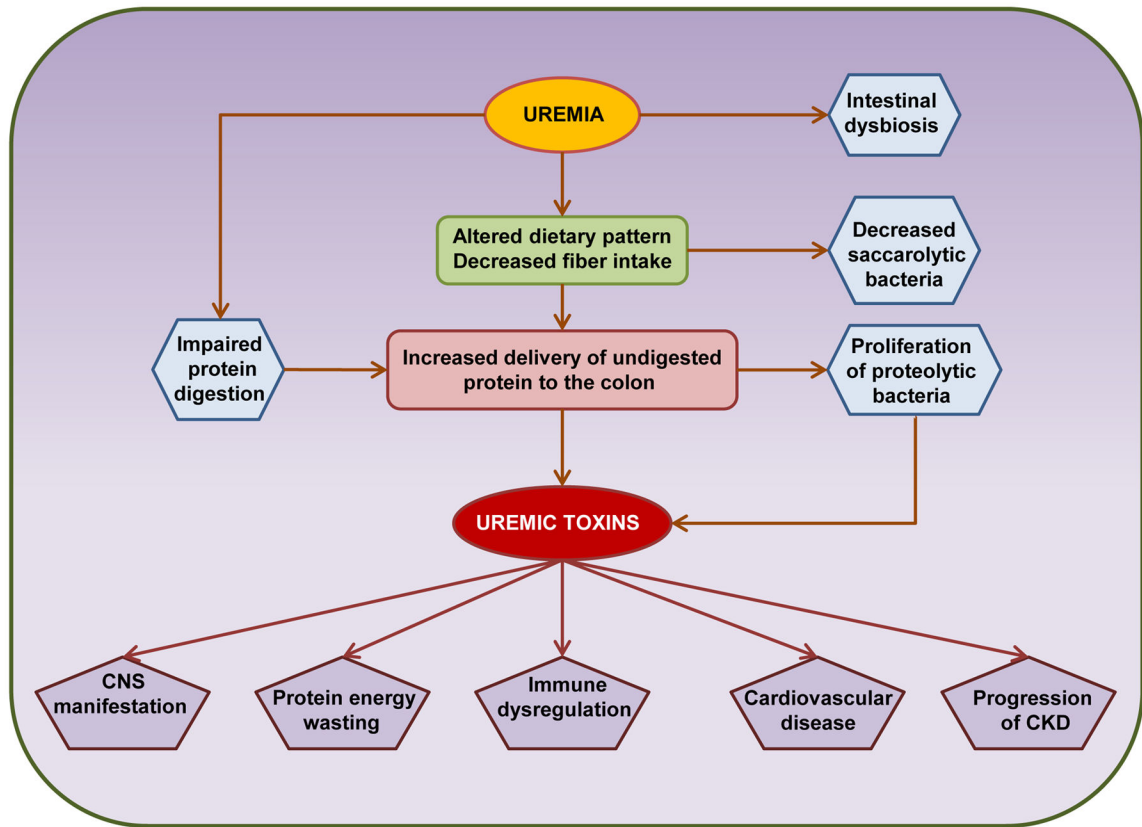


Figure 1. Schematic representation of the association between uremia, dysbiotic gut microbiome, gut-derived uremic toxins, and the clinical manifestations of these uremic toxins.

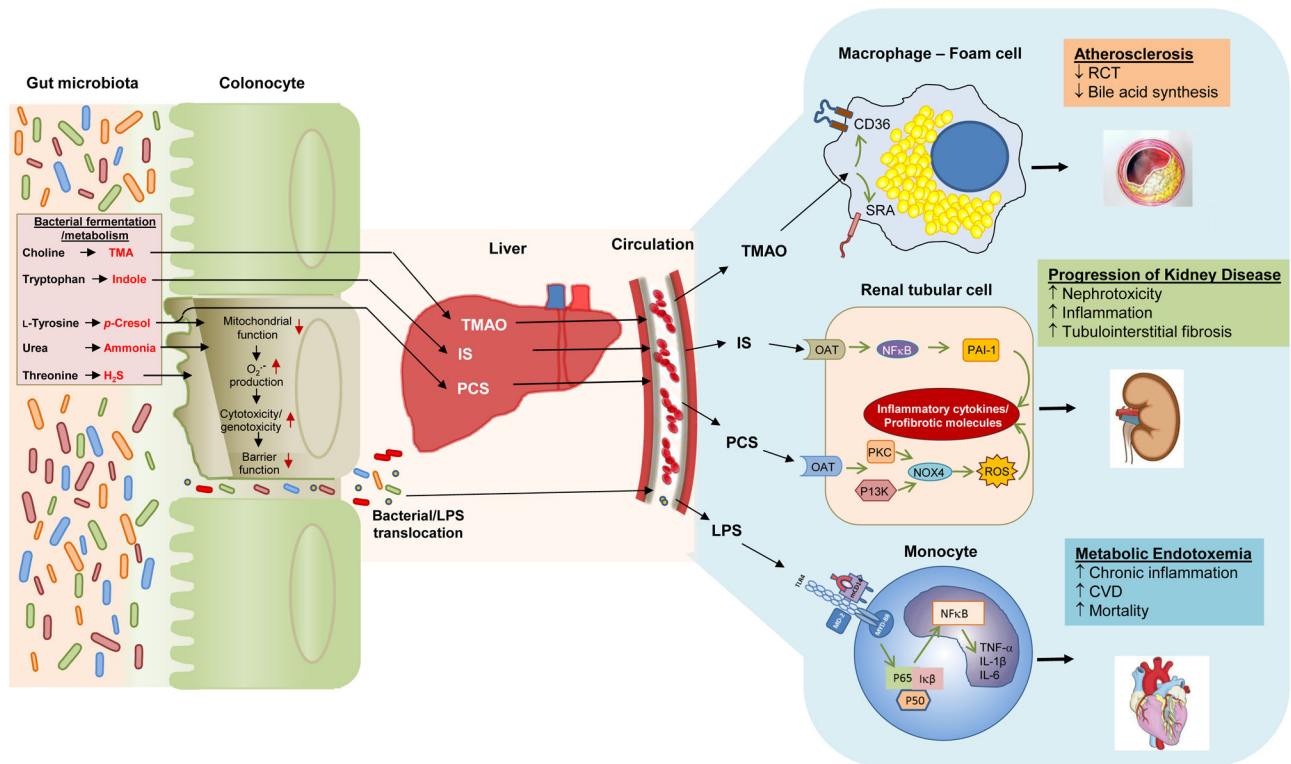


Figure 2.

Schematic showing some of the major toxic metabolites originating from synthesis by dysbiotic gut microbiome and potential pathways linking their accumulations to pathophysiological consequences in CKD, including in ESRD. Increased intestinal concentration of uremic toxins associated with the progression of CKD leads to microbial dysbiosis. *p*-Cresol, produced by the intestinal microbiota from the amino acid tyrosine, inhibits colonocyte respiration and proliferation, and at higher concentrations, increases DNA damage and becomes genotoxic towards colonocytes.¹⁵¹ Overgrowth of pathogenic bacteria, the loss of barrier integrity, and the breach in the epithelia barrier lead to endotoxemia. Circulating endotoxin, also referred to as lipopolysaccharide (LPS), activates production of inflammatory cytokines. Endotoxin translocation from the gut has been suggested as one of the causes of inflammation in CKD.^{103, 113} Amongst the most studied uremic toxins are phospholipid metabolites and bacterial products of choline degradation, such as TMAO, which has been associated with CVD. TMAO causes alteration of cholesterol and sterol metabolism, promotes foam cell formation by increasing expression of scavenger receptors on macrophages, and leads to alternations in bile acid metabolism and sterol transporters both within the liver and intestine.¹⁰³ Other uremic toxins include cometabolites of phenols (eg, PCS), and indoles (eg, IS), which have been associated with progression of CKD, CVD, and mortality in hemodialysis patients. Accumulation of PCS in human tubular cells leads to Nox4-dependent ROS generation via upregulation of Nox4 and p22phox, which subsequently enhance the expression of inflammatory cytokines and profibrotic factors, resulting in cell injury.¹⁵² Activation of PI3-K and PKC mediate the stimulatory effect of PCS on Nox4 and NADPH oxidase. IS induces nephrotoxicity via

organic anion transporter–mediated uptake in the basolateral membrane of renal proximal tubular cells, where it activates NF- κ B and plasminogen activator inhibitor type 1 expression.⁵⁴ ROS, reactive oxygen species

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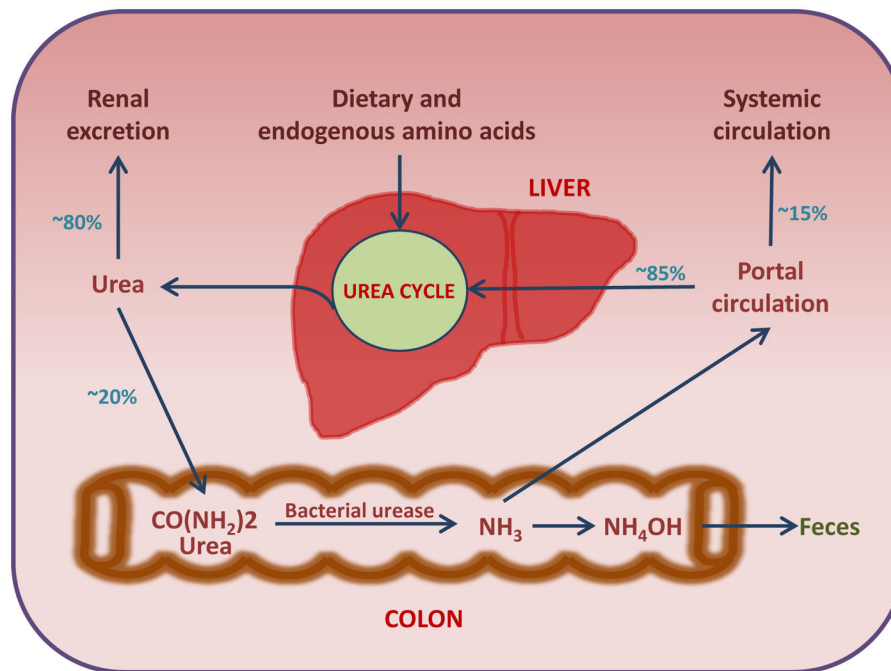


Figure 3. Schematic illustration of amino acid, ammonia (NH_3), and urea flux between the gastrointestinal tract and liver. Urea is produced via the urea cycle in the liver from dietary amino acids and by their catabolism in peripheral tissues. Urea is then excreted into the gastrointestinal system and into the urine. Within the intestinal tract, gut bacteria, particularly coliforms and anaerobes in the colon and cecum, convert dietary amino acids and urea into ammonia and CO_2 , using microbial urease. Some of this ammonia is, in turn, converted to ammonium hydroxide which raises the luminal fluid's pH before being excreted in feces. The remaining ammonia is absorbed into the portal circulation and converted back to urea via the urea cycle in the liver. Of the total ammonia produced, the majority enters the urea cycle, with the remaining smaller proportions being metabolized by peripheral tissues.

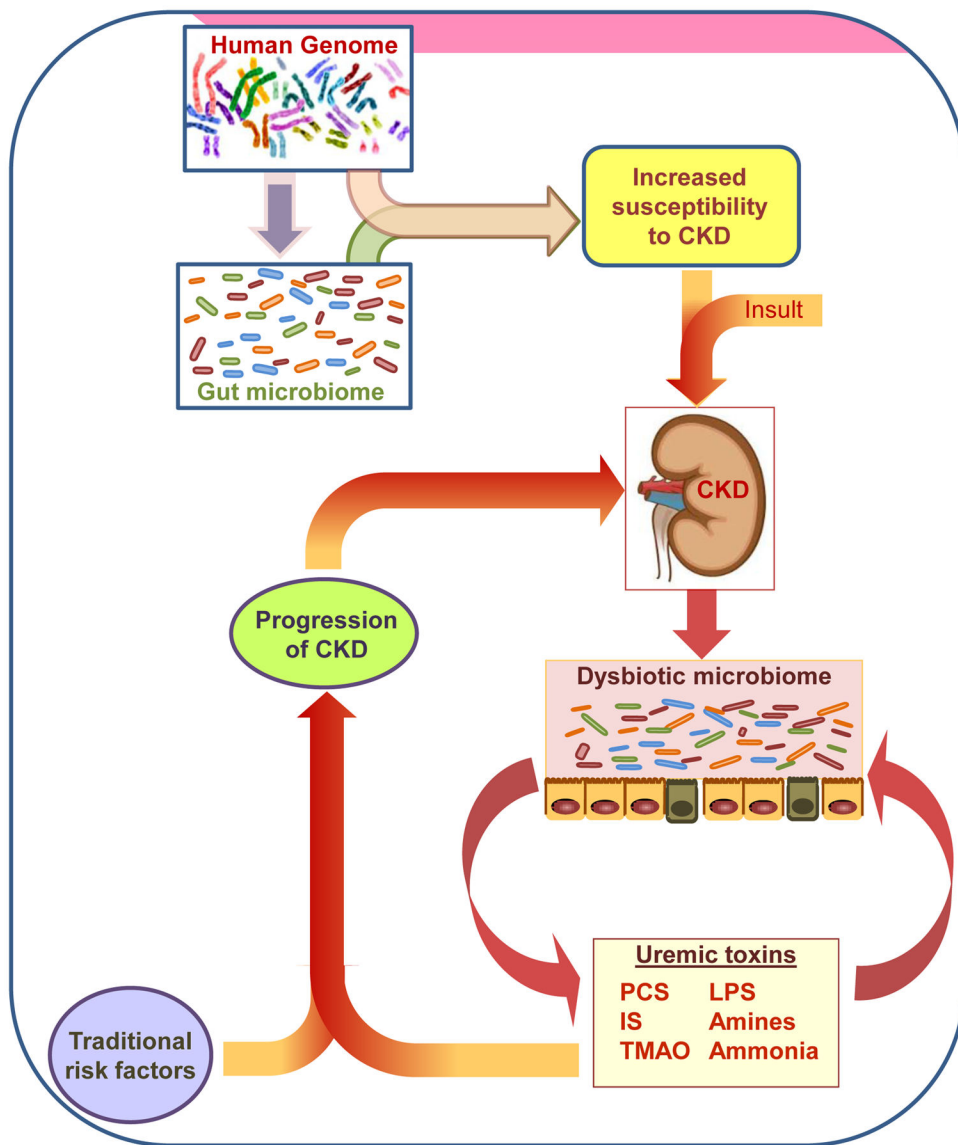


Figure 4.

The cause-consequence relationship between gut dysbiosis and CKD. Human genome affects gut microbiome, and together, in presence of a specific dysbiotic microbiome or absence of protective microbiome, they can increase the susceptibility to kidney disease when exposed to insult. Subsequent maladaptive changes of microbiome in response to uremic state, and in association with traditional risk factors, lead to further increased generation of uremic toxins and disease progression.

Table 1

Alterations of gut microbiome in CKD

Study group	Methods	Gut microbiota features associated with CKD	Ref.
Human studies			
CKD patients (n=22), with (n=12) and without (n=10) GI symptoms	Antroduodenaljejunal manometry, culture	Abnormal motility and bacterial overgrowth in small intestine of CKD patients	153
Hemodialysis patients (n=8)	Bacterial cell count	Increased counts of both aerobic (~ 10 ⁶ bacteria/ml) and anaerobic (~ 10 ⁷ bacteria/ml) organisms in the duodenum and jejunum (which are normally not colonized heavily by bacteria in healthy individuals)	154
ESRD patients (n=24) vs. healthy persons (n=12)	Phylogenetic microarray	Highly significant differences between the ESRD and the healthy control groups in the abundance of over 200 bacterial operational taxonomic units belonging to 23 bacterial families	23
ESRD patients (n=24) vs. healthy persons (n=12)	Phylogenetic microarray and Hypergeometric distribution tests	Expansion of bacterial families possessing urease, uricase, and <i>p</i> -cresol- and indole-forming enzymes; depletion of bacteria possessing short-chain fatty acid forming enzymes	155
ESRD patients (n=10) vs. healthy persons (n=8)	16S rDNA amplification and DNA pyrosequencing	Disturbed composition of the microbiota characterized by an overgrowth of aerobic bacteria such as Enterobacteria and Enterococci species (~100 times higher in ESRD patients); of the anaerobic bacteria, ESRD patients had significantly lower numbers of Bifidobacteria and higher numbers of <i>Clostridium perfringens</i>	24
ESRD patients (n=52)	16S rDNA amplification and pyrosequencing	Increased bacterial translocation in plasma of ESRD patients; bacterial DNA concentration positively correlated with plasma levels of CRP and IL-6	156
Experimental animal studies			
5/6 nephrectomy (n=6) or sham-operated rats (n=5)	Phylogenetic microarray	Significant difference in the abundance of 175 bacterial operational taxonomic units between the uremic and control animals, most notably as decreases in the Lactobacillaceae and Prevotellaceae families	23
Uremic (n=20) and control (n=20) Wistar rats	Culture	Impaired intestinal mucosa barrier with significantly more bacterial translocation in uremic than in control animals	157

Abbreviation: CKD, chronic kidney disease; ESRD, end-stage renal disease; GI, gastrointestinal; rDNA, ribosomal DNA; CRP, C-reactive protein; IL, interleukin

Table 2

Gut microbiome derived uremic toxins

Solute (MW)	Group	Source	Related bacteria	Pathogenies/Mechanism	Ref.
Free, water-soluble, low MW molecules (<0.5 kDa)					
Ammonia (17 Da)		Bacterial hydrolysis of urea by urease ; bacterial fermentation of glutamine, serine, threonine and glycine	Urease is produced by diverse bacterial species; <i>Clostridium spp.</i> , <i>Enterococcus</i> , <i>Shigella</i> and <i>Escherichia coli</i>	High concentration of ammonia changes the luminal pH causing uremic enterocolitis; amino acid catabolism leads to the formation of sulfides, phenolic compounds and amines, which are inflammatory and/or precursors to the formation of carcinogens	158, 159
1- Methyl guanidine (73 Da)	Guanidin e	Metabolism of creatinine	<i>Pseudomonas stutzeri</i>	Accumulate in CKD and considered a uremic toxin; administration to rats with kidney failure demonstrates a dose- dependent increase in mortality	43-45
TMAO (75 Da)	Amine	Endogenous; bacterial metabolism of dietary lipid phosphatidylcholine	<i>Faecalibacterium prausnitzii</i> , <i>Bifidobacterium</i>	↑ progression of kidney disease and mortality in CKD; ↑ in tubulointerstitial fibrosis and collagen deposition; ↑ phosphorylation of Smad3, which regulates the profibrotic TGFβ/Smad3 signaling	160-162
Homocysteine (135 Da)	Amino acid	Endogenous; intestinal bacteria lower homocysteine by production of folic acid	<i>Bifidobacterium spp.</i>	↑ CVD and death; ↑ oxidative stress (through the production of reactive oxygen species), binds to nitric oxide, produces homocysteinylated proteins, and leads to the accumulation of its precursor, S-adenosylhomocysteine, a potent inhibitor of biological transmethylylations	163
D-lactic acid (90 Da)	D-amino acid	Ingestion; endogenous; bacterial production	<i>Enterococcus</i> and <i>Streptococcus spp.</i>	D-lactic acidosis; neurotoxic effects; encephalopathic symptoms	100, 101
Oxalate (90 Da)		Ingestion; endogenous; certain intestinal bacteria have oxalate-degrading potency	<i>Oxalobacter formigenes</i> , <i>B. lactis</i> , <i>E. faecalis</i> , and <i>Eubacterium lentum</i>	Hyperoxaluria leading to urolithiasis; ↓ endothelial cell replication and migration leading to atherosclerosis	164
Protein-bound solutes					
p-Cresol sulfate (188 Da)	Phenol	Bacterial metabolism of tyrosine and phenylalanine	<i>Clostridium difficile</i> , <i>F. prausnitzii</i> , <i>Bifidobacterium</i> , <i>Subdoligranulum</i> , <i>Lactobacillus</i>	↑ progression of CKD, CVD, and mortality in hemodialysis patients; ↓ cytokine-stimulated expression of endothelial adhesion molecules; ↑ endothelial permeability	78-81, 165, 166
Indoxyl sulfate (213 Da)	Indole	Bacterial metabolism of tryptophan	<i>Clostridium sporogenes</i> , <i>E. coli</i>	↑ vascular stiffness, aortic calcification, and cardiovascular mortality; ↑ oxidative stress in endothelial cells, ↑ vascular smooth muscle cell proliferation; ↑ expression of genes related to tubulointerstitial fibrosis; ↑ nephrotoxicity	53, 55, 56
Indole-3-acetic acid (175 Da)	Indole	Endogenous; bacterial metabolism of tryptophan	<i>Clostridium sporogenes</i> , <i>C. bartlettii</i> , <i>E. coli</i>	Induce glomerular sclerosis and interstitial fibrosis in subtotally nephrectomized rats, thus contributing to progression of CKD; Serum indole-3-acetic acid is a significant predictor of	71, 72

Solute (MW)	Group	Source	Related bacteria	Pathogenes/Mechanism	Ref.
Phenylacetic acid (136 Da)		Endogenous; bacterial metabolism of tryptophan	<i>Clostridium spp.</i> , <i>Bacteroides spp.</i>	mortality and cardiovascular events in patients with CKD; induces pro-inflammatory enzyme COX-2 and oxidative stress Toxic; induces nausea, vomiting, diarrhea, and convulsion; associated with impaired immunoregulation, increased oxidative stress, and osteoblast dysfunction; shown to cause renal tubular damage in dogs	88, 88-91
Hippuric acid (179 Da)	Hippurate	Ingestion, bacterial metabolism of aromatic compounds and polyphenols	<i>Clostridia spp.</i>	Non-toxic; ↑ the anion gap acidosis; may cause glucose intolerance and interfere with erythropoiesis and platelet cyclo-oxygenase activity	94-96

COX-2 cyclooxygenase 2; CVD, cardiovascular disease; CKD, chronic kidney disease; MW, molecular weight; TMAO, Trimethylamine N Oxide

Table 3

Treatment options for uremic toxins and inflammation

Intervention	Patient type	Comments	Ref.
Probiotic			
Oligofructose-enriched inulin	Healthy participants (n=50)	↓ Urinary excretion of <i>p</i> -cresol	72
Inulin/oligofructose	Obese women (n=30)	↓ Endotoxemia	64
Oligosaccharides	Elderly participants (n=74)	↓ TNF- α mRNA and IL-6 mRNA; Serum soluble CD14	73
Prebiotic			
<i>Bifobacterium bifidum</i> , <i>B. catenulatum</i> , <i>B. longum</i> , and <i>Lactobacillus plantarum</i>	Peritoneal dialysis patients (n=39; 21 in the probiotics group and 18 in the placebo group)	↓ TNF- α , IL-5, IL-6, and endotoxin; ↑ IL-10; Preserve residual kidney function	139
Oligofructose-enriched inulin	Hemodialysis patients (n=22)	↓ Serum <i>p</i> -cresyl sulfate and generation rate	58
Synbiotic			
Probiotic along with inulin, oat bran, pectin, and resistant starch	Trauma patients (n=65)	↓ Rate of systemic inflammatory response, syndrome, infections, severe sepsis, and mortality	74
Galacto-oligosaccharides and <i>L. casei</i> , and <i>B. breve</i>	Hemodialysis patients (n=7)	↓ Serum <i>p</i> -cresol	144, 167
Probinul neutro®	CKD patients stages 3–4 (n=30)	↓ Total plasma <i>p</i> -cresol	140
Oral adsorbent			
AST-120	Adult patients with moderate to severe CKD (n=132) ¹⁴⁷ ; Adult patients with moderate to severe CKD (n=2035) ¹⁴⁹	↓ Indoxyl sulfate in a dose-dependent manner ¹⁴⁷ ; Unable to demonstrate a beneficial effect of AST-120 on progression of CKD ¹⁴⁹	147, 149
α-galactosidase inhibitor			
Acarbose (an inhibitor of the α -glucosidase enzymes)	Healthy volunteers (n=9)	↓ Serum concentrations of <i>p</i> -cresol; ↓ Urinary excretion of <i>p</i> -cresol; ↑ Fecal excretion of nitrogen increased	168
Genetically engineered bacteria			
Microencapsulated genetically engineered live <i>E. coli</i> /DH5 cells	Uremic rats	↓ Plasma urea concentration; ↓ Plasma ammonia concentration	146
Ongoing studies			
SYNERGY - HMW inulin, fructooligosaccharides and galactooligosaccharides plus strains from the <i>Lactobacillus</i> , <i>Bifidobacteria</i> and <i>Streptococcus</i> genera	Patients with moderate to severe CKD stages 4–5 (n=37)	Ongoing study aiming to assess the effectiveness of synbiotics on the synthesis of uremic toxins indoxyl sulfate and <i>p</i> -cresol sulfate	150
Arabinoxylan-oligosaccharides (AXOS)	Patients with CKD stage 3b-4	Ongoing study aiming to assess whether AXOS can decrease intestinal generation and serum concentrations of microbial metabolites in patients with CKD	169

CKD, chronic kidney disease; HMW, high-molecular-weight; IL, interleukin; mRNA, messenger RNA; TNF- α , tumor necrosis factor α ;