Symposium Report

Gut microbiome in chronic kidney disease

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New Findings

- What is the topic of this review?
  This review addresses the contribution of the altered gut microbiome to uraemic syndrome, with specific reference to gut microbiome-derived uraemic toxins. It also discusses the potential treatment options to normalize the disturbed microbiome in chronic kidney disease (CKD).

- What advances does it highlight?
  This review highlights the importance of the gut–kidney connection and how the altered microbial landscape in the intestine contributes to dysmetabolism and inflammation in CKD. Recent findings linking gut-derived uraemic toxins to progression of CKD, cardiovascular disease and mortality are also discussed. Finally, we briefly explain targeted therapies that have been studied to restore intestinal symbiosis in CKD.

The human intestine is now recognized as an important metabolic organ powered by gut microbiota. This review addresses the alteration in the gut microbiome in patients with chronic kidney disease (CKD) and its consequence. We describe the major uraemic toxins, p-cresol sulfate, indoxyl sulfate and trimethylamine N-oxide, which are produced by the gut microbiome, and how these metabolites contribute to progression of CKD and associated cardiovascular disease. Translocation of endotoxin from the gut into the systemic circulation contributes to inflammation in CKD. Targeting the gut microbiome to restore symbiosis may prove to be a potent strategy in reducing inflammation and production of these uraemic toxins.

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What constitutes our gut microbiome?

A healthy adult harbours about 100 trillion bacteria in the gut alone, which is 10 times the number of human cells. The genome of the gut microbiota is 150 times larger than the human genome and contributes over 3 million genes. The gut microbiome has co-evolved with humans, and this symbiotic relationship has expanded our metabolic and biosynthetic capabilities well beyond what is coded in our genomes. The metabolic potential of the gut microbiome is enormous and is claimed to be equal to that of the liver.

In 2007, the National Institutes of Health invested 170 million dollars in the Human Microbiome Project (HMP) in order to characterize the human microbiome (Turnbaugh et al. 2007). The investigators reported substantial variation between individuals in the microbiome profile and also intra-individual variation between different body sites. The large intestine is 150 cm long and has a surface area of 1.3 m², giving a home to billions of bacteria. More than 50 bacterial phyla are known to colonize the gut, with Bacteroidetes and Firmicutes being the most common. The abundance and diversity of the bacteria in the gut increases from the proximal to distal parts of the intestine. Proteolytic bacteria, such as Bacteroidetes, are observed in the distal colon, whereas saccharolytic bacteria are located in the proximal colon. Proteolysis generates toxins; therefore,
the distribution of proteolytic bacteria in the distal colon minimizes the exposure to toxin.

What are the factors that modulate the gut microbiome?

The gut microbiome is relatively stable, but adapts dynamically to changing environment and health. A short-term controlled-feeding experiment study showed that short-term changes to the diet did not significantly alter the gut microbiome (Wu et al. 2011a). De Filippo and colleagues investigated the long-term effect of dietary pattern by comparing the faecal microbiota of Caucasian children and children in rural Africa, where the diet is plant based and high in fibre content (De Filippo et al. 2010). African children showed a significant enrichment of Bacteroidetes and a depletion of Firmicutes when compared with the European children. In addition, the bacteria present in the African children expressed genes related to cellulose and xylan hydrolysis. These results illustrate that these bacteria co-evolved with the polysaccharide-rich diet in Africans, which allowed them to maximize the energy intake from fibres.

Antibiotics can have a profound effect on the gut microbiome. For example, ciprofloxacin treatment results in a profound and rapid loss of diversity and richness of the microbiome, with a shift in the community composition as early as 3–4 days after drug initiation (Dethlefsen & Relman, 2011). One week after stopping antibiotics, the microbial communities began to return to their initial state, but the recovery was often incomplete. Epidemiological studies have shown that the decline in infectious diseases over the last 50 years has been accompanied by a steady rise in the incidence of allergic and autoimmune diseases in developed countries. The ‘hygiene hypothesis’ claims that alterations in the gut microbiome may be related to this phenomenon. In 2000, Nobel Laureate Lederberg called for an end to the ‘hygiene hypothesis’ to describe the altered pathogenic bacteria in the gut. A more current definition is a state in which intestinal flora have qualitative and quantitative changes in their metabolic activity and local distribution, when compared with a ‘normal’ functioning gut (Holzapfel et al. 1998). Mounting evidence indicates that an individual’s microbiome plays a role in risk for obesity, kwashiorkor, insulin resistance, atherosclerosis, immune dysregulation and susceptibility to infection in the general population.

A number of interesting and provocative associations between disease and microbiome are emerging, which remains to be confirmed. For instance, circulating levels of Proteobacteria in the Epidemiological Study on the Insulin Resistance syndrome (DESIR) cohort was associated with increased risk for cardiovascular events (Amar et al. 2013). Patients with progressive IgA nephropathy have a distinct microbiome profile, with higher percentages of some genera/species of Ruminococcaceae, Lachnospiraceae, Eubacteriaceae and Streptococcaceae (De Angelis et al. 2014). The microbiome has also been implicated in kidney stone formation. In 2008, nearly 300,000 infants in China acquired kidney stones from milk formula tainted with melamine, a plastics additive that was used illegally to bulk up the apparent protein content of the formula. It was shown that kidney stones could be formed from melamine and its co-crystallizing chemical derivative, cyanuric acid. Studies in rats showed that cyanuric acid can be produced in the gut by microbial (Klebsiella) transformation of melamine. Cyanuric acid was detected in the kidneys...
of rats administered melamine alone; the concentration increased significantly after *Klebsiella* colonization, and the melamine-induced toxicity in rats was attenuated by antibiotic treatment (Zheng *et al.* 2013).

The gut microbiome can also produce beneficial metabolites, such as short-chain fatty acids (SCFAs) that could be kidney protective. The role of SCFAs in acute kidney injury was examined in a mouse model of ischaemia–reperfusion injury (Andrade-Oliveira *et al.* 2015). Treatment with the SCFAs acetate, propionate and butyrate improved renal dysfunction caused by acute kidney injury and reduced local and systemic inflammation. Treatment with SCFAs also improved mitochondrial function and shifted the cell death pathway from apoptosis to autophagy. Germ-free mice are known to have increased susceptibility to acute kidney injury. Mice treated with acetate-producing bacteria also had better outcomes after acute kidney injury.

**Why do individuals with chronic kidney disease have dysbiosis?**

Vaziri *et al.* (2013a) studied the gut microbiome profile in patients and animal models with CKD using phylogenetic microarrays. Rats with CKD had a decrease in total richness of bacterial populations, with 175 operational taxonomic units difference between rats with CKD and control animals. The bacterial community structure was distinctive between the two groups, with some Bacteroidetes and Firmicutes less prevalent in rats with CKD. In humans, 24 patients with end-stage renal disease (ESRD) had 190 operational taxonomic units that had different abundances when compared with 12 control subjects. End-stage renal disease patients also had differences in the distribution of bacterial species, with increases in Firmicutes, Actinobacteria and Proteobacteria and decreases in bifidobacteria and lactobacilli.

A myriad of factors contribute to dysbiosis in patients with CKD, such as slowing of intestinal transit, decreases in digestive capacity, and secretion of ammonia and urea into the gut (Ramezani & Raj, 2014; Fig. 1). The cause of slow colonic transit time and frequent constipation observed in CKD and particularly haemodialysis patients seems to be multifactorial. Dietary restriction and low fibre consumption, lack of activity, use of phosphate binders and co-morbidities, such as diabetes and heart disease, might all contribute to the greater prevalence of constipation in these patients (Wu *et al.* 2004). Slowing of the intestinal transit permits proliferation of bacteria. Impaired protein digestion results in undigested protein being delivered to the colon, which also causes proliferation of proteolytic bacteria.

There is a minimal increase in the serum concentration of uric acid in advanced CKD owing to CKD-induced adaptive secretion of uric acid by the colon (Alderman *et al.* 1999). As a result of the urea/uric acid secretion in the gut, bacterial families possessing urease, uricase, phenol- and indole-forming enzymes are expanded, whereas the SCFA-forming bacteria are contracted in ESRD patients (Wong *et al.* 2014). Furthermore, increased secretion of ammonia and urea into the gut changes the pH, leading to the growth of pH-sensitive bacteria. End-stage renal disease (ESRD) had 190 operational taxonomic units that had different abundances when compared with 12 control subjects. End-stage renal disease patients also had differences in the distribution of bacterial species, with increases in Firmicutes, Actinobacteria and Proteobacteria and decreases in bifidobacteria and lactobacilli.

**Figure 1. The impact of intestinal dysbiosis in chronic kidney disease**

Delivery of undigested protein to the colon results in the proliferation of proteolytic bacteria. These bacteria ferment proteins and amino acids to generate potential uraemic toxins, including *p*-cresol, indoxyl sulfate and trimethylamine *N*-oxide. Impaired gut barrier function allows translocation of uraemic toxin into systemic circulation. This contributes to chronic kidney disease (CKD) progression, cardiovascular disease, protein energy wasting and CNS manifestations.
disease patients also have changes in diet, including a decrease in fibre intake, which decreases the bifidobacteria. Finally, frequent use of antibiotics and iron delivered in either oral or intravenous form could alter the microbial landscape in the gut.

**Does the gut microbiome generate uraemic toxins?**

Changes in the gut microbiome also contribute to the accumulation of uraemic toxins in CKD patients (Ramezani & Raj, 2014; Fig. 1). An interesting study examined the metabolomics profile of nine ESRD patients with an intact colon and six ESRD patients who had undergone colectomy (Aronov et al. 2011). Mass spectrometry detected >1000 metabolites that were different between the two groups; specifically, indoxyl sulfate, p-cresol sulfate and hippurate. High-throughput liquid chromatography confirmed the colonic origin of p-cresol sulfate and indoxyl sulfate. We will briefly discuss the most important uraemic toxins generated by gut bacterial metabolism. Several of these toxins are protein bound; hence, they are resistant to removal by dialysis.

**Indoles:** indoxyl sulfate. Indolic compounds are produced by bacterial tryptophanase from tryptophan. The most extensively studied uraemic indole, indoxyl sulfate, is normally cleared by proximal tubules of the kidneys, but accumulates in CKD patients because of the impaired renal function (Watanabe et al. 2011). Cellular transport of indoxyl sulfate in the proximal tubules is mediated by the organic anion transporter (OAT) 1 and OAT3, expressions of which are shown to be reduced in experimental models of renal failure (Enomoto et al. 2002). A prospective, observational study performed in 268 patients with CKD indicated that the baseline concentration of indoxyl sulfate was a predictor of CKD progression (Wu et al. 2011b). Animal studies suggest that this uraemic toxin may damage renal tubular cells (Satoh et al. 2003). In uraemic rats, administration of indoxyl sulfate mediates the renal expression of genes related to tubulointerstitial fibrosis, such as TGF-β1 and tissue inhibitor of metalloproteinases (Miyazaki et al. 1997).

Indoxyl sulfate induces oxidative stress in endothelial cells, increases shedding of endothelial microparticles, increases vascular smooth muscle cell proliferation and impairs the endothelial cell repair mechanism. Barreto et al. (2009) showed that an elevated level of indoxyl sulfate is associated with vascular stiffness, aortic calcification and higher cardiovascular mortality.

**Phenols:** p-cresol sulfate. Partial breakdown of tyrosine and phenylalanine by intestinal bacteria genera, including *Bacteroides*, *Bifidobacterium*, *Lactobacillus*, *Enterobacter* and *Clostridium*, generates phenols, such as phenylacetic acid and p-cresol. Most of the phenols produced in the colon are absorbed and modified by sulfate or glucuronide conjugation in the liver. Meijers et al. (2010a) measured p-cresol levels in 499 patients with mild-to-moderate CKD and showed that p-cresol sulfate levels increased with decreasing estimated glomerular filtration rate. Multivariate analysis showed that a higher baseline concentration of p-cresol was an independent predictor for cardiovascular events, even after adjusting for estimated glomerular filtration rate and Framingham risk factors. Likewise, an elevated p-cresol concentration was associated with increased risk of death in ESRD patients treated with maintenance haemodialysis (Bammens et al. 2006).

**Amines:** trimethylamine N-oxide (TMAO). Trimethylamine N-oxide is a metabolite that was recently found to be associated with atherosclerosis and increased risk for major cardiovascular events (Wang et al. 2011). Gut bacteria convert choline and betaine present in food into trimethylamine, which is then oxidized into TMAO. The role of TMAO in CKD has also been examined. In a cohort with ~500 CKD patients, Tang et al. (2015) found that TMAO concentrations were elevated in patients with CKD. These elevated concentrations were associated with a 70% higher risk for all-cause mortality even after adjusting for traditional risk factors and C-reactive protein. Elevated TMAO concentrations in animal models were associated with corresponding increases in tubulointerstitial fibrosis and collagen deposition. Animals with increased TMAO levels also had increased fibrosis and phosphorylation of Smad3, an important regulator of fibrosis. Further studies are needed to see whether TMAO plays a role in progression of CKD.

**Does dysbiosis contribute to inflammation in CKD?**

Postnatal colonization of the intestine by bacteria educates our immune system and reduces allergic responses to food and environmental antigens. The segmented filamentous bacteria live in the distal ileum and recruit T-helper 17 cells, which increase production of interleukin-17 and interleukin-22 in animals. The maturation of the immune system seems to be co-directed by segmented filamentous bacteria in the ileum and *Clostridia* in the colon. The segmented filamentous bacteria have not been detected in humans, but *Bacillus fragilis* secretes a polysaccharide that regulates maturation of T cells. There is evidence that SCFAs are important in regulating the immune response by influencing T-cell differentiation and proliferation and reducing pro-inflammatory cytokine expression initiated by Toll-like receptor signalling (Smith et al. 2013; Barrows et al. 2015).
Microbial stimuli are known to alter immune and inflammatory responses. Jang et al. (2009) observed that normal kidneys of germ-free mice exhibited more natural killer T (NKT) cells and lower interleukin-4 levels, whereas postischaemia, more CD8 T cells trafficked into postischaemic kidneys of germ-free mice with more severe renal structural injury and functional decline following ischaemia–reperfusion injury compared with control mice. Wingender et al. (2012) analysed the invariant natural killer T (iNKT) cells in germ-free mice and found that intestinal microbes can affect the iNKT cell phenotypes and functions in mice. Moreover, they showed that the effects of intestinal microbes on iNKT cell responsiveness did not require Toll-like receptor signals.

Patients with CKD have immune dysregulation accompanied by evidence of systemic inflammation (Gupta et al. 2012). In a series of in vivo and in vitro studies using rats with CKD and human colonocytes, Vaziri and colleagues demonstrated that breakdown of the colonic epithelial tight junction by NH$_3$/NH$_2$OH causes dissociation, retraction and degradation of transcellular tight junction proteins, impairing the barrier function and enabling translocation of endotoxin and other noxious luminal contents to the intestinal wall and systemic circulation (Vaziri et al. 2012a,b, 2013b). Translocated endotoxin from the gut has been suggested as one of the specific causes for inflammation in CKD. Raj et al. (2009) measured the plasma concentrations of endotoxin and its soluble receptor sCD14 in patients with ESRD and showed that sCD14 is an independent predictor of mortality. Whether altered gut microbial community is a cause for the state of micro-inflammation in CKD remains to be elucidated.

**Is it possible to establish symbiosis in CKD?**

A number of interventions have been proposed to establish symbiosis and adsorptive removal of gut-derived uraemic toxins, with variable success (Ramezani & Raj, 2014). Probiotics are defined as ‘live micro-organisms’ that, when administered in adequate amounts, confer a health benefit on the host. In a double-blind placebo randomized controlled trial, Guida et al. (2014) showed that a 4 week synbiotic treatment (Probinul neutro®) reduced the plasma level of p-cresol in CKD patients. However, treatment with an enteric capsule preparation of *Bifidobacterium longum* had a minimal effect on progression of CKD (Ando et al. 2003).

A prebiotic is a non-digestible food ingredient that has a beneficial effect through its selective stimulation of the growth or activity of one or more types of bacteria in the colon. Meijers et al. (2010b) showed that oligofructose inulin significantly reduced p-cresol sulfate generation rates and serum concentrations in haemodialysis patients but had no effect on indoxyl sulfate. When given to rats, a diet high in amylose-resistant starch slowed the progression of CKD and attenuated oxidative stress and inflammation (Vaziri et al. 2014). Synbiotic (combining *Lactobacillus casei*, *Bifidobacterium breve* and galacto-oligosaccharides) therapy also decreased the plasma p-cresol concentration in maintenance haemodialysis patients (Nakabayashi et al. 2011). An adsorbent, AST-120, was shown to decrease plasma indoxyl sulfate in a dose-dependent manner (Schulman et al. 2006). Although small randomized controlled trials suggested a renoprotective effect for AST-120, the subsequent large-scale multicentre randomized controlled trial could not confirm it (Schulman et al. 2015).

**Where do we go from here?**

Emerging science indicates that interaction of the human host with our resident microbes could have significant impact on health and disease. The structure and composition of the gut flora could be due to an adaptive response to the uraemic state, which might have become maladaptive and lead to increased generation of uraemic toxins. The Human Microbiome Project was launched by the National Institutes of Health as a ‘roadmap’ for discovering the role of the microbiome in human health and disease. It is evident that the gut microbiome is altered in patients with kidney disease, but meticulous characterization using state-of-the-art metagenomics approaches and integrating it with metabolomics will lead to novel discoveries that could pave the way for future targeted therapies.

**References**


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Additional information

Competing interests

None declared.

Author contributions

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