Second Prize Essay

METABOLOME AND MICROBIOME IN KIDNEY DISEASE

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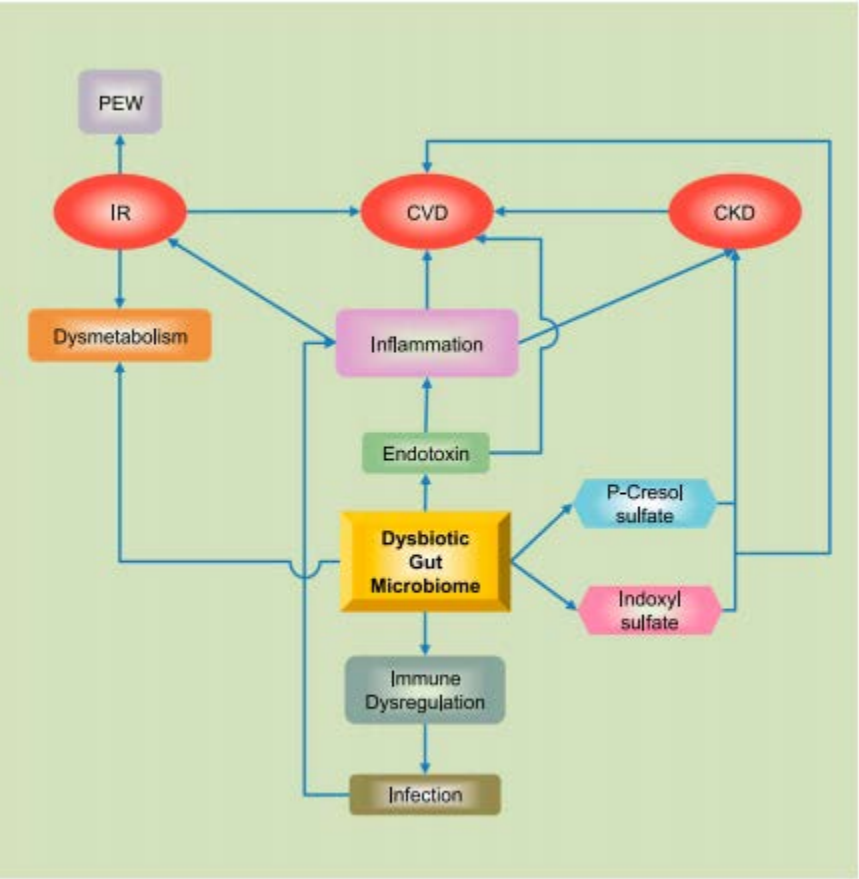
Alteration in the metabolism and gut microbiome have big impacts in the development of kidney disease. The application of metabolomics in nephrology research is slowly expanding and is useful in identifying decreased mitochondrial function in diabetic nephropathy and a preference for aerobic glucose metabolism in Polycystic kidney disease.

The human gut harbors more than 100 trillion microbial cells, which influence the nutrition, metabolism, physiology, and immune function of the host. The gut microbiota has coevolved with humans for a mutually beneficial coexistence and plays an important role in health and disease [1]. Normal gut microbiota influences the well-being of the host by contributing to its nutrition, metabolism, physiology, and immune function [2]. Disturbance of normal gut microbiota (dysbiosis) has been implicated in the pathogenesis of diverse illnesses, such as obesity, type 2 diabetes, inflammatory bowel disease, and cardiovascular disease [3].

Gut microbiota: An endogenous organ

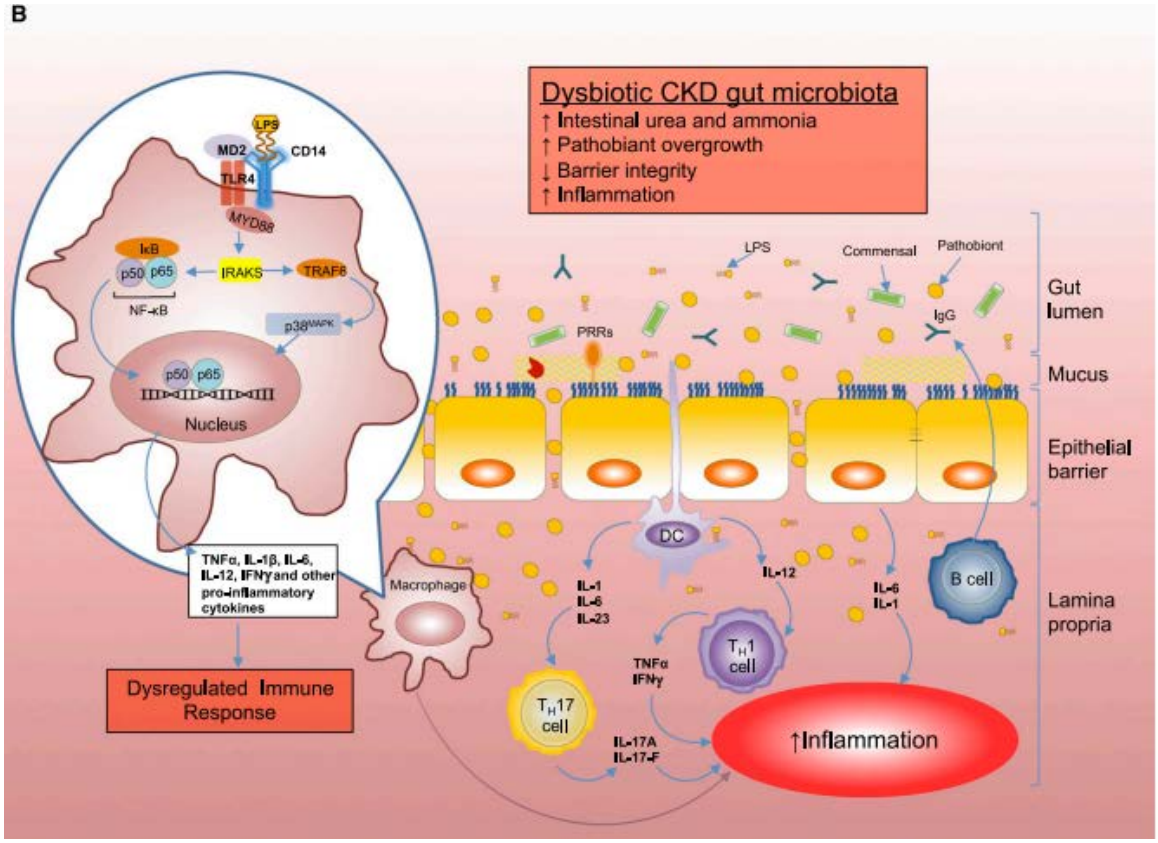
The combined microbial genome of the gut microbiota is known as the gut microbiome. In general, the adult gut is dominated by two bacterial phyla, Firmicutes and Bacteroidetes; other phyla, including Actinobacteria, Proteobacteria, Verrucomicrobia, Cyanobacteria, Fusobacteria, Spirochaetes, and TM7, are present in smaller proportions [4]. Each species of bacteria colonizes a specific niche, leading to different bacterial compositon along the intestinal tract. Gut microbiota performs a multitude of functions and can be considered a metabolically active endogenous organ. It participates in complementary metabolic activities such as breakdown of undigestible plant polysaccharides, synthesis of certain vitamins [5], biotransformation of conjugated bile acids and degradation of dietary oxalates. Quantitative and qualitative alterations in gut microbiota are noted in patients with CKD and ESRD. Endotoxin derived from gut bacteria incites a powerful inflammatory response in the host organism. Furthermore, protein fermentation by gut microbiota generates myriad toxic metabolites, including p-cresol and indoxyl sulfate. Disruption of gut barrier function in chronic kidney disease (CKD) allows translocation of endotoxin and bacterial metabolites to the systemic circulation, which

contributes to uremic toxicity, inflammation, progression of CKD and associated cardiovascular disease.



Gut Microbiome in CKD/ESRD –

Uremic patients show greatly increased counts of both aerobic and anaerobic organisms in the duodenum and jejunum, normally not colonized heavily by bacteria in healthy persons. Lower intestinal microbial flora is found to be altered in patients with CKD, most notably with decreases in both Lactobacillaceae and Prevotellaceae families. Analysis of the fecal microbiota in hemodialysis patients revealed a disturbed composition of the microbiota characterized by an overgrowth of aerobic bacteria [6]. The number of aerobic bacteria, such as Enterobacteria and Enterococci species, was approximately 100 times higher in hemodialysis patients. Of the anaerobic bacteria, hemodialysis patients had significantly lower numbers of Bifidobacteria and higher Clostridium perfringens. Patients with ESRD were also at a high risk of Clostridium difficile–associated diarrheas [7].



The intestinal dysbiosis may be due to iatrogenic causes or uremia per se. Loss of kidney function leads to secretion of urea into the gastrointestinal tract. Subsequent hydrolysis of urea by urease expressed by some gut microbes, results in the formation of large quantities of ammonia, which could affect the growth of commensal bacteria [8]. Other contributing factors include decreased consumption of dietary fiber, frequent use of antibiotics, slow colonic transit, metabolic acidosis, intestinal wall edema, and possibly oral iron intake.

Gut Barrier Function in Chronic Kidney Disease –

The gastrointestinal system is at the interphase between the blood and the potentially toxic contents of the gut. Histologic changes, including reduction of villous height, elongation of the crypts, and infiltration of lamina propria with inflammatory cells are noted in CKD . Uremia increases intestinal permeability in patients with CKD [9]. The disruption of colonic epithelial tight junction could subsequently lead to translocation of bacteria and endotoxin across the intestinal wall. Studies in uremic rats have shown marked azotemia, systemic oxidative stress,

and marked depletion of the key protein constituents of the epithelial tight junction (claudin-1, occludin, and ZO1) in the stomach, jejunum, and ileum, as well as penetration of bacteria across the intestinal wall and localization in the mesenteric lymph nodes. Hemodialysis induced systemic circulatory stress and recurrent regional ischemia may also damage the mechanical barrier of the gut. In addition, factors that promote intestinal dysbiosis may also contribute to the leaky gut in CKD. Gut microbiome dysbiosis is associated with bacterial translocation, thereby contributing to microinflammation in patients with ESRD.

Gut-Derived Uremic Toxins –

Certain intestinal bacteria can generate uremic toxins that are absorbed into the blood and are normally cleared by the kidney. Protein fermentation by gut microbiota results in the generation of different metabolites, including phenols and indoles. The studies have compared plasma from hemodialysis patients with and without colon and confirmed the colonic origin of indoxyl sulfate and p-cresol [10]. These are prototype members of a large group of protein bound uremic toxins that are resistant to clearance by dialysis. P-cresol, a 108-Da protein-bound solute, is a colonic fermentation product of the amino acid tyrosine and phenylalanine. Most of the p-cresol generated by the intestinal flora is conjugated to p-cresol sulfate in the intestinal wall and to p-cresol glucuronide in the liver. Intestinal bacteria also have tryptophanase that converts tryptophan to indole, which is subsequently absorbed and metabolized to indoxyl sulfate in the liver. It has been shown that an elevated level of indoxyl sulfate is associated with vascular stiffness, aortic calcification, and higher cardiovascular mortality [11]. Indoxyl sulfate is a potential vascular toxin that induces oxidative stress in endothelial cells, increases shedding of endothelial microparticles, impairs endothelial cell repair mechanism, and increases vascular smooth muscle cell proliferation. It is reported that free serum levels of p-cresol is associated with mortality in hemodialysis patients [12]. Thus, gut-derived uremic toxins contribute to progression of CKD as well as cardiovascular disease.

Targeted interventions to treat intestinal dysbiosis –

Most therapies targeting the colonic microenvironment in CKD aim to modulate gut microbiota, block LPS or attenuate inflammation, or target adsorption of uremic toxin end products of microbial fermentation.

Prebiotic - A prebiotic is a nondigestible (by the host) food ingredient that has a beneficial effect through its selective stimulation of the growth or activity of one or a limited number of bacteria in the colon. The candidate prebiotics include inulin, fructo-oligosaccharides, galacto-

oligosaccharides, soya-oligosaccharides, xylo-oligosaccharides, and pyrodextrins. Prebiotics promote the growth of Bifidobacteria and Lactobacilli species at the expense of other groups of bacteria in the gut, such as Bacteroides species, Clostridia species, and enterobacteria. Preliminary evidence indicates that prebiotic oligofructose-enriched inulin (p-inulin) promotes growth of Bifidobacteria species, mediates weight loss, reduces inflammation, and improves metabolic function [13]. High dietary fiber intake is associated with lower risk of inflammation and reduced mortality in patients with CKD.

Probiotics- Probiotics are defined by the United Nations' Food and Agriculture Organization and the World Health Organization as "live microorganisms" that when administered in adequate amounts confer a health benefit on the host. Probiotics consist of living bacteria, such as Bifidobacteria species, lactobacilli, and streptococci, that can alter gut microbiota and affect the inflammatory state. Treatment with Bacillus pasteurii and Sporlac slowed the progression of kidney disease and prolonged the life span of 5/6 nephrectomized rats. Hemodialysis patients treated with oral Lactobacillus acidophilus showed decreased serum dimethylamine, a potential uremic toxin [14].

Acarbose – it is an inhibitor of alpha-glucosidase enzymes in the intestinal brush-border that blocks the hydrolysis of poly- and oligosaccharides to glucose and other monosaccharides. The undigested oligosaccharides that enter the colon act as fermentable carbohydrates. Studies have showed that treatment with acarbose reduces the colonic generation of p-cresol in healthy persons [15.]

Blocking of LPS/Attenuation of Inflammation - Sevelamer is a large cationic polymer phosphate binder that binds endotoxin in both invitro and invivo studies [16]. Endotoxin level is found to be lower in hemodialysis patients on sevelamer.

Metabolomics in kidney disease -

Metabolomics, or metabolite profiling, refers to the systematic analysis of metabolites (i.e., sugars, amino acids, organic acids, nucleotides, bile acids, acylcarnitines, lipids, etc.) in a biologic specimen [17]. Metabolomic approaches are particularly promising in nephrology research because of the broad impact kidney function has on circulating metabolite levels and because metabolites may themselves play functional roles in CKD pathogenesis and its complications.

The technological advances in chromatography and mass spectrometry, have enabled more metabolites to be measured simultaneously and more rapidly in smaller sample volumes. This has enabled unbiased examinations of blood and urine from individuals with kidney disease, in some cases highlighting novel metabolite perturbations. Second, they have catalyzed examination of large cohorts, enhancing statistical power for biomarker studies of cross-sectional phenotypes and longitudinal outcomes.

Metabolomics of Clinical Diabetic kidney disease (DKD) –

Many recent studies have aimed at identifying metabolites that are differentially expressed in DKD using a variety of metabolomic approaches and biosamples. An overall conclusion from recent studies is that robust changes in metabolites of the TCA cycle, lipid metabolism, amino acid metabolism, urea cycle and nucleotide metabolism are associated with DKD. The metabolites including amino acids, aspartic acid, citruline, symmetric dimethylargine (SDMA) and kynurenine were found to be increased in DKD. Aspartic acid and citrulline are involved in the production of urea in the urea cycle. SDMA and asymmetric dimethylarginine (ADMA) are formed by the enzymatic methylation of arginine. While kidneys further metabolize ADMA, SDMA is excreted directly into the urine [18]. Tryptophan is metabolized to kynurenine and further metabolized to acetyl-CoA and NAD in the tryptophan-kynurenine pathway. Several of these metabolites have also been identified in CKD without diabetes, suggesting that these could be markers for renal dysfunction [19] .

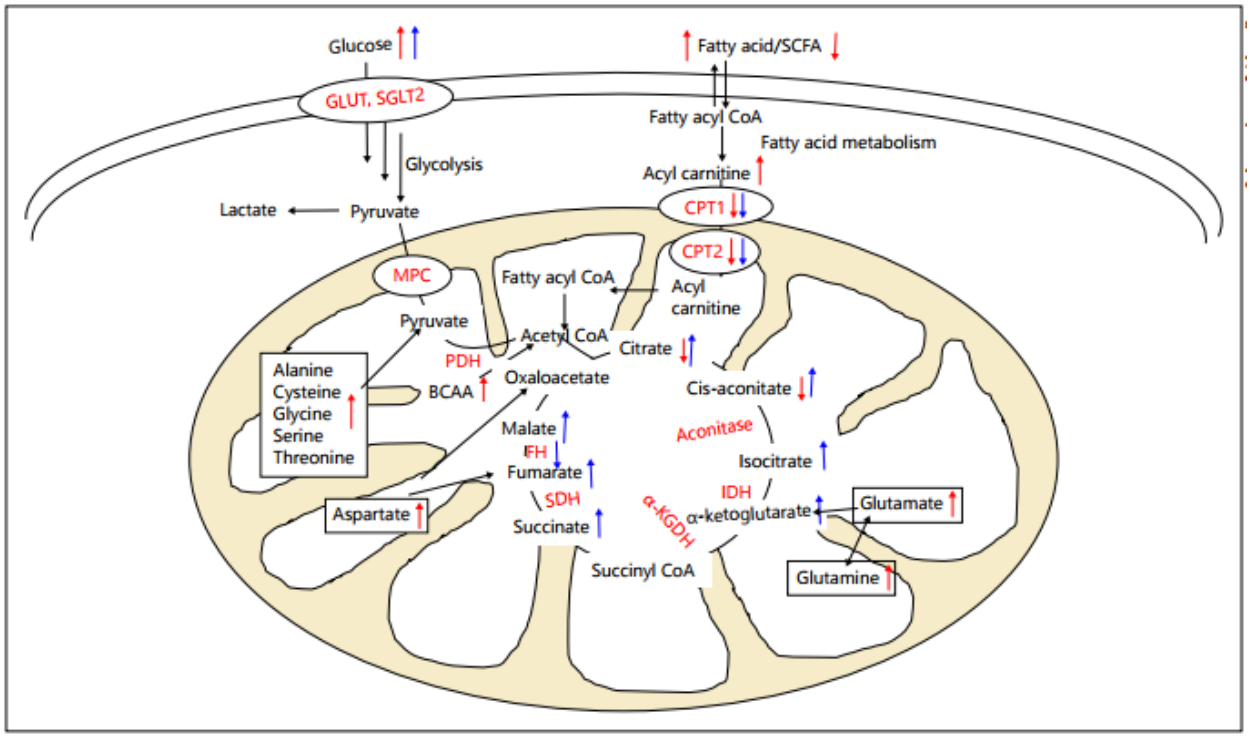


Figure 1 -- Overview of urinary metabolite changes in TCA cycle, fatty acid and amino acid metabolism, and affected enzymatic pathways in DKD clinical and preclinical studies. MPC = Mitochondrial pyruvate carrier; PDH = pyruvate dehydrogenase complex; IDH = isocitric dehydrogenase; α-KGDH = alpha ketoglutarate dehydrogenase

A few metabolies like octanol, oxalic acid, phosphoric acid, benzamide, creatinine, 3,5-dimethoxymandelic amide and N-acetylglutamine were selected as the best predictors of eGFR decline [20]. Indoxyl sulfate, a well-known uremic toxin, with significant correlations with low eGFR. Amino acids and acyl carnitines have been identified as predictors of progression of DKD in several studies. Elevated concentrations of branched chain and aromatic amino acids were identified as predictors of diabetes 12 years prior to the onset of kidney disease in a longitudinal plasma metabolomic analysis [21]. Acyl carnitines, acyl-glycines and intermediates of tryptophan metabolism were found to correlate with changes in albuminuria and are associated with the progression of DKD in urine samples of Type 1 diabetic patients [22]. Plasma metabolites histidine, butenoylcarnitines, and urine metabolites hexose, glutamine and tyrosine were identified as predictors for progression to micro- and macro-albuminuria in T2 diabetic and hypertensive patients [23]

Metabolomics Technologies –

Nuclear magnetic resonance spectroscopy (NMR) uses the magnetic properties of select atomic nuclei to determine the structure and abundance of metabolites in a specimen. It requires relatively little sample preparation and does not require up-front chromatography. However, because of limited sensitivity and high data complexity, unambiguous identification is typically limited to less than 100 metabolites. Mass spectrometry (MS)-based approaches, generally coupled to an array of separation techniques including liquid chromatography (LC) and gas chromatography (GC), have higher sensitivity and rely on a combination of chromatographic separation and mass-to-charge ratio (m/z) resolution for metabolite identification.

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Disclosure

The author reports no disclosures relevant to the manusript

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