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# Chronic kidney disease alters intestinal microbial flora

Nosratola D. Vaziri[1], Jakk Wong[2], Madeleine Pahl[1], Yvette M. Piceno[2], Jun Yuan[1], Todd Z. DeSantis[3], Zhenmin Ni[1], Tien-Hung Nguyen[2] and Gary L. Andersen[2]

*[1]Division of Nephrology and Hypertension, UC Irvine Medical Center, Irvine, California, USA; [2]Center for Environmental Biotechnology, Lawrence Berkeley National Laboratory, Berkeley, California, USA and [3]Second Genome, San Bruno, California, USA*

**The population of microbes (microbiome) in the intestine is a symbiotic ecosystem conferring trophic and protective functions. Since the biochemical environment shapes the structure and function of the microbiome, we tested whether uremia and/or dietary and pharmacologic interventions in chronic kidney disease alters the microbiome. To identify different microbial populations, microbial DNA was isolated from the stools of 24 patients with end-stage renal disease (ESRD) and 12 healthy persons, and analyzed by phylogenetic microarray. There were marked differences in the abundance of 190 bacterial operational taxonomic units (OTUs) between the ESRD and control groups. OTUs from Brachybacterium, Catenibacterium, Enterobacteriaceae, Halomonadaceae, Moraxellaceae, Nesterenkonia, Polyangiaceae, Pseudomonadaceae, and Thiothrix families were markedly increased in patients with ESRD. To isolate the effect of uremia from inter-individual variations, comorbid conditions, and dietary and medicinal interventions, rats were studied 8 weeks post 5/6 nephrectomy or sham operation. This showed a significant difference in the abundance of 175 bacterial OTUs between the uremic and control animals, most notably as decreases in the Lactobacillaceae and Prevotellaceae families. Thus, uremia profoundly alters the composition of the gut microbiome. The biological impact of this phenomenon is unknown and awaits further investigation.**

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**Correspondence:** *Nosratola D. Vaziri, Division of Nephrology and Hypertension, UC Irvine Medical Center, Suite 400, City Tower, 101 City Drive, Orange, California 92868, USA. E-mail: ndvaziri@uci.edu*

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The large community of microbes residing in the intestinal tract (microbiome) constitutes a dynamic and symbiotic ecosystem that is in constant interaction with the host metabolism.[1–3] Under normal conditions, the gut microbiome provides trophic[2] and protective[4] functions. In addition, the normal microbial flora influences energy metabolism[5] by facilitating absorption of complex carbohydrates and contributes to the nitrogen[6] and micronutrient homeostasis via synthesis of amino acids, such as lysine and threonine,[7] and various vitamins, such as vitamin K[6] and group B vitamins.[8]

Alteration in the functions or signaling pathways of the commensal flora contributes to the pathogenesis of diverse illnesses such as inflammatory bowel disease,[9] chronic inflammation, dyslipidemia, diabetes,[10] atopic disorders,[11] cardiovascular diseases, neoplasms,[12] and obesity.[13] The biochemical milieu has a decisive part in shaping the structure, composition, and function of the microbial flora. Uremia can profoundly modify the biochemical milieu of the gut via heavy influx of urea into the gastrointestinal tract and secretion of uric acid and oxalate by the colonic epithelium.[14–16] In addition, therapeutic interventions, including dietary restriction of fruits, vegetables, and high-fiber products to prevent hyperkalemia and oxalate overload, use of phosphate-binding agents to manage hyperphosphatemia, and administration of antibiotics to treat vascular access and other infections can modify the luminal milieu of the gut and impact its microbial flora. Alteration of microbial flora in inflammatory bowel diseases contributes to and may be exacerbated by the disruption of the gut epithelial barrier function and structure. This enables leakage of the luminal antigens and other noxious contents into the intestinal wall and the systemic circulation.[17]

Several observations suggest that uremia impairs intestinal barrier function and promotes inflammation throughout the gastrointestinal tract. This is based on the reported increase in intestinal permeability to high-molecular-weight polyethylene glycols in uremic humans and animals,[18,19] penetration of bacteria across the intestinal wall and their detection in the mesenteric lymph nodes in uremic rats,[20] the presence of endotoxemia in patients with end-stage renal disease (ESRD),[21,22] recent demonstration of the disruption of colonic epithelial tight-junction apparatus in the uremic

rats,[23] and histological evidence of chronic enterocolitis in ESRD patients maintained on dialysis.[24,25] These events can clearly contribute to systemic inflammation and oxidative stress, which are constant features of advanced chronic kidney disease (CKD) and the major mediators of cardiovascular disease, cachexia, anemia, and numerous other morbidities in this population.[26–30]

As noted above, uremia and its treatment can significantly alter the biochemical milieu of the intestinal tract and, as such, may alter the structure, composition, and function of microbial flora. This may disturb the symbiotic relationship that prevails under normal conditions and lead to the production and absorption of proinflammatory and otherwise harmful byproducts, and simultaneously limit the beneficial functions and products conferred by the normal flora. Such events can contribute to uremic toxicity, inflammation, and cardiovascular, nutritional, and other complications of CKD. The present study was designed to test the hypothesis that the biochemical modification of the gut milieu in advanced CKD can lead to significant changes in composition of the gut microbial flora.

## RESULTS

### General data

***Patients and controls.*** As expected, compared with the healthy control group, the ESRD patients had a significant increase in plasma concentrations of creatinine (8.6 ± 2.9 vs. 0.8 ± 0.1 mg/dl, $P<0.0001$) and urea nitrogen (70 ± 18.0 vs. 24.0 ± 9.9 mg/dl, $P<0.0001$) concentrations. All patients were treated with phosphate binders, erythropoiesis-stimulating agents, intravenous iron compounds, and multivitamin preparations. Strict dietary fluid and sodium, phosphorus, and potassium restrictions were prescribed to minimize fluid overload, hyperphosphatemia, and hyperkalemia. Patients received hemodialysis therapy for 3 h three times weekly using cellulose triacetate dialyzers. Systemic heparinization was used for anticoagulation during hemodialysis. The Kt/V in the ESRD group was 1.5 ± 0.3, reflecting adequacy of the dialysis regimen. The ethnic background of the ESRD group (9 Caucasians, 13 Hispanics, and 2 Asians) was similar to that of the control group (4 Caucasians, 7 Hispanics, and 1 Asian) Similarly, the body mass index in the ERSD group (30.4 ± 8.3) was comparable to that of the control (29.2 ± 6.1 kg/m$^2$, $P=0.65$).

***CKD and control rats.*** Data are summarized in Table 1. Compared with the sham-operated control group, the CKD group exhibited significant elevation of arterial pressure, increased urinary protein excretion, elevated plasma urea and creatinine concentrations, reduced hematocrit, and lower body weight.

### Microarray data

***Human data.*** Relative richness (the number of bacterial taxa in a sample) was assessed for subfamilies found in samples in each group. Although the mean relative richness (summarized at subphylum) for ESRD and control groups was similar (Figure 1a), the relative abundances (i.e., probe intensities) of bacterial groups within the subfamilies differed significantly. Significant increases (adjusted $P<0.02$) in relative abundance were found for 190 bacterial operational taxonomic units (OTUs) in the ESRD group compared with the control group. Many (159) of the OTUs that were

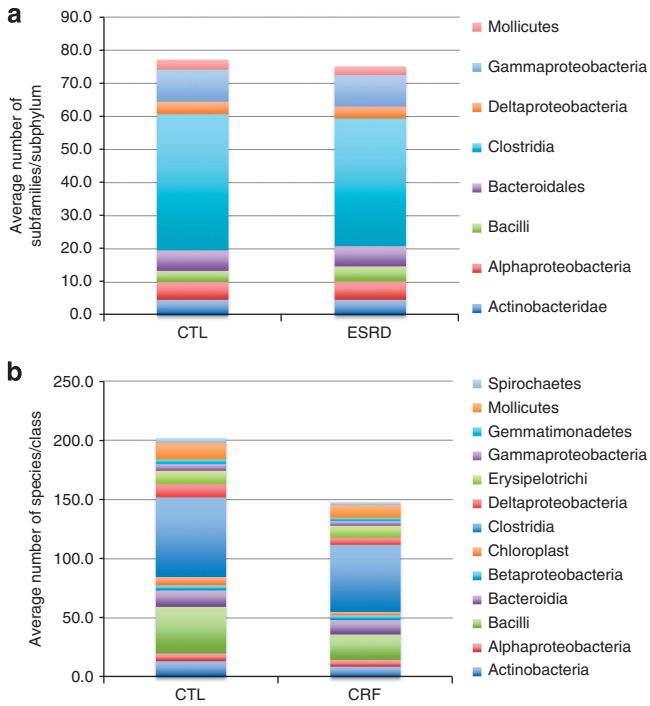


**Figure 1 | Relative richness of the gut microbiome in the study groups.** Relative richness comprised of the average number of (**a**) subfamilies per subphylum for control (CTL) or end-stage renal disease (ESRD) patients or (**b**) species per class for control (CTL) and chronic renal failure (CRF) rats. A subfamily or species had to be present in at least three replicates of a treatment group and also had to have an average of four subfamilies or species present in a subphylum or class to be included in the figure.

**Table 1 | BW, blood pressure, Hct, serum creatinine and urea concentration, Ccr, and urinary protein excretion in normal control rats and rats with CRF**

| | BW (g) | BP (mm Hg) | Hematocrit (%) | Creatinine (mg/dl) | Urea (mg/dl) | Ccr (ml/min/kg) | U Protein (mg/mg Cr) |
|---|---|---|---|---|---|---|---|
| CTL | 407 ± 5.9 | 120 ± 2.1 | 48 ± 1.2 | 0.61 ± 0.2 | 48 ± 3.3 | 8.8 ± 0.05 | 7.4 ± 0.5 |
| CRF | 374 ± 4.4 | 155 ± 2.5* | 35 ± 0.7* | 1.14 ± 0.2* | 93 ± 7.4* | 3.4 ± 0.03* | 81.5 ± 5.6* |

Abbreviations: BP, tail arterial pressure; BW, body weight; Ccr, creatinine clearance; Cr, plasma creatinine; CRF, chronic renal failure; CTL, control; Hct, hematocrit; U protein, urine protein excretion in the CRF and control rats.
Values are mean ± s.d.
*$P<0.05$ compared with CTL.