Colon

- It plays an important role in the homeostasis of electrolytes and minerals.
- Role in disposal of nitrogenous waste products.
- It is an important source of uremic toxins.
- Possible target as adjuvant therapy to decrease burden in CKD.
Colon in health

• ~ 150 cm long, SA: 1.3 m²
• colonic transit time: 20-140 hours

Role:
• disposal of waste products of digestion
• Absorption of salt and water (electrogenic via the epithelial sodium channel or electroneutral via parallel sodium/hydrogen and chloride/bicarbonate-exchange)
• Acid base homeostasis
• Potassium excretion
Gut microbiome

- Is a symbiotic ecosystem with different functions:
  1. protection against pathogenic organisms
  2. development and modulation of the human gut immune system
  3. facilitating absorption of complex carbohydrates
  4. participates in nitrogen homeostasis
  5. synthesizing amino acids (e.g., lysine and threonine)
  6. Synthesizing vitamins (e.g., vitamin K and group B vitamins).
Colonic bacteria

Saccharolytic: ferment carbohydrates

- production of short-chain fatty acids: Butyrate

Proteolytic: ferment protein

- Production of shorter branched-chain fatty acids, ammonia, amines, thiols, phenols, and indoles

uremic retention solutes

**p-cresyl sulfate**
- The end-product of the combined actions of bacterial fermentation of tyrosine to p-cresol and endogenous sulfate conjugation.
- Highly protein-bound,
- Linked to overall mortality
- Cardiovascular disease
- CKD progression
- Uremic concentrations:
  - Elicit oxidative stress
  - Induce endothelial dysfunction and cardiac remodeling
  - Accelerate CKD progression

**Indoxyl sulfate**
- The end-product of bacterial fermentation of tryptophan to indole followed by endogenous oxidation and sulfate conjugation.
- Highly protein-bound,
- Linked to overall mortality
- Cardiovascular disease
- CKD progression
- Uremic concentrations:
  - Elicit oxidative stress
  - Induce endothelial dysfunction and cardiac remodeling
  - Accelerate CKD progression

In healthy individuals, the phyla Bacteroidetes and Firmicutes contribute 90% of all species.

Yiang X et al. PloS One 2009 Jun 29;4(6)
Bacteroidetes

- Gram negative,
- involved in degradation of high molecular weight organic matter, i.e., proteins and carbohydrates.

Firmicutes

<table>
<thead>
<tr>
<th>Major groups within the Firmicutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacillales</td>
</tr>
<tr>
<td>Mollicutes, Planococaceae, Caryophanaceae</td>
</tr>
<tr>
<td>Bacillaceae</td>
</tr>
<tr>
<td>Staphylococcaceae</td>
</tr>
<tr>
<td>Sporolactobacillaceae</td>
</tr>
<tr>
<td>Listeriaceae</td>
</tr>
<tr>
<td>Brevibacillus, Poenibacillus</td>
</tr>
<tr>
<td>Ammoniphilus, Aeurinibacillus, Oxalophagus</td>
</tr>
<tr>
<td>Alicyclobacillaceae</td>
</tr>
<tr>
<td>Thermoactinomycetaceae</td>
</tr>
<tr>
<td>Lachnospiraceae</td>
</tr>
<tr>
<td>Peptostreptococcaceae</td>
</tr>
<tr>
<td>Eubacteriaceae</td>
</tr>
<tr>
<td>Clostridiaceae (with Veillonellaceae and Selenomonads)</td>
</tr>
<tr>
<td>Haloanaerobiales</td>
</tr>
<tr>
<td>Acidaminococcaceae</td>
</tr>
<tr>
<td>Moorella, Sulfobacillus</td>
</tr>
<tr>
<td>Thermoanaerobacter, Thermoanaerobium</td>
</tr>
</tbody>
</table>

- Gram positives
- Involved in obesity

Vollmer W. Nature Chemical Biology 8, 14–18 (2012)
Change in microbiome

- Inflammatory bowel disease
- Chronic inflammation
- Dyslipidemia
- Diabetes mellitus
- Cardiovascular diseases
- Neoplasms
- Obesity
- Atopic disorders
Microbiome change in CKD

Potential causes

• Dietary restrictions
• Drug therapy (e.g., phosphate binders, frequent antibiotic use)
• Volume overload
• Metabolic acidosis
Impaired protein assimilation in the small intestine $\rightarrow$ more protein available in colon

Colonic transit time increased $\rightarrow$ segments of carbohydrate-deprived colon

Bacterial mediated hydrolysis of urea $\rightarrow$ High levels of ammonia $\rightarrow$ high pH $\rightarrow$ overgrowth of proteolytic species.

Saccharolytic to a proteolytic fermentation

Symbiosis: pathobionts are kept in check, barrier integrity maintained

Dysbiosis: pathobiont overgrowth — promotes loss of barrier integrity

Translocation of bacteria and bacterial products activates systemic inflammation (and CKD/ESRD complications?)

Uremia induces dysbiosis

Proinflammatory cytokines

Macrophage

Defensins, mucus, IgA

Intestinal lumen

Intestinal epithelial barrier

Homeostatic signals

Healthy kidney

B cell

DC

Chronic kidney disease alters intestinal microbial flora

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

Humans: 24 patients (6M, 18F) with end-stage renal disease (ESRD) and 12 healthy persons (4M, 8F)
• Analysis of microbial DNA isolated from the stools by phylogenetic microarray.

Animals: 11 Male Sprague–Dawley rats used.
• fed a standard laboratory diet and water ad libitum.
• 6 rats underwent 5/6 nephrectomy by surgical resection of the upper and lower thirds of left kidney followed by right nephrectomy 7 days later.
• 5 rats underwent sham operation
16S rRNA gene regions for bacteria

- 16S rRNA gene sequences is used to study bacterial phylogeny and taxonomy.
- rDNA gene sequences are highly conserved within living organisms of the same genus and species,
- they differ between organisms of other genera and species.

- Widely used:
  (i) present in almost all bacteria
  (ii) the function of the 16S rRNA gene over time has not changed, suggesting that random sequence changes are a more accurate measure of time (evolution)
  (iii) the 16S rRNA gene (1,500 bp) is large enough for informatics purposes
PCR amplification
Microarrays

Amplify 16s rRNA gene sequence

Hybridize to proprietary library of 16S probes

Labeling target DNA with fluorescent dye

Attaching the probe DNA to the chip

Probe DNA

Hybridization and cleaning of target DNA

Capturing images with the CCD sensor

Identifying the hybridized probes by image processing
In patients

- mean relative richness (summarized at subphylum) for ESRD and control groups was similar.
- the relative abundances (i.e., probe intensities) of bacterial groups within the subfamilies differed significantly.
- Significant increases ($P < 0.02$) in relative abundance were found for 190 bacterial operational taxonomic units (OTUs) in the ESRD group compared with the control group.
- 159 of the OTUs that were significantly different between the study groups belonged to the Pseudomonadaceae family.
In rats

- Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria had the greatest number of species.
- Total richness was significantly greater (\(P < 0.0086\)) in the control group compared with the CRF group.
- Analysis of the number of species showed weak differences between groups (\(R = 0.356\)), with class Bacilli contributing the most (19%) to the difference between groups.
Diversity within and between groups

The control group samples showed tighter clustering than did the ESRD samples.
Diversity within and between groups

- Bacterial community structure was distinctive between normal and CRF rats

- Less prevalence of Bacteroidetes and Firmicutes families in the CRF rats, esp. Lactobacillaceae and Prevotellaceae

- Of the significantly different OTUs, 81 had at least a two-fold change in average probe intensity.

- Much less variability among samples within each group for rats compared with humans
X: rat fecal microbiota composition
Y: OTUs

decreased abundance
Conclusion

• significant differences in the abundance of 190 microbial OTUs between the ESRD and the normal control individuals.
• These OTUs were classified mostly in the families containing aerobic and facultative anaerobic bacteria.
• These observations point to a significant difference in the composition of the gut microbiome between the ESRD patients and healthy control individuals.
• Impact of uremia on the composition of the gut microbiome (differences in relative abundance of 175 microbial OTUs btw CRF and Control rats)
Conclusion

• the transition in the intestinal environment from healthy to CRF $\rightarrow$ a shift of the microbiota from an evenly distributed and complex community to one a simple and more dominated community.

• the profound alteration in the microbial flora found in the ESRD patients is, at least in part, due to uremia.