HIGH THROUGHPUT PYROSEQUENCING ANALYSIS OF UREMIC TOXINS PRODUCING GUT MICROBIOTA

Mami Kikuchi[1], Ryoko Tateoka[1], Yoshiharu Itoh[1], Wataru Suda[2], Masahira Hattori[2],
1 KUREHA CORPORATION, Pharmaceuticals Division, Tokyo, JAPAN,
2 The University of Tokyo, Graduate School of Frontier Sciences, Chiba, JAPAN.

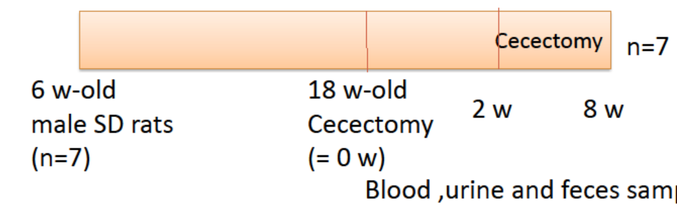
INTRODUCTION AND AIMS:

The gut microbiota plays an important role in health and the pathogenesis of various diseases including chronic kidney disease (CKD). It is also known to be involved in the production of uremic toxins (UTs). To elucidate the production of UTs by gut microbiota, we performed the analysis of the production of UTs and pyrosequencing of bacterial 16S rRNA gene of the gut microbiota in the cecectomy rats and the CKD model rats. We also examined the intervention effect of the oral spherical carbon adsorbent AST-120, which adsorb small molecular weight UTs and/or their precursors in intestine, to evaluate the role of UTs for gut microbiota.

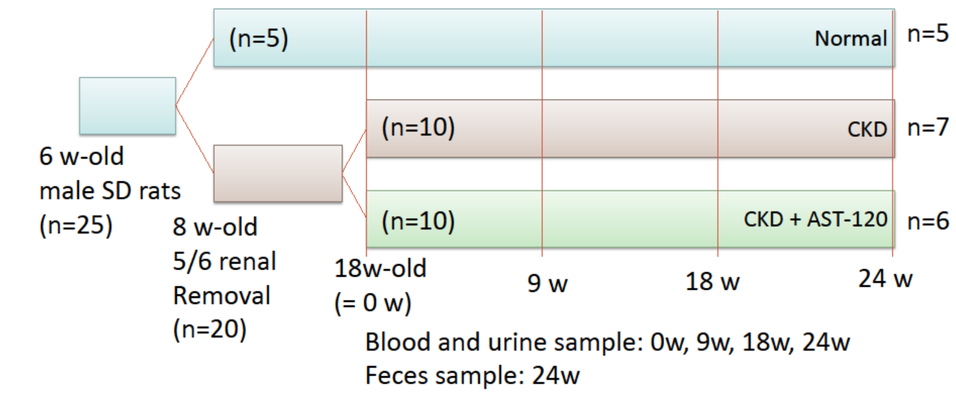
METHODS:

The collection of blood, urine and feces samples were carried in normal rats, CKD rats and cecectomy rats. Serum and urine levels of Indoxyl sulfate (IS) and p-cresyl sulfate (PCS) were quantified by SRM of LC/ESI-MS/MS. Gut microbiota from feces were analyzed using 454-pyrosequencing of a hypervariable V1-V2 region of the 16S rRNA gene in combination with barcode sequences. Operational taxonomic unit clustering and UniFrac analysis were performed. Relative abundances were calculated from taxonomic assignment.

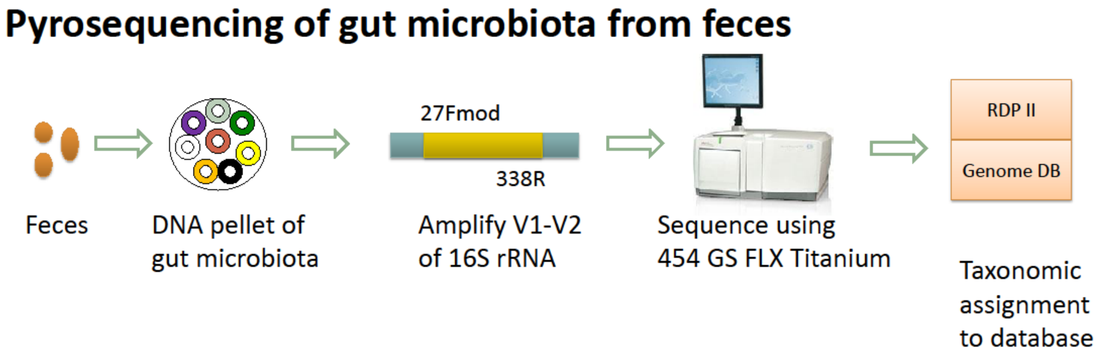
Experiment 1 (Cecectomy)



Experiment 2 (CKD)



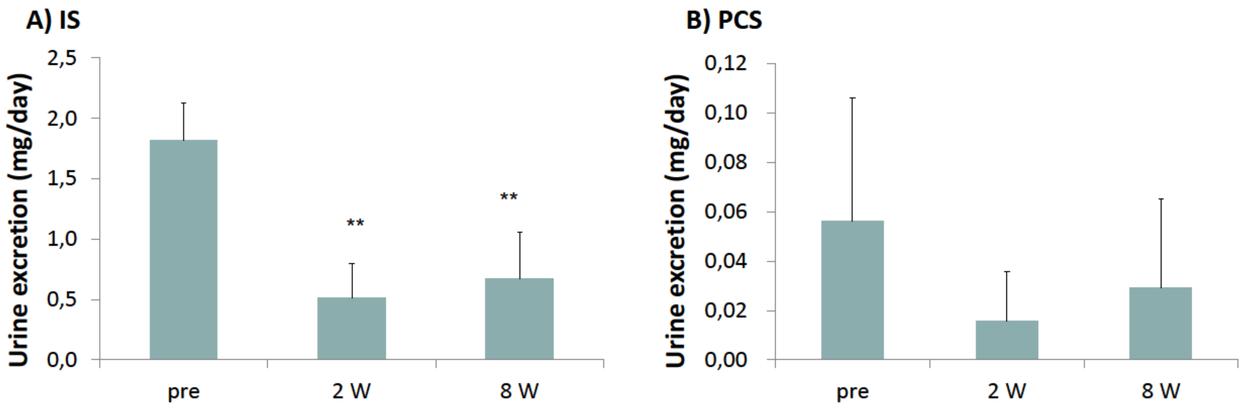
Pyrosequencing of gut microbiota from feces



RESULTS:

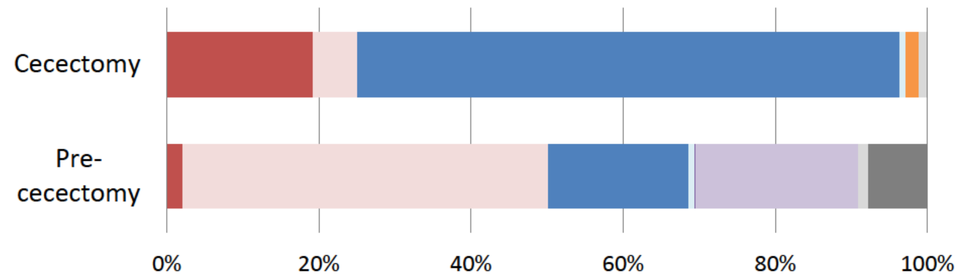
Experiment 1 (Cecectomy)

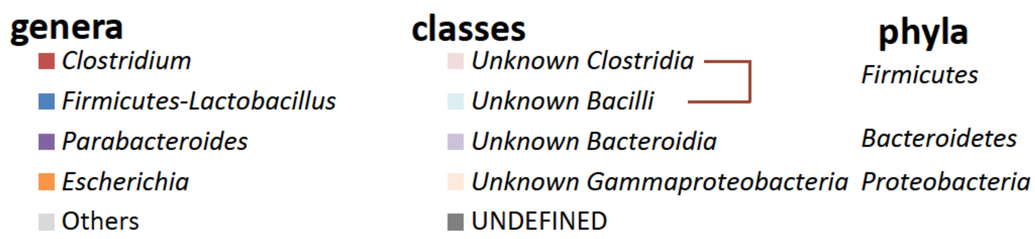
(1) Urinary excretions of UTs



Results represent mean ± SD. Student t-test was used, comparing post- and pre-operation, **P<0.01.

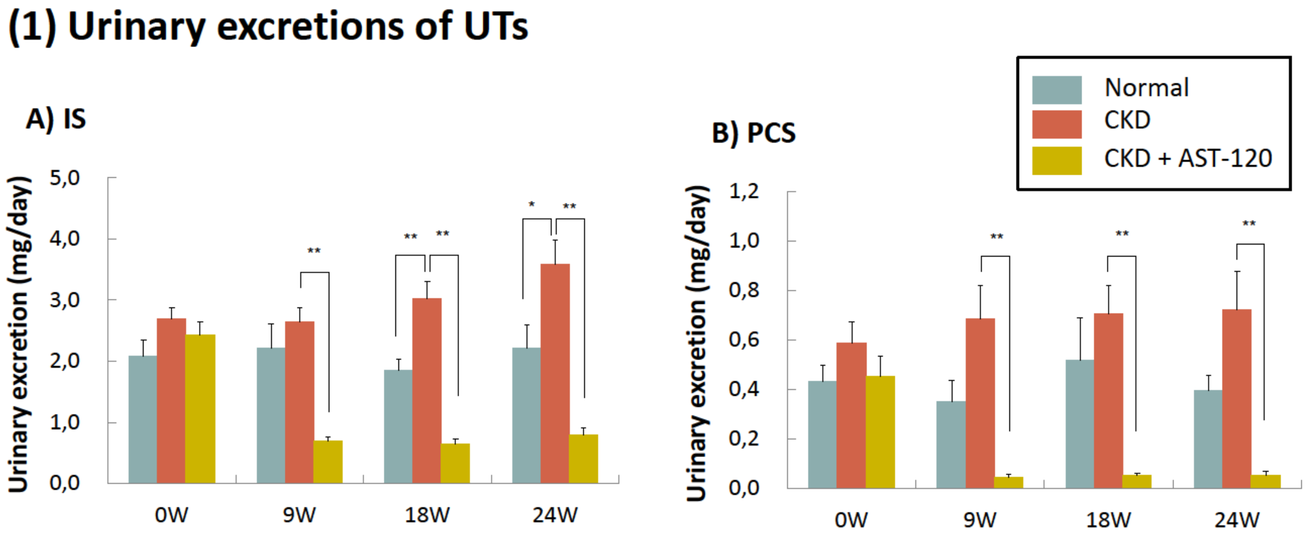
(2) Relative abundance of genera and classes





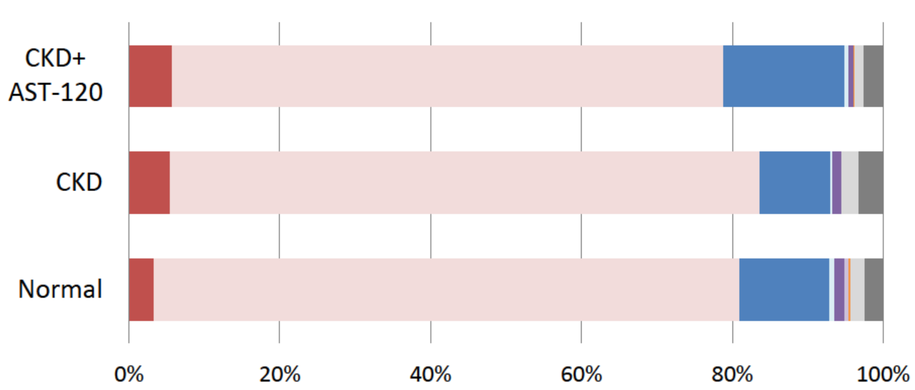
Experiment 2 (CKD)

(1) Urinary excretions of UTs

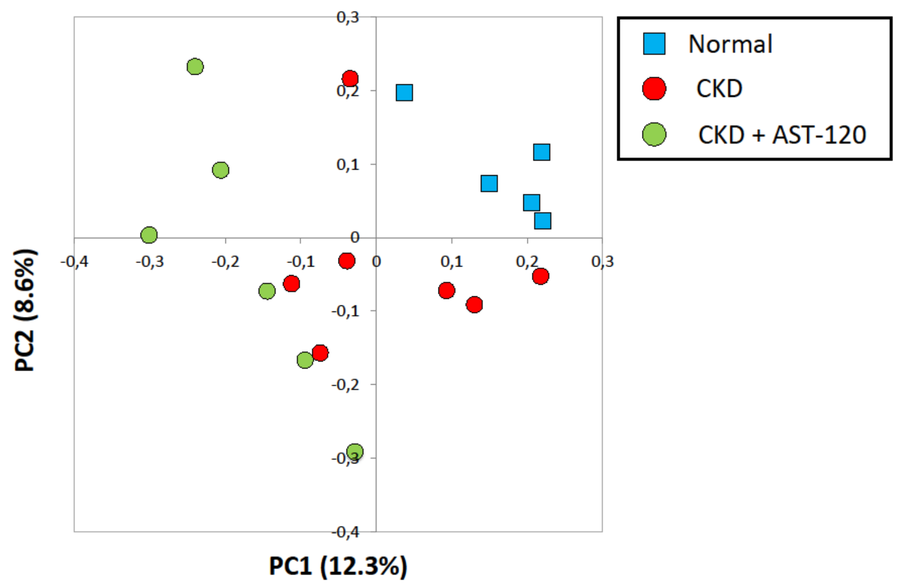


Results represent mean ± SD. Student t-test was used, control rats vs. CKD rats, CKD rats vs. CKD rats with AST-120, *P<0.05, **P<0.01.

(2) Relative abundance of genera and classes



(3) PCoA plot of UniFrac (Similarity of gut microbiota)



CONCLUSIONS:

1. The production of IS and PCA decreased and the fecal microbiome composition changed drastically by cecectomy. These showed that productions of UTs are correlated with a subset of indigenous gut microbe, such as unknown *Clostridia* (Phy. *Firmicutes*) and unknown *Bateroidia* (Phy. *Bacteroidetes*).
2. The productions of IS and PCS increased in CKD rats. PCoA plot of UniFrac showed the composition of gut microbiota changed.
3. Administration of AST-120 reduced UTs productions and may affect to the composition of gut microbiota.

DOI: 10.3252/pso.eu.52era.2015