FR-OR58

Association Between the Gut Microbiota and Kidney Function

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Background: The human gut microbiota is composed of the bacteria, fungi and other microorganisms that live in the lower intestines in a symbiotic relationship with the host. Disruption of the gut microbiota has been associated with cardiovascular and metabolic diseases, but the association with kidney disease is still largely unknown.

Methods: We studied the composition and predicted function of the gut microbiota based on shotgun whole-genome sequencing of microbial DNA in fecal samples collected from 9,788 adults enrolled in the longitudinal, population-based Swedish SCAPIS cohort study. Linear regression adjusted for technical variables, age, sex, Shannon diversity index and (in sensitivity analysis) established kidney disease risk factors was used to identify associations between the log(x)-transformed relative frequencies of 1,900 eukaryotic species and estimated glomerular filtration rate (eGFR). Additional sensitivity analyses included stratified analyses for gender, hypertension and diabetes mellitus. The Benjamini-Hochberg false discovery rate (FDR) multiplicity correction was used.

Results: We included 5,130 women (57.5±4.3 years) and 4,658 men (57.6±4.4 years). The mean eGFR was 86.5±11.3 for men, and 85.5±8.7 for women. Amongst all participants, 42% had an eGFR above 90, 39% had an eGFR between 75-90, 17% had an eGFR between 60-75, 2% had an eGFR between 45-60, and less than 0.1% had an eGFR below 45. In the age- and sex-associated model, we identified four bacteria that were associated with an eGFR < 0.05. Additional adjustment for kidney disease risk factors rendered one of the associations no longer significant. The kidney function-associated bacteria could be identified down to the species level and belonged to the Coriobacteriales, Coriobacteriaceae and Veillonellales. Genes sex-enrichment analysis indicated significant (FDR < 0.05) enrichment in 48 metabolic pathways.

Conclusions: In the largest gut microbiome association study of kidney function on human adults to date, four bacteria whose abundance was associated with glomerular filtration rate. The functional enrichment of kidney function-associated microbiota provides further insights into its possible role in kidney health.

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Genetic Determinants of Serum Calcification Propensity and Mortality Risk in CKD

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Background: Serum calciprotein particle maturation time (τm), a measure of calcification propensity, is associated with cardiovascular morbidity and mortality in patients with chronic kidney disease (CKD). Here, we aimed to identify genetic loci for serum τm and examine whether these loci are linked with adverse outcomes.

Methods: We performed a genome-wide association study (GWAS) of serum τm in 2,739 community-dwelling individuals of mostly European descent. Subsequently, we used the community-based Rotterdam study (RS) to examine the association between the identified variants and all-cause mortality in the general population and in a subgroup of CKD patients, applying multivariate logistic regression analysis.

Results: We identified three independent genome-wide significant single nucleotide polymorphisms (SNPs), rs4197 (p=1.7x10^-8), rs2077119 (p=3.3x10^-8), and rs7063776 (p=1.3x10^-7) in the HSGG gene encoding fetuin-A. The three SNPs together explained 18.3% of the variation in serum τm. Quantitative trait locus analysis revealed that all three SNPs have effects detectable at blood protein level of fetuin-A. Associations with these SNPs were studied in 8,556 RS participants (age 65±9.9 y, 57% female, 63% hypertension, BMI 27.3±4.2 kg/m²), of whom 833 had CKD (age 75.8±8.7, 59% female, 85% hypertension, BMI 27.7±4.1 kg/m²). The minor allele of rs9870756, linked with a reduced τm, and thus a higher calcification propensity, was significantly associated with a higher risk of all-cause mortality, both in the general population [OR (95% CI)=1.14 (1.00-1.30)] and in the CKD subgroup [OR (95% CI)=1.60 (1.05-2.42)]. In the fully adjusted model, the minor allele of rs9870756 was only associated with all-cause mortality in the CKD subgroup [OR (95% CI)=1.93 (1.21-3.08)]. The other two variants were not associated with all-cause mortality.

Conclusions: We identified three independent SNPs in the fetuin-A gene as strong genetic determinants of calcification propensity. The minor allele of rs9870756 was significantly associated with a higher risk of all-cause mortality in CKD patients. Our findings connect genetic susceptibility to calcification with adverse outcome in CKD patients.

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FR-OR60

Decision Aid for Renal Therapy (DART) Reduces Decisional Conflict and Improves Knowledge Among Older Adults with Advanced CKD: A Randomized Clinical Trial

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Background: For older adults, making decisions about kidney failure treatments is challenging, and dialysis may be inconsistent with life goals. Greater decisional conflict is associated with regret, poor outcomes, and worse satisfaction. The DART trial assessed the effectiveness of an interactive, web-based decision aid on decisional conflict and knowledge among older CKD patients facing dialysis decisions.

Methods: Randomized trial evaluating the web-based DART versus usual education, enrolling adults age 70+, English-fluent, with CKD stage 4-5 from 4 US sites. The primary outcome was change in decisional conflict scale (DCS) score from baseline to first follow-up (~3 months) compared using ANCOVA. The validated 16-question DCS (100 point scale; lower score indicates less decisional conflict) measures personal perception of uncertainty in choosing among treatment options and modifiable factors contributing to uncertainty. Twelve knowledge questions about CKD and treatment options were assessed at both visits.

Results: Among 363 participants, 180 were randomized to education and 183 to DART; 162 (89%) completed DART. Mean age was 78 years, mean eGFR was 23 mL/min/1.73 m², 78% were white and 48% had diabetes. Groups were balanced at baseline. At first follow-up, DCS score improved significantly more among the DART group [mean difference (95% CI)=7.1 (2.9, 11.3)]. Both groups improved from baseline knowledge among older adults facing kidney failure treatment decisions. Knowledge among older adults facing kidney failure treatment decisions.

Conclusions: DART reduced decisional conflict and improved knowledge among older adults facing kidney failure treatment decisions, emphasizing that the decision-making process for older adults with advanced CKD can be improved with use of this effective educational intervention. Funded by PCORI, CDR-2017C1-6297

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Decisional Conflict by Randomization Group

Table: The associations of individual metabolites with CKD progression using Cox proportional hazard models.

| Metabolite | Adjusted p-value | Fully adjusted p-value |
|------------|------------------|------------------------|
| CKD 1 (plasma) | 0.043 | 0.025 |
| CKD 2 (plasma) | 0.049 | 0.033 |
| CKD 3 (plasma) | 0.057 | 0.041 |
| CKD 4 (plasma) | 0.065 | 0.050 |

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral; PO - Poster; PUB - Publication Only

Underline represents presenting author.
SA-OR03

The Spike Protein of the Causative COVID-19 Virus Induces Heme Oxygenase-1: Pathophysiological Implications
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Background: Acute kidney injury (AKI) is both a consequence and determinant of outcomes in COVID-19. The kidney is one of the major organs infected by the causative virus SARS-CoV-2. The spike protein of SARS-CoV-2 is required for viral entry into cells and is present in the urine of patients with COVID-19 and AKI. The present study examined cellular effects that result from transfecting the spike protein of SARS-CoV-2 in HEK293 kidney cells.

Methods: HEK293-AE2+ cells stably overexpressing ACE2 were used. Codon optimized pcDNA encoding SARS-CoV-2 spike (S788bp) or empty vector (4033bp) plasmid was transfected using Lipofectamine LTX. For studies examining the effect of quercetin (an inducer of heme oxygenase-1, HO-1), full media containing quercetin or vehicle was added at 4-6 hours post transfection. mRNA and protein expression was assessed by quantitative real-time RT-PCR and western blot respectively. Syncytium formation was assessed by acquiring phase contrast images using Olympus CK40 microscope and the area covered by syncytia was measured using ImageJ software.

Results: HEK293-AE2+ cells expressed SARS-CoV-2 spike protein upon spike transfection. Such expression led to syncytia formation, the sloughing of sheets of cells, and focal denudation of the cell monolayer. Spike protein expression upregulated potentially nephrotoxic genes such as TNF-α, MCP-1, and ICAM1. Spike protein expression also upregulated potentially cytoprotective genes such as HO-1, as demonstrated by HO-1 mRNA and protein expression and relevant signaling pathways (p-Akt, p-STAT3, and p-p38) involved in inducing the HO-1 gene. Quercetin, a naturally occurring compound that induces HO-1, markedly reduced syncytia formation and spike protein expression.

Conclusions: These findings introduce a clinically relevant, spike protein-induced, in vitro model for the study of AKI in COVID-19. The major conclusions of the study are: 1) Spike protein expression in kidney cells provides a useful and timely model for the study of maladaptive and adaptive responses in these cells relevant to AKI observed in COVID-19; 2) spike protein expression in kidney cells upregulates HO-1; and 3) quercetin, an inducer of HO-1, may provide a clinically relevant/feasible protective strategy in AKI occurring in the setting of COVID-19.

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SA-OR04

A Novel Soluble ACE2 Protein Protects from Lethal SARS-CoV-2 Infection
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Background: Severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) uses full-length angiotensin converting enzyme 2 (ACE2) as the main receptor to enter the target cells. A novel soluble ACE2 protein with increased duration of action and binding capacity to exert a decoy effect as a way to intercept SARS-CoV-2 from binding to membrane-bound ACE2 was generated. The protein was administered to a lethal mouse model of COVID-19 to examine its efficacy.

Methods: A human soluble ACE2 protein variant fused with a 5kD albumin binding domain (ABD) was linked via a dimerization motif hinge-like 4-cysteine dodecapeptide to uses full-length angiotensin converting enzyme 2 (ACE2) as the main receptor to enter the target cells. A novel soluble ACE2 protein with increased duration of action and binding capacity to exert a decoy effect as a way to intercept SARS-CoV-2 from binding to membrane-bound ACE2 was generated. The protein was administered to a lethal mouse model of COVID-19 to examine its efficacy.

Results: Infected mice that received ACE2-1:618-DCD-ABD (1:618-DCD-ABD) was administered intranasally and intraperitoneally prior to viral inoculation and on the two following consecutive days. Infected animals were observed for weight, clinical score and mortality in a BSL-3 facility. Upon sacrifice, lung histopathology was evaluated, and viral loads were measured by plaque assay.

Conclusions: This study demonstrates for the first time in vivo the preventative/therapeutic efficacy of a soluble ACE2 protein in a preclinical animal model.

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