injury and inflammation. Acute treatment of AS mice with hyaluronidase reduced excess glomerular and endothelial glycosylation and blocked immune cell homing and albumin leakage through the GFB.

**Conclusions:** We identified the central roles of glomerular capillary mechanical strain, endothelial and immune cell activation early in AS in both mice and humans that may be therapeutically targeted to reduce local tissue injury and improve kidney function.

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**FR-OR41**

**Comparative Human and Mouse Kidney Transcriptomics Identify ELF4 as Potential Therapeutic Target**

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**Background:** Mouse models provide an excellent tool to study kidney disease pathogenesis, but little is known how well mouse models recapitulate molecular changes of human CKD.

**Methods:** Here we created four different mouse kidney disease models a) unilateral ureteral obstruction, b) folic acid injection c) tubular specific overexpression of Nelly1 and d) podocyte specific overexpression of risk variant APOL1. We performed detailed phenotyping and molecular profiling by RNA Sequencing of mouse models. We also generated RNA Sequencing for 95 human kidney samples. We used the CRISPR technology to generate mice with ELF4 deletion. We used antisense oligonucleotides for test the therapeutic potential of ELF4 inhibition.

**Results:** Using comparative bioinformatics approaches we identified 1256 genes and 47 transcription factors that were commonly regulated in all mouse CKD and in patient with CKD. In particular we identified ELF4 and ELF4 transcription factors as they were elevated in both all mouse models and patient samples. Mice with genetic deletion of ELF4 was healthy at baseline and showed protection from FA and cisplatin induced kidney fibrosis and disease. We found that ELF4 is mostly expressed in immune cells and influenced inflammation. Therapeutic inhibition of ELF4 was tested by injection of siRNA, which showed similar protection of kidney disease.

**Conclusions:** Comparative transcriptomics identified ELF4 as one of the key conserved transcription factor in human and mouse CKD. Genetic deletion or pharmacological inhibition of ELF4 protected mice from fibrosis.

**FR-OR42**

**Proteome-Wide and Transcriptome-Wide Association Studies of Kidney Function**

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**Background:** Large-scale genome-wide association studies (GWAS) have implicated 424 loci associated with eGFR based on creatinine (eGFRcr), including 320 genes and proteins affecting kidney function.

**Methods:** We applied proteome-wide and transcriptome-wide association studies (PWAS) for eGFRcr and eGFRcys using summary-statistics from the CKDGen Consortium (EA, N, GFRcr = 11,004,041; N, GFRcys = 460,826) and 1,318 genetic protein level prediction models developed in the Atherosclerosis Risk in Communities (ARIC) study (N = 7,213 European American (EA) and African American (AA)) and 61 conducted proteome-wide association studies (TWAS) based on prediction models developed in 49 human tissues (GTEx) and from 121 kidney tubule samples.

**Results:** We identified 62 proteins which were associated with eGFRcr and with eGFRcys (p<0.05/1,318). Of these, 19 were associated with both kidney function measures in a directionally consistent manner, nominating novel gene annotations in 18 of the 19 genetically associated regions. The enzyme isopentenyl-diphosphate delta isomerase 2 (ID2) showed the strongest associations (p=4.3e-37, p<1.0e-15). Two hours after infusion of ID2 transcripts for eGFRcr and eGFRcys, respectively (p=0.05/235,763). Of these, 544 were associated with both kidney function measures, including 13 of the 19 PWAS genes. There were also 27 identified in kidney tubule expression, including DACH1 and MANBA, which were recently identified as contributors to kidney fibrosis.

**Conclusions:** We were able to consistently implicate 13 genes/proteins for eGFRcys and eGFRcys across both PWAS and TWAS. Based on our human in vivo data these proteins are excellent candidates for downstream functional studies and for potential drug repurposing in the context of chronic kidney disease.

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**FR-OR43**

**CTGF Aggravates the Oxidative Stress-DNA Damage-Cellular Senescence Sequence Following Renal Ischemia-Reperfusion Injury**

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**Background:** Recent data suggest that AKI to CKD progression may be driven by cellular senescence evoking from prolonged DNA damage response following oxidative stress. Connective tissue growth factor (CTGF; CCN2) is a major contributor to CKD development and was found to aggravate DNA damage and the subsequent DNA damage response (DDR)-Cellular Senescence-Fibrosis sequence following renal ischemia reperfusion injury (IRI). Here, we investigated the impact of CTGF inhibition on the immediate (4 hours) and early (3 days) renal response to IRI.

**Methods:** We induced AKI by bilateral IRI in wild type and conditional CTGF-KO mice and euthanized the mice 4 hours and 3 days after reperfusion. We performed full transcriptome RNA sequencing to identify major dysregulated pathways and validated the findings by qPCR and immunohistochemistry.

**Results:** IRI resulted in upregulation of CTGF 4 hours and 3 days after reperfusion (Figure 1A,C). Four hours after reperfusion, CTGF-dependent differentially regulated genes were enriched in multiple signaling pathways related to oxidative stress and DNA damage. Consistently, decreased staining for h2AX and p-p53 (Figure 1B) indicated reduced DNA damage response in tubular epithelial cells of CTGF-KO mice, although decline in kidney function, acute tubular damage score, and Kim1- and NGAL expression were not influenced. Three days after IRI, oxidative stress response markers (HINE, nitrotyrosine, and Nrg2 target genes HMox1 and Nqo1), DDR markers (p-12AXS, p-p53, p21), and anti-apoptotic factors (Bel-1L, Hmgb1) were less elevated in CTGF-KO than in wild type mice.

**Conclusions:** Together, our observations suggest that CTGF inhibition might mitigate AKI to CKD progression by reducing oxidative stress induced DNA damage and the subsequent DDR-cellular senescence-fibrosis sequence response.

**FR-OR44**

**Urinary mRNA Expression of Glomerular Podocyte Markers in Glomerular Disease and Renal Transplant**

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**Background:** The search for urinary markers to monitor the progression of kidney disease is still ongoing and previous works have demonstrated the useful of quantifying mRNA expression of urinary cells. It is well known that in podocytes the slit diaphragm integrity and the function of the molecules shared with neuronal signaling pathways are essential for the maintenance of cell physiology. Our study focuses on the identification of the urinary mRNA expression of a panel of podocytes genes useful to identify possible biomarkers of glomerular pathology.

**Methods:** We studied the urine obtained from patients, native and renal transplant, affected by renal disease and undergone, with clinical indication, to renal biopsy (Rx). We investigated the presence and the morphology of podocytes by immunocytochemistry and measured the expression of genes responsible for their structure and function by RT-qPCR.

**Results:** We detected the increase of mRNA for protein expression in the urine of both patients. After all kinds of normalization for the number of podocytes, there was a tendency to increase, compared to healthy controls, of the most of the tested genes; in particular, we obtained a significant rise of TRPC6 expression.

**Key:** TH - Thursday; FR - Friday; SA - Saturday; OR - Oral; PO - Poster; PUB - Publication Only Underline represents presenting author.
Conclusions: We suggest the expression of WT1 mRNA as a surrogate for qualitative detection for urinary podocytes. We propose the increase of TRPC5 and GRM1 mRNA in urinary podocytes as a marker helpful to provide complementary information to Rbx. These genes are useful for monitoring actin cytoskeleton remodeling in podocytes that contributes to glomerular damage in course of renal disease.

FR-OR45
FKBP12 Interacts with 14-3-3 and Synaptopodin to Maintain Actin Cytoskeleton and Processes in Podocytes
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Background: FKBP12 is identified as a binding protein of Tacrolimus (Tac). We reported that FKBP12 is highly expressed in podocytes in kidney, and FKBP12 in podocytes is localized along the actin cytoskeleton. We also reported that FKBP12 is decreased in injured podocytes, and Tac ameliorates podocyte injury by restoring FKBP12 in the actin cytoskeleton (ASN 2019). However, the interaction of FKBP12 with actin-associated proteins and the molecular function of FKBP12 in podocyte are not elucidated yet.

Methods: The localization of FKBP12 with actin-associated proteins was analyzed by dual-labelling immunostaining in glomerular and human cultured podocytes. The subcellular distribution of FKBP12 was analyzed by western blot in the cultured podocytes. The interaction of FKBP12 with F-actin was analyzed by actin-binding assay with the cell lysate. The interaction of FKBP12 with the actin-associated proteins was analyzed by immunoprecipitation (IP) assay with the lysate of cultured podocytes and HEK293 transfected cells. The effect of FKBP12 siRNA and Tac treatment was analyzed in cultured podocytes.

Results: FKBP12 staining was co-localized with the actin-associated proteins 14-3-3p and synaptopodin (Synp) in glomerular. The subcellular distribution of FKBP12 was similar to that of 14-3-3p in cultured podocytes. FKBP12 was co-localized and associated with F-actin in the podocytes. FKBP12 interacted with 14-3-3p in cultured podocytes. The IP assay with the HEK expression system also showed FKBP12 interacted with endogenous 14-3-3p. FKBP12 interacted with Synp in the HEK cells co-transfected with 14-3-3p and Synp. The interaction of FKBP12 with Synp was not altered by the treatment of 14-3-3p siRNA. Tac enhanced the interaction of FKBP12 with Synp. The expression of 14-3-3p was decreased (63.0% to normal, P<0.01), the structure of F-actin was deranged (staining score, 2.0 vs. 2.9 of normal, P<0.05), and the process formation was impaired (40.4% to normal, P<0.005) in the podocytes treated with FKBP12 siRNA. Tac treatment to normal cells increased the expression of FKBP12 at F-actin in processes and impaired (2.6% vs. normal, P<0.05) in the podocytes treated with FKBP12 siRNA.

Conclusions: FKBP12 interacts with 14-3-3 and Synp to maintain the actin cytoskeleton and processes in podocyte. The enhanced interactions of FKBP12 with Syn and 14-3-3p by Tac treatment restores FKBP12 at actin cytoskeleton in podocyte.

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FR-OR46
Soluble Flt1 Binds to Anti-Inflammatory Macrophages in the Kidney
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Background: Soluble Flt1 (sFlt1), a decoy receptor of VEGF ligands, is a key regulator of angiogenesis. High systemic levels of sFlt1 have been linked to the pathogenesis of preeclampsia. However, we have previously reported that treatment with low concentrations of sFlt1 ameliorates kidney damage and inflammation. Specifically, sFlt1 targets macrophages, suggesting that sFlt1 has nephroprotective immunomodulating effects. Here, we studied the presence of sFlt1 in human kidney diseases and investigated the expression and direct binding of sFlt1 to macrophages.

Methods: Renal biopsies of patients with various kidney diseases (IgA, LN, DN, FSGS, MCD) and pre-transplant control biopsies were stained for sFlt1, CD163 and CD68. Cultured macrophages were incubated with increasing concentrations of sFlt1-His, after which membrane binding was measured using flow cytometry. For this, THP-1 macrophages were differentiated with PMA and activated with IFN-γ and LPS or IL-4, primary macrophages were differentiated using GM-CSF or M-CSF.

Results: A patchy pattern of sFlt1 staining colocalizes with CD163/CD68-positive cells in subepithelial areas and with CD68-positive cells in glomeruli. No quantitative differences in renal sFlt1 levels were observed in patients with kidney disease and controls. Flow cytometric analysis revealed that sFlt1 binds to PMA-differentiated THP-1 macrophages but does not bind to THP-1 monocytes. Activation with IFN-γ and LPS upregulates sFlt1 binding to THP-1 macrophages. However, IL-4 activation of THP-1 macrophages strongly increases membrane sFlt1 binding. Furthermore, IL-4 activation upregulates sFlt1 mRNA expression in THP-1 macrophages. In primary macrophages, sFlt1 binding was higher in macrophages differentiated with GM-CSF compared to M-CSF.

Conclusions: Our results suggest that sFlt1, while typically associated with angiogenesis, binds to anti-inflammatory macrophages in the human kidney. Alternative activation of macrophages by IL-4 strongly induces sFlt1 production and increases direct binding of sFlt1 to the cell surface membrane. We infer that sFlt1 functions as an autocrine stimulus of anti-inflammatory macrophages, independent of its antiangiogenic properties. Since anti-inflammatory macrophages mediate repair after kidney injury, our work suggests the potential of sFlt1 as a therapeutic tool.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral; PO - Poster; PUB - Publication Only
Underline represents presenting author.