O1.
The calcium-dependent proteinase calpain-1 links TRPC6 activity to podocyte injury

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Objective: The hallmark of Focal Segmental Glomerular Sclerosis (FSGS) is podocyte injury resulting in proteinuria and glomerular scarring. Transient Receptor Potential channel C6 (TRPC6) is calcium-conducting ion channel that is expressed at the slit-diaphragm that interconnects foot processes of individual podocytes. TRPC6 gain-of-function mutations and glomerular TRPC6 overexpression are associated with hereditary FSGS as well as acquired proteinuric diseases. However, the underlying mechanisms that link TRPC6 to podocyte injury remain elusive. Activation of the calcium-dependent protease calpain-1 was suggested to mediate renal injury in ischemia reperfusion injury and anti-glomerular basement membrane glomerulonephritis. We hypothesize that calpain-1 is involved in TRPC6-mediated renal injury.

Methods: Immortalized mouse podocytes were transfected with either a scrambled, inducible TRPC6 knockdown (KD), or calpain-1 KD construct. Thereafter, podocytes were injured using adriamycin or treated with the TRP channel activator 1-oleoyl-acetyl-sn-glycerol (OAG), the calpain inhibitor calpeptin or the TRPC channel blocker 2-APB. We measured TRPC6-dependent calcium influx using the calcium-sensitive dye FURA-2. Calpain activity and expression of the podocyte cytoskeletal protein talin-1, a substrate molecule of calpain, was determined using a calpain activity assay or western blot, respectively. To evaluate the importance of these findings in vivo, healthy rats were injected with adriamycin, a model for human FSGS, and treated with a calpain inhibitor. Urine, blood and kidneys were harvested, and urinary as well as cortical calpain activity was assessed. To localize the increased calpain activity in the cortex, in situ zymography was used on frozen kidney slides. Furthermore, glomerular calpain-1, TRPC6, nephrin and talin-1 expression were determined. Importantly, calpain activity and localization, talin-1 and TRPC6 expression were also determined in kidneys of human FSGS patients.

Results: Adriamycin and OAG increased cellular calpain activity in podocytes. Concomitant treatment with calpeptin or 2-APB prevented this increased calpain activity. Using stably transfected TRPC6 KD cells, we demonstrated that the increased calcium influx after adriamycin pre-treatment in podocytes is TRPC6-dependent, and that calpain activation did not occur in TRPC6 KD podocytes. Furthermore, we showed that the TRPC6-dependent calpain activation leads to talin-1 cleavage, which was previously shown to induce podocyte injury and proteinuria in FSGS. In an animal model for human FSGS, increased calpain activity in urine, glomeruli and tubuli was associated with increased proteinuria, which could be prevented by calpain inhibition. Urine as well as glomeruli from FSGS patients showed enhanced calpain activity, along with increased glomerular TRPC6 and reduced talin-1 and nephrin abundance.

Conclusions: We have elucidated a novel mechanism that directly links TRPC6-dependent calcium influx to calpain-1 activation, talin-1 cleavage and podocyte injury. Therefore, calpain-1 and/or TRPC6 inhibition could be a novel future therapeutic option to treat FSGS.
O2. Microbiota derived phenylacetylglutamine associates with survival and cardiovascular disease in patients with CKD

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Objective: Lately, there has been increasing interest in gut microbiota derived uremic retention solutes as driving force behind adverse outcome in CKD. Both p-cresyl sulfate and indoxyl sulfate are considered representatives of this group of solutes, also commonly referred as to protein-bound solutes due to their high protein binding and dependence on active tubular secretion for renal clearance. Phenylacetylglutamine is another microbial metabolite subjected to high tubular secretion, although protein binding is rather low. We questioned whether this solute also relates to adverse outcome in CKD patients not yet on dialysis.

Methods: We performed a single-center prospective study in patients with CKD stage 1-5. Baseline serum levels of phenylacetylglutamine were determined using LC-MS. Correlation between eGFR and serum phenylacetylglutamine was explored using Spearman's rank correlation analysis. The relationship between phenylacetylglutamine, survival and cardiovascular disease was examined using Kaplan Meier and Cox proportional hazard analysis.

Results: 488 CKD patients were followed from November 2005 until December 2010. Median serum level of phenylacetylglutamine was 6.2 µM (IQR 3.0–13.2). We observed a highly significant inverse correlation between eGFR and serum phenylacetylglutamine (rho -0.76, P < 0.0001). During follow-up, we noted a total of 51 deaths and 75 cardiovascular events with a gradual and significant increase with higher tertiles of phenylacetylglutamine (both log rank P < 0.0001, see figure). In univariate cox proportional hazard analysis, phenylacetylglutamine was a significant predictor of mortality (HR per SD increase of 2.165 (1.570–2.985), P < 0.0001) and cardiovascular disease (2.209 (1.702–2.868), P < 0.0001), even after adjustment for renal function, Framingham risk factors, markers of mineral bone metabolism, CRP and albumin (HR (1.705 (1.156–2.513), P 0.007) for mortality and HR 1.818 (1.362–2.428), P <0.0001 for cardiovascular disease).

Conclusion: Serum levels of microbiota derived phenylacetylglutamine are elevated in patients with more advanced CKD. In addition, serum phenylacetylglutamine is independently associated with mortality and cardiovascular disease. Further analysis of 24h urinary samples is ongoing to differentiate between the effect of higher urinary excretion rate as a surrogate of intestinal generation vs. the effect of lower tubular secretion.
O3.
A blunted response to thiazide diuretics is not specific for patients with Gitelman syndrome

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Objective:
Magnesium and potassium wasting disorders can result from excessive urinary loss of these cations due to mutations in genes encoding specific tubular transporters or ion channels. In Gitelman syndrome (GS), classically a defective sodium-chloride co-transporter (NCCT) in the distal tubule eventually leads to hypokalemia, hypomagnesemia and hypocalciuria. In Bartter syndrome (BS), defective sodium-potassium-chloride co-transport in the loop of Henle leads to a similar salt-losing nephropathy. A challenge with thiazide diuretics, testing the functional presence of NCCT, has been shown to differentiate GS from BS and normal controls. However, the performance of the thiazide test in renal magnesium wasting disorders other than GS and BS has not been studied. Therefore, we studied thiazide responsiveness in a case series of patients with renal magnesium loss.

Methods:
Eleven patients who presented to our department between 2010-2014 with hypomagnesemia due to renal magnesium wasting and in whom a thiazide test was performed were included. An abnormal test result is defined as a maximal delta fractional chloride excretion (FeCl) <2.3% (Colussi 2007). Genetic testing for SLC12A3, CLCNKB, KCNJ1, FXYD2 and HNF1B mutations was performed by Sanger sequencing and MLPA.

Results:
In three patients a mutation in SLC12A3 (GS) was identified, in one patient a mutation in CLCNKB and KCNJ1 (BS), in one patient a mutation in FXYD2 and in five patients a deletion of one whole HNF1B gene. In one patient a molecular diagnosis was not identified. Responses to thiazide diuretics were diverse: The three patients with GS showed a blunted response to thiazide diuretics, whereas the BS patient showed a normal response, the single patient with a FXYD2 mutation showed a blunted response and the five patients with HNF1B-associated nephropathy showed different responses ranging from normal to blunted. A delta FeCl of 2.4 was measured in the patient without a genetic diagnosis. The thiazide test in eight healthy volunteers at our clinic showed normal responses, with a mean delta FeCl 3.12 ± 0.48% and the lowest value being higher than 2.3%.

Conclusions:
A blunted response to thiazide diuretics is not pathognomic for GS and can also be found in other renal magnesium wasting disorders, such as patients with mutations in FXYD2 or HNF1B. Hypothetically, in contrast to what has been assumed until now, these mutations do not cause isolated distal tubular magnesium wasting but also seem to affect NCCT function. Although not specific for diagnostic purposes, performing a thiazide test in patients with tubulopathies may improve patient phenotyping and increase our understanding of the interrelationship between transcription factors, transporters and ion channels.
O4. Haemodynamic response to oxygen supplementation in CKD: evidence against the existence of a kidney hypoxia induced pressor effect in chronic kidney disease

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Background
Renal hypoxia is thought to be an important factor in the progression of CKD and a likely culprit in CKD associated systemic sympathetic hyperactivity and hypertension. In a recent study among stage IV CKD patients, supplementation with 100% oxygen reduced muscle sympathetic nerve activity and lowered pulse pressure compared to healthy controls. The current study aimed to elaborate on these findings and assess the underlying haemodynamic modulation. We hypothesized a decreased systemic vascular resistance (SVR) caused by the observed decrease in sympathetic activity.

Methods
19 stage IIIb and IV CKD patients (14 males/5 females, age 62 ± 10, eGFR 23.6 ± 7.2, blood pressure: 128 ± 24 mmHg systolic, 72 ± 19 diastolic) and 8 young healthy controls (6 males/2 females, age 26 ± 3, blood pressure: 114 ± 7 mmHg systolic, 63 ± 7 diastolic) were studied by measuring finger artery blood pressure (Nexfin, BMEye) continuously in supine position while at 12 minute intervals the partial oxygen pressure was increased stepwise from room air (0.21 atm) via 0.5 to 1.0 atm over a non-rebreathing mask. Using pulse wave analysis (Nexfin@PC, BMEye) we derived heart rate and indexes of cardiac output (CO) and SVR.

Results
In all patients, systolic and diastolic pressure rose dose dependently. In patients, systolic blood pressure rose from 132 ± 25 to 141 ± 23 mmHg (p=0.007), diastolic pressure rose from 73 ± 20 to 80 ± 21 mmHg (p=0.004), heart rate dropped from 62 ± 8 to 57 ± 7 bpm (p<0.001), CO dropped from 4.6 ± 1.3 to 4.2 ± 1.3 L/min (p=0.009), SVR rose from 1440 ± 546 to 1745 ± 710 dyn·s/cm⁵ between 0.21 to 1.0 atm oxygen. In healthy subjects blood pressure did not change. Heart rate dropped from 59 ± 11 to 55 ± 7 bpm (NS), CO dropped from 7.3 ± 1 to 6.8 ± 1 L/min (NS), SVR rose from 902 ± 174 to 985 ± 179 dyn·s/cm⁵ between 0.21 to 1.0 atm oxygen.

Conclusion
Contrary to previously published data, and our hypothesis we found a blood pressure increase in CKD patients in response to oxygen supplementation that was fully explained by an increased SVR. The simultaneous decrease of heart rate indicates baroreflex loading, that occurs both in CKD patients and healthy subjects. This implies that oxygen supplementation leads to a (toxic, possibly endothelial dysfunction mediated) non-sympathetically mediated systematic vasoconstriction, followed by a baroreflex loading and subsequent baroreflex mediated sympathetic de-activation. The data suggest that this effect is larger in CKD patients. The previously reported decrease of sympathetic nerve activity during oxygen supplementation in CKD patients should therefore not be considered a proof of the existence of an exaggerated kidney based pressor response, but rather proof of intact baroreflex function. In the clinical care for CKD patients, oxygen supplementation beyond the physiological range should be a considered a cardiovascular stressor.
O5. Calcineurin inhibitor Tacrolimus impairs host immune response against urinary tract infection by affecting TLR4 negative regulators.

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Background: Calcineurin inhibitor Tacrolimus (TAC), is a potent immunosuppressive drug widely used for preventing acute graft rejection. However, long term use of TAC increases susceptibility to bacterial infections. Urinary tract infection (UTI) is the most frequent infectious complication in renal transplant patients. The mechanism by which TAC suppresses the adaptive immunity is well known. However, it remains largely unknown how TAC affects host innate immune response against UTI.

Methods: To study this, we performed experimental UTI model by intravesical inoculation of uropathogenic E. coli in C57Bl/6 female wild type (WT) mice (n=8/group) pre-treated with TAC or solvent. Mice were sacrificed after 24 and 48 hours to determine bacterial loads and inflammatory mediators (MPO and TNF-α) in kidney and bladder homogenates. Ex-vivo and in vitro experiments with pre-treated WT mice or primary granulocytes were performed to investigate the effects of TAC on granulocyte phagocytic function with FACS and TLR4 negative regulators with qPCR.

Results: Our results show that TAC pre-treated mice display higher bacterial loads than vehicle pre-treated mice (CTR) in kidney and bladder after 24 and 48 hours. However, MPO and TNF-α levels in kidney and the bladder are similar between TAC and CTR group, indicating that TAC pre-treated mice respond relatively less to E. coli than CTR mice. Indeed, granulocytes from TAC pre-treated mice phagocytize less E. coli ex-vivo compared to CTR. Furthermore, whole blood of TAC pre-treated mice respond less to a LPS stimulus ex-vivo. This tolerant state can be explained by upregulation of TLR4 negative regulators (IRAKM, SOCS1, A20, ATF3, BCL3 and TRIM30A) on mRNA level as seen in TAC pre-treated bone-marrow derived granulocytes.

Conclusion: We show that TAC affects TLR-mediated responses in granulocytes by enhancing expression of negative regulators. This so called TAC-induced tolerant state can explain the impaired response against UTI in TAC pre-treated mice.
O6. Outcomes of renal transplantation from deceased donors for recipients over the age of 75 compared with recipients between 65 and 74 of age – A Dutch Cohort Study


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Objective: The age of candidates awaiting kidney transplantation has been increasing in the Netherlands. In 2014 the number of elderly (≥65y) waitlisted patients for renal transplantation increased almost four-fold since 2002. An increased age at time of transplantation has impact on graft survival and death-with-a-functioning-graft is a more common event. The aim was to compare the results of kidney transplantation from deceased donors for recipients older than 75 years to the results of patients between 65 and 75 years of age.

Methods: In this retrospective cohort study, we used the Dutch Organ Transplantation Registry (NOTR) to include recipients (≥65y) from all Dutch centers, transplanted from 2002 to 2012 with a first DBD or DCD kidney (Maastricht category III). Evaluated outcomes were primary non-function, delayed graft function, incidence of acute rejection treatments within three months and one year, estimated glomerular filtration rate after three months and one year, and one and five year graft loss and patient mortality.

Results: The five year death-censored graft survival was lower for recipients aged 75+ (n=42) compared with recipients aged 65-74 (n=672) (66.1% vs 82.0%, log rank p=.053). The five year graft survival (no censoring for death) and patient mortality were comparable between recipients over the age of 75 and recipients between 65 and 75 of age (51.9% vs 54.8%, log rank p=.659; 60.0% vs 62.5%, log rank p=.937, respectively). Donor age, number of DCD donors, number of Eurotransplant senior program allocations, donor smoking, cold ischemia time, HLA mismatch levels, dialyses vintage prior to transplantation, and original disease of the recipient were comparable between the recipient age groups. Primary non-function and delayed graft function rate were not statistically different between recipients aged 75+ and 65-74 (11.9% vs 8.0%, and 37.2% vs 33.3%, respectively). The incidence of acute rejection treatments within 3 months and within 1 year after transplantation were comparable between recipients aged 75+ and 65-74 (4.8% vs 13.8%, and 4.1% vs 10.8%, respectively). Estimated glomerular filtration rate after three months one year were comparable between recipients age groups.

Conclusion: Our findings should be interpreted with care. The elderly recipient older than 75 years probably represent a selected group, and already survived the age of the comparative group (aged 65-74). Although patient mortality was comparable, the older aged recipients (75+) are accompanied with higher graft loss on the long-term irrespective of patient death. The results indicate that the current practice of transplanting deceased donors kidneys in patients over the age of 75 results in fair outcomes regarding graft and patient survival.
O7. The epigenetic signature of older donor kidneys

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Objective: Advanced donor age is one of the key factors associated with inferior long-term outcome after kidney transplantation. Yet, donor shortage obliges transplant teams to increasingly use grafts from older donors. Research has focused on the several phenomena intrinsically linked to advanced age. Only very recently, it has become clear that senescence is also accompanied by DNA methylation alterations. In addition, DNA methylation changes are involved in the process of fibrosis. In this study, we investigated aging-associated changes in DNA methylation in kidney transplants.

Methods: Whole-genome array-based methylation analysis using the Infinium HumanMethylation 450K Beadchips was performed on 29 kidney allograft biopsies obtained during transplantation surgery, at time of implantation. Inadequate samples were filtered and correction for batch effect was performed. The association between donor age and methylation of each CpG dinucleotide was measured with linear regression, adjusted for gender, cold ischemia time and batch, and using Benjamini-Hochberg correction for multiple testing. The genes mapped to these significant CpGs were selected for gene ontology analyses.

Results: Donor age associated significantly with methylation levels at 11 289 CpG dinucleotides after adjustment for other covariates and correction for multiple testing. These 11 289 CpGs represents more than 3000 unique genes. Several of these differentially methylated CpG dinucleotides were in concordance with the CpGs included in a DNA methylation age model that has previously been described on peripheral blood DNA. With a lasso approach combining CpGs with similar methylation differences, we could observe 210 significant differentially methylated regions. When we entered the most significant genes (those with q-value < 0.001) in the functional annotation platform DAVID, several pathways were enriched. One of the most significantly enriched pathways was the Wnt signalling pathway, which is involved in kidney injury, repair and fibrosis.

Conclusion: There is a striking and profound independent association between donor age and DNA methylation changes in the kidney transplant. These DNA methylation changes occur preferentially at genes involved in fibrosis, which might provide a link between advanced donor age and chronic allograft dysfunction.
O8.
Mitochondrial feedback is a key feature of kidney graft senescence


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Background
Progressive DNA damage is considered one of the key instigators of ageing. Different models for ageing where DNA damage leads to accelerated senescence have been proposed.

Methods
A test cohort of 40 consecutive kidney donors, with pre-implantation renal allograft biopsies, was included in this study. Intrarenal and donor leucocyte telomere length, and mitochondrial DNA content was assessed using quantitative RT-PCR. In these same samples (N=40), whole genome microarray mRNA expression analysis was performed using Affymetrix Gene 2.0 ST arrays (N=40). The associations between mRNA gene expression and the biomarkers of replicative senescence were investigated using multiple regression models, adjusted for calendar age, gender and batch number. For biological interpretation, Ingenuity Pathway Analysis and Consensus software were used to identify overrepresented pathways. An independent cohort of 160 implantation biopsies was used for validation.

Results
In total, 1180 transcripts significantly associated with intrarenal telomere length, of which 611 were significantly upregulated with shorter telomeres. Pathway analysis revealed enrichment of transcripts coding for proteins of the citric acid cycle (q=1.06x10⁻¹³), transcripts involved in respiratory electron transport (q=1.06x10⁻¹³) and transcripts involved in oxidative phosphorylation (q=4.48x10⁻¹⁰). Also mitochondrial DNA content correlated highly significantly with telomere length (r= 0.3; p=0.0005). Independent replication of these findings is on-going on a separate cohort of 160 pre-implantation biopsies.

Conclusion
This unbiased study suggests that mitochondrial alterations (DNA content and mitochondrial gene expression) are key features of replicative senescence of human kidneys. Upregulation of mitochondrial gene expression in baseline kidneys for transplantation is a protective feedback mechanism and a potential therapeutic target for improving renal allograft viability in the perioperative faze.
Objectives: Hypertension after kidney transplantation is common and associated with poorer graft and recipient outcomes. Recently, we and others showed that tacrolimus activates the thiazide-sensitive sodium chloride cotransporter to cause hypertension. This suggests that thiazide diuretics may be especially effective drugs in this context, but prospective data are lacking. We assessed the antihypertensive effect of chlorthalidone compared to amlodipine, the drug of choice in our center, in hypertensive kidney transplant recipients using tacrolimus.

Methods: We conducted a single-center, non-inferiority crossover trial to compare chlorthalidone (CT, 12.5-25 mg) with amlodipine (AML, 5-10 mg). Patients were invited for ambulatory blood pressure measurement (ABPM) if office BP >140/90 mmHg. Other criteria included eGFR >30 ml/min, proteinuria < 1 g, and no use of glucocorticoids (glucocorticoids are discontinued three months after transplantation in our center). The treatment periods were randomized, lasted 8 weeks (allowing dose titration after 2 weeks), and were separated by a 2-week wash-out. Background anti-hypertensive drugs were allowed except for other diuretics.

Results: 82 patients underwent initial ABPM of whom 49 patients (59%) with average wake SBP >140 mmHg were enrolled (median 2.6 years after transplantation). 38 patients completed the study (6 patients stopped during CT mainly due to electrolyte disorders vs. 1 during AML, p=0.4). CT and AML both markedly reduced ABPM after 8 weeks (151/85 ± 11/9 to 141/81 ± 12/9 mmHg vs. 151/84 ± 13/9 to 138/79 ± 14/7 mmHg). There was no statistical difference in blood pressure response between the two drugs (p=0.3 by 2-way ANOVA). Dose titration rates were similar (42% for CT vs. 37% for AML, p=0.8). CT decreased eGFR (53 ± 17 to 46 ± 15 ml/min), whereas amlodipine increased it (50 ± 16 to 53 ± 17 ml/min, P<0.001). The first post-CT eGFR returned to baseline (51 ± 17 ml/min). Treatment with CT resulted in less proteinuria (median 14 vs. 19 mg/mmol, p=0.03) and less edema (8 vs. 31%, p=0.02). Regression analysis showed that a higher aldosterone to renin ratio, lower baseline serum potassium, and higher baseline serum bicarbonate predicted a better anti-hypertensive response to CT.

Conclusions: Thiazide diuretics effectively lower blood pressure in kidney transplant recipients using tacrolimus. Thiazides were especially effective in patients with more aldosterone effect.
O10. Effects of Dietary Sodium Restriction in Renal Transplant Recipients on RAAS blockade: a Randomized Clinical Trial

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**Background:** In chronic kidney disease (CKD) patients on blockade of the renin-angiotensin-aldosterone system (RAAS), dietary sodium restriction is an established therapy to reduce blood pressure and albuminuria. Whether these beneficial effects extend to renal transplant recipients (RTR) is unknown.

**Objective:** To study the effects of dietary sodium restriction on blood pressure (BP) and urinary albumin excretion (UAE) in RTR on RAAS blockade.

**Methods:** We performed a randomized, cross-over trial in stable outpatient RTR with creatinine clearance >30 ml/min, BP >120/80 mmHg, and on stable RAAS blockade therapy. Exposures consisted of a 6-week regular sodium (RS) diet (target: 150 mmol/24h) and 6-week low sodium (LS) diet (target: 50 mmol/24h). Main outcome parameters were systolic and diastolic BP, and UAE at the end of each diet period. Dietary compliance was assessed by 24h urinary sodium excretion.

**Results:** We randomized 23 RTR, of which 22 (age 58±8 yrs, 50% men, eGFR 51±21 ml/min) completed the study. One patient withdrew from the study, because of orthostatic complaints on the LS diet. Sodium intake was 156 [130-193] versus 68 [55-86] mmol/24h on RS versus LS diet respectively (P<0.0001). Sodium restriction significantly reduced systolic BP from 140±14 to 129±12 mmHg (-10%, P<0.0001), diastolic BP from 86±8 to 79±8 mmHg (-9%, P<0.0001), and non-significantly reduced UAE from 29 [11-99] to 22 [13-94] mg/24h (-22%, P=0.3). In a **per protocol** analysis with 17 RTR, excluding RTR with protocol violations or dietary non-compliance, sodium restriction reduced BP to a similar extent, and significantly reduced UAE from 48 [14-101] to 16 [10-82] mg/24h (-38%, P=0.04).

**Conclusions:** In stable RTR on RAAS blockade, dietary sodium restriction effectively reduces BP and, with adequate compliance, UAE as well. Dietary sodium restriction is relevant to blood pressure management in RTR on RAAS blockade.
O11.
Urine of premature neonates as source of nephron progenitor cells

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Background: Recently, subpopulations of cells from amniotic fluid (AF) and adult urine were shown to express kidney progenitor cell features with multipotential of differentiation. We aimed to study urine of preterm neonates born before the completion of nephrogenesis for the presence of kidney neonatal stem/progenitor cells (nKSPC) and the ability of these cells to differentiate into functional podocytes.

Methods: Clonal cell lines were characterized as KSPC and KSPC-derived podocytes by gene expression analyses using quantitative rt-PCR and protein expression by flow cytometry and immunofluorescence. Podocytes differentiation was induced by incubation of cells in medium containing retinoic acid and vitamin D. Function of podocytes was assessed by albumin endocytosis and calcium influx assays. Results were compared to conditionally immortalized podocytes (ciPodocytes) and podocytes differentiated from amniotic fluid stem cells (AFSC) and adult urine progenitor cells (aUPC).

Results: nKSPCs expressed mesenchymal stem cell markers and kidney progenitor cell markers as SIX2, CD24, CD133, Vimentin and CITED1 maintaining these characteristics up to passage 17. nKSPC-derived podocytes presented mesenchymal-to-epithelial transition and acquired arborized cytoplasm comparable to ciPodocytes. Cells presented up-regulation of podocyte-specific genes and proteins and were able to endocytose albumin and uptake calcium via transient receptor potential cation channel, subfamily C, member 6 (TRPC6).

Conclusions: Preterm neonatal urine represents a novel noninvasive source of self-reviewing kidney progenitor cells with potential to differentiate into functional podocytes and may be a promising tool for regenerative medicine aiming kidney repair and a model for studying renal disease.
O12.
Neutrophil Extracellular Traps convert endothelial cells to a mesenchymal phenotype in Systemic Lupus Erythematosus

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Objective. The increased production and impaired degradation of neutrophil extracellular traps (NETs) has been linked to the development of lupus nephritis in patients with systemic lupus erythematosus (SLE). However, how NETs incite renal injury in SLE remains to be fully characterized. The present study was undertaken to evaluate the effects of NETs on microvascular glomerular endothelial cells (ciGEnCs) and macrovascular umbilical vein endothelial cells (HUVECs).

Methods. Neutrophils were isolated from anticoagulated whole blood by routine Ficoll density gradient centrifugation and stimulated with phorbol myristate acetate (PMA) to form NETs. NETs were isolated by partial digestion with micrococcal nuclease. Subsequently, NET-containing supernatants were co-cultured with HUVECs or ciGEnCs, after which cell morphology, the expression of cell surface markers and gene expression was evaluated with immunofluorescence microscopy, flow cytometry and quantitative RT-PCR, respectively. For in vivo experiments, diseased MRL/lpr mice were sacrificed and divided into three groups based on their degree of proteinuria: no proteinuria (albustix < 300 µg/ml), short proteinuria (albustix > 1000 µg/ml, proteinuria < 7 days) or long proteinuria (albustix > 1000 µg/ml, proteinuria > 14 but < 21 days). Age-matched CBA mice were included as control. Kidney sections were stained for citrullinated H3, a NET-specific marker, and Ly6G to detect neutrophils. RNA was isolated from kidney cortices using TRIzol® reagent.

Results. We show that the receptor of advanced glycation endproducts (RAGE) on endothelial cells promotes uptake of NETs via clathrin-mediated endocytosis. Internalization of NETs by endothelial cells occurred in a relative immunologically silent manner, since elastase derived from internalized NETs managed to cleave the endothelial NFκB subunit p65, thereby dampening pro-inflammatory NFκB signaling. RAGE-mediated endocytosis of NETs is saturable and exceeding the phagocytic capacity of endothelial cells resulted in the enduring extracellular presence of NETs. Here, NETs rapidly altered endothelial cell-cell contacts and induced transendothelial albumin passage through elastase-mediated proteolysis of the intercellular junction protein VE-cadherin. Furthermore, NET-derived elastase promoted nuclear translocation of junctional β-catenin and induced endothelial-to-mesenchymal transition (endMT). In vivo, we identified NETs in glomeruli of diseased MRL/lpr mice, particularly in the proteinuric stage of disease, whose presence coincided with markers of endMT.

Conclusion. We conclude that an excess of NETs in SLE patients may overload the phagocytic capacity of glomerular endothelial cells for NETs and alter vascular integrity through proteolysis of VE-cadherin, activation of β-catenin signaling and induction of endMT. Inhibition of elastase is potentially beneficial in the prevention of NET-mediated glomerular damage in patients with SLE.
O13.
Acetazolamide: an improved treatment for Lithium-Induced Nephrogenic Diabetes Insipidus?

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Objective: Lithium causes nephrogenic diabetes insipidus (Li-NDI) and hydrochlorothiazide (HCTZ) forms, together with amiloride, the mainstay treatment for Li-NDI patients. The antidiuretic action of HCTZ in Li-NDI is generally ascribed to increased proximal sodium and water uptake, compensating for sodium loss due to NaCl-co-transporter (NCC) inhibition. Earlier, we found that HCTZ also reduces Li-NDI in NCC knockout mice, coinciding with alkalinized urine, suggesting that inhibition of carbonic anhydrase by HCTZ plays a role. To test whether inhibition of only carbonic anhydrases could be a useful alternative therapy, the effect of acetazolamide (ACZ) in Li-NDI was tested.

Methods: Polarize-d mouse cortical collecting duct (mpkCCD) cells were cultured in 2D transwell model and exposed to lithium chloride and ACZ, HCTZ or amiloride or used in siRNA assays. 8-10 weeks old female C57Bl6 mice received normal diet and water ad libitum for 10 days. For the experiments, mice were divided into four groups (n = 8), which were treated as follows: Group 1: Control mice given a normal diet; Group 2: Normal diet with 40 mmol LiCl /kg of dry food. Group 3: diet of group 2 with 200 mg amiloride and 350 mg hydrochlorothiazide per kg dry food. Group 4: diet of group 2 with 180 mg acetazolamide /kg dry food.

Results: Treatment of mpkCCD cells with ACZ attenuated the lithium-induced downregulation of endogenous AQP2. ACZ did not affect transcellular voltage, and, upon co-incubation with amiloride, resulted in significantly higher levels of AQP2, indicating that the action mechanism of ACZ differs from that of amiloride. Treatment of Li-NDI mice with ACZ revealed a significant antidiuresis and increased urine osmolality, which was indifferent from Li-NDI mice treated with HCTZ/amiloride. However, unlike HCTZ/amiloride, ACZ treatment did not result in hyponatremia, hyperkalemia, hypercalcaemia, metabolic acidosis and increased serum lithium concentrations. Moreover, ACZ rescued AQP2 expression over the entire length of the collecting duct, whereas HCTZ/amiloride only increased AQP2 levels in the renal papilla. Finally, acetazolamide appeared to reduce the glomerular filtration rate and prostaglandin E2 release, partially explaining the acetazolamide-induced antidiuresis.

Conclusions: In conclusion, our data reveal that inhibition of carbonic anhydrases attenuates lithium-induced downregulation of AQP2 and NDI development. Moreover, as ACZ appeared as effective as the conventional treatment to rescue Li-NDI, but caused fewer side effects, ACZ may represent a better therapeutic agent than HCTZ/amiloride to treat Li-NDI.
TOLVAPTAN DECREASES THE INCIDENCE OF RENAL PAIN EVENTS IN PATIENTS WITH AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE: RESULTS FROM THE TEMPO 3:4 TRIAL


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Objective: Renal pain affects a large proportion of patients with autosomal dominant polycystic kidney disease (ADPKD). Pain can sometimes be severe, adversely affecting quality of life, and difficult to manage. Data from the TEMPO 3:4 ADPKD trial suggested that tolvaptan may reduce episodes of renal pain. The aim of the present study was to investigate risk factors for renal pain and the effect of tolvaptan on renal pain incidence.

Methods: Renal pain was assessed during the 3-year TEMPO 3:4 trial, and studied as a composite score as well as categorized in 5 groups according to severity (mild = prescription of paracetamol; moderate = prescription of non-narcotic analgesics; moderately severe = limitation in physical activity; severe = prescription of narcotic analgesics; most severe = need for hospitalization and/or invasive intervention). Total kidney volume (TKV) was measured by MRI and eGFR was calculated with the CKD EPI formula.

Results: 1445 ADPKD patients were included (746 males, age 39±7 year, eGFR 81±22 ml/min/1.73m², TKV 1692 (750 – 7555) mL. Overall 50.9% of participants had a history of renal pain. A history of urinary tract infection, renal stones and macroscopic hematuria was associated with a history of renal pain (all p<0.0001). Other associations were found with female sex (p=0.0005), lower height (p=0.01) and lower urine osmolality (p=0.04). No associations were found with eGFR, TKV nor height corrected TKV (p=0.15, p=0.70 and p=0.47, respectively).

In the placebo group (n=484), 20.2% of the patients experienced an episode of renal pain during 3 years of follow up, and 1.03% needed surgical intervention or hospitalization for pain. In the placebo treated patients a history of urinary tract infection, renal stones, macroscopic hematuria, renal pain and female sex tended to be associated with incident renal pain events (p=0.002, p=0.05, p=0.09 and p=0.0003, p=0.03 respectively).

Tolvaptan use resulted in a significantly lower incidence of renal pain when compared to placebo (11.9%, p<0.0001; relative risk reduction 42% (95% CI 25-54%). When pain was defined more strictly, similar reductions in incidence with tolvaptan were noted. The pain incidence lowering effect of tolvaptan was independent of baseline characteristics predisposing for renal pain (p for interaction all NS).

Conclusion: Tolvaptan decreased the incidence of renal pain events, independent of patient characteristics predisposing for renal pain.
P1.
Progressive decline in tacrolimus clearance after renal transplantation is partially explained by decreasing CYP3A4 activity and increasing hematocrit

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Objective The long-term disposition of tacrolimus following kidney transplantation is characterized by a gradual decrease in dose-requirements and increase in dose-corrected exposure. This phenomenon has been attributed to a progressive decline in CYP3A4-activity, although this has never been demonstrated in vivo.

Methods Sixty-five tacrolimus and 10 cyclosporine treated renal transplant recipients underwent pharmacokinetic testing at day 7 and months 1, 3, 6 and 12 after transplantation, including 8 h tacrolimus or cyclosporine AUC curves and assessment of Cyp3A4 activity using oral and intravenous midazolam drug probes.

Results Tacrolimus clearance decreased gradually throughout the entire first year but only in CYP3A5*3/*3 homozygous recipients (25.6±11.1 l/h at day 7; 17±9.1 l/h at month 12; p<0.001). In mixed model analysis, decreasing CYP3A4 activity, measured by apparent oral midazolam clearance (924±443 ml/min at day 7 vs. 730±344 ml/min at month 1; p<0.001), explained 55.4% of the decline in tacrolimus clearance in the first month. CYP3A4-activity decreased by 18.9 ml/min for every milligram of methylprednisolone dose tapering within the first month; beyond this point it remained stable. A gradual rise in hematocrit throughout the entire first year explained 31.7% of decrease in tacrolimus clearance in the first month and 23.6% of the decrease between months 1 and 12. Cyclosporine clearance did not change over time.

Conclusion The maturation of tacrolimus disposition in the first year after renal transplantation observed in CYP3A5*3/*3 homozygous patients can partly be explained by a (steroid tapering related) decline in CYP3A4 activity and a progressive increase in hematocrit.
P2
Histological scoring system predicts renal outcome of post transplantation acute tubular necrosis.

* Contributed equally

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Introduction
Acute Tubular Necrosis (ATN) is a common cause of Delayed Graft Function (DGF) after renal transplantation (RTX). Currently no histological model is available to predict renal outcome. Recovery of ATN is the result of the balance between damage and repair. In this study we evaluated the predictive value of immunohistochemical parameters of renal damage and regeneration and compared these to an accepted clinical prediction model for cadaveric renal transplantation.

Methods
Retrospectively we identified 25 patients that underwent cadaveric RTX with DGF caused by ATN only, as shown in a renal biopsy 1 week after RTX. Biopsies were evaluated for histological tubular damage (atrophy, edema, casts, vacuolization), DNA damage (γH2AX staining) and apoptosis (cC3 staining). Regeneration was assessed by staining for stem cell marker CD133 and proliferation marker Ki67. The correlation between these parameters and renal outcome was assessed individually as well as a combined, because damage may be a confounder for regeneration. Clinical parameters for renal outcome were collected as previously described in the Deceased Donor Score (DDS). The relation between these parameters and renal outcome, defined as eGFR at 6 months, was assessed using regression or one-way ANOVA. A corrected analysis for regenerative markers was performed to eliminate potential confounding by the amount of renal damage.

Results
Our histological damage score significantly predicted renal outcome (R:-0.52 P:0.01), whereas the DDS only tended to correlate with renal outcome (F:3.12 R: P:0.05). Neither parameters for DNA damage, nor for apoptosis correlated to renal outcome significantly (R:-0.24 P:0.91 and R:-0.16 P:0.44 respectively). In addition, the investigated parameters for regeneration (CD133 and Ki67) did not predict renal outcome (R:-0.25 P:0.23 and R:-0.10 P:0.63 respectively), also not after correction for renal damage.

Conclusion
We are the first to show that histological parameters can predict renal outcome of post transplantation ATN. Importantly, our histological damage score correlated better with renal outcome than the DDS. Despite the crucial role of regeneration in recovery after ATN, no relation was found between stem cell marker CD133, proliferation marker Ki67 and renal outcome.
Abstracts – BENELUX Kidney Meeting 2015

P3. Renal Concentrating Capacity and Copeptin Concentration in Patients with ADPKD and IgA Nephropathy with Impaired Renal Function

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Objective: ADPKD patients have an impaired maximal urine concentrating capacity (Umax). Whether this is an aspecific effect of renal function impairment, or specific for ADPKD is yet unknown. We hypothesized that ADPKD patients have a more severely impaired Umax in comparison with non-ADPKD renal disease patients, which leads to an exaggerated vasopressin (AVP) response that may be damaging to the kidney.

Methods: 15 ADPKD (eGFR<60) and 15 IgAN patients, matched for age, sex and eGFR, underwent a water deprivation test to determine Umax. Urine and plasma osmolality (Uosm and Posm), albuminuria (ACR) and plasma copeptin (surrogate marker for AVP in pmol/L), were measured at baseline and after water deprivation (average 18 hours). Height adjusted total kidney volume (htTKV) was measured by MRI.

Results: Umax was lower in ADPKD compared with IgAN patients. Upon water deprivation Posm increased in ADPKD (p=0.003), but not in IgAN (p=0.1), whereas copeptin increased in both groups similarly (ADPKD: p=0.001; IgAN: p=0.02). Copeptin after water deprivation was negatively associated with Umax in both groups (ADPKD: R=-0.72, p=0.002; IgAN: R=-0.70, p=0.004). In ADPKD, copeptin and albuminuria were correlated after water deprivation (R=0.71, p=0.003), independently of eGFR or htTKV. Furthermore, htTKV in ADPKD was associated after water deprivation with Posm (R=0.52, p=0.048), copeptin (R=0.58, p=0.03) and Umax (R=-0.54, p=0.04).

<table>
<thead>
<tr>
<th></th>
<th>ADPKD</th>
<th>IgAN</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
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<tr>
<td>eGFR</td>
<td>43±9</td>
<td>44±14</td>
<td>0.74</td>
</tr>
<tr>
<td>Posm</td>
<td>289±5</td>
<td>294±10</td>
<td>0.14</td>
</tr>
<tr>
<td>Uosm</td>
<td>378±157</td>
<td>498±144</td>
<td>0.04</td>
</tr>
<tr>
<td>Copeptin</td>
<td>14.0 (6.6-29.1)</td>
<td>11.9 (7.3-26.1)</td>
<td>0.98</td>
</tr>
<tr>
<td><strong>Umax</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Posm</td>
<td>293±6</td>
<td>295±10</td>
<td>0.51</td>
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<tr>
<td>Uosm</td>
<td>533±138</td>
<td>642±148</td>
<td>0.046</td>
</tr>
<tr>
<td>Copeptin</td>
<td>26.6 (12.8-43.0)</td>
<td>20.7 (10.3-43.0)</td>
<td>0.84</td>
</tr>
</tbody>
</table>

Conclusions: ADPKD patients have a lower Umax compared with IgAN patients with similar renal function. Remarkably, this is not accompanied with an exaggerated increase in AVP. Notwithstanding, ADPKD severity was associated with stronger increases in Posm, copeptin and albuminuria during water deprivation. This suggests that in ADPKD water deprivation may be deleterious and should be avoided.
P4. Role of Skin and Endothelial Surface Layer Heparan Sulfates in Blood Pressure Regulation

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Objective: Besides the skin, the endothelial surface layer (ESL) contains many glycosaminoglycans (GAGs) that can osmotically inactivate Na⁺ and may affect blood pressure (BP). Exostosin (EXT) genes regulate polymerization of heparan sulfate (HS), the predominant ESL GAG. In mice with heterozygous loss of EXT-1 and EXT-2 (EXT-1-2+/−), and wildtype (WT) mice, we investigated the role of HS GAGs in the skin and ESL in BP regulation after an acute and chronic NaCl load.

Methods: We investigated BP effects during a 7-day normal (0.3%, NSD) and high (8.0%, HSD) NaCl diet with tail cuff measurements, and during an acute 1.8% NaCl load (8 µl/g) with intracarotid measurements. We used intravital microscopy to estimate ESL thickness in <40 µm cremaster vessels on both diets. We used high performance liquid chromatograph-mass spectrometry to measure skin HS disaccharide concentration and sulfation.

Results: Baseline BP was equal in WT and EXT-1-2+/− mice (p=0.9), with no difference between NSD and HSD. Relative to WT, acute NaCl infusion increased BP in EXT-1-2+/− mice (p=0.02), while heart rate remained equal (p=0.5). After a NSD and HSD, EXT-1-2+/− mice had a 78% reduction of ESL thickness compared to WT mice (Fig A). HSD increased ESL thickness in WT mice, especially in 20-40 µm vessels, but not in EXT-1-2+/− mice (Fig BC). Skin HS concentration and sulfation patterns were equal between diets in WT mice (Fig DE). On a NSD, EXT-1-2+/− mice had more highly sulfated HS compared to WT (Fig DE). EXT-1-2+/− mice on a HSD had the highest skin HS concentration, of which most were low-sulphated.

Conclusion: An intact ESL is pivotal to prevent a BP increase during acute NaCl excess. Skin GAGs may be particularly important to prevent detrimental NaCl effects on the long-term, especially when the ESL is damaged.
P5.
METFORMIN TREATMENT PROTECTS AGAINST RENAL FAILURE DEVELOPMENT AND PRESERVES NORMAL PHOSPHORUS AND CALCIUM BALANCE
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Objective: Metformin, an oral anti-hyperglycemic agent used for type II diabetes mellitus as a standard treatment, has been shown to attenuate neointimal hyperplasia and to reduce atherosclerosis and vascular senescence in experimental studies. One in vitro study revealed that Metformin inhibited β-glycerophosphate-induced calcification in rat aortic smooth muscle cells along with the prevention of phenotypic reprogramming towards osteoblast-like cells. These findings are the impetus to further in-depth in vivo research into the effect of Metformin on the onset and progression of vascular calcification in the setting of chronic kidney disease (CKD).

Methods: To induce CKD-related vascular calcification, rats received a 0.25% adenine/low vitamin K diet for 8 weeks. Animals were daily treated with 200 mg/kg Metformin or vehicle (1% carboxymethylcellulose) from 1 week after CKD induction onwards, until the end of the study at week 8 by oral gavage. To follow renal function and the mineral balance over time creatinine, calcium and phosphorus were measured in the serum and urine at various time points throughout the study period. Vascular calcification was evaluated by measurement of the total calcium content in the abdominal aorta and the left carotid and femoral arteries by atomic absorption spectrometry. Renal histology was evaluated semi-quantitatively on Periodic Acid Schiff stained sections at the end of the study.

Results: Continuous adenine dosing resulted in severe, stable CKD along with serious hyperphosphatemia and hypocalcemia in vehicle treated rats. Daily Metformin treatment protected adenine dosed rats from the evolution towards severe CKD and as such, serum phosphorus and calcium concentrations remained within the normal range. In contrast to the vehicle-treated animals no calcification in the arteries was found in CKD rats treated with Metformin. The calcium content in the aorta and the carotid and femoral arteries was lower in Metformin treated CKD rats as compared to vehicle treated ones, however a significant difference was only noted in the femoral artery. Although overall renal damage (particularly dilatation) was extensive in both groups, Metformin treated animals clearly presented less tubulo-interstitial infiltration.

Conclusion: Metformin treatment led to, at least partial, protection of renal failure development and concomitant preservation of normal circulating phosphorus and calcium levels. These beneficial effects on the kidney and mineral metabolism in turn prevented the onset of vascular calcification in the Metformin study group. Further studies are required to gain more insight into the mechanism by which Metformin preserves kidney function.
P6.
INTERLEUKIN-37 DIMINISHES THE INFLAMMATORY RESPONSE OF ISCHEMIA/REPERFUSION-SUSCEPTIBLE RENAL TUBULAR EPITHELIAL CELLS

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**Objective:** Renal ischemia and subsequent reperfusion (IR) is the primary cause of developing acute kidney injury. IR induces excessive local inflammation that results in tubular injury and renal dysfunction. Therapeutic strategies aiming to dampen inflammation might therefore provide new opportunities to diminish renal IR injury. The human cytokine Interleukin (IL)-37 inhibits inflammation via nuclear as well as cell-surface receptors. IL37 is expressed by different cell types, including renal epithelium and circulating monocytes. Cell type-specific effects of IL37 in renal IR remain however unknown.

**Methods:** Primary tubular epithelial cells (PTECs) and bone marrow-derived macrophages (BMDMs) were isolated from WT and transgenic mice expressing human IL37 (hIL37tg) and cultured. In vitro, cells were pretreated with different concentrations of recombinant human IL37 protein (rhIL37) or vehicle and subsequently stimulated with LPS for 4 or 24hrs. Cytokine release (ELISA) and mRNA expression (quantitative RT-PCR) were determined.

**Results:** After 24hrs of LPS stimulation, the release of both CXCL1 and IL6 was reduced in hIL37tg PTECs as compared to WT PTECs. This was preceded by diminished CXCL1 and IL6 mRNA levels after 4hrs of LPS stimulation. rhIL37 pretreatment of WT PTECs reduced CXCL1 mRNA, but not IL6 mRNA expression after 4hrs.

In hIL37tg BMDMs CXCL1 mRNA levels and protein release were both reduced after 4 and 24hrs of LPS stimulation, as compared to WT BMDMs. IL6 release was only diminished after 24hrs, whereas IL6 mRNA levels did not significantly differ. In contrast, rhIL37 pretreatment of WT BMDMs reduced IL6 mRNA expression after 24hrs of LPS stimulation, but neither affected CXCL1 nor IL6 release.

**Conclusion:** Our data indicate that the human cytokine IL37 diminishes inflammatory responses of renal epithelial cells and macrophages, both central players in the pathophysiology of renal IR injury.
P7.
Exploring the Exocyst and its association with the Cilium and Retinal-Renal Ciliopathies

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Introduction: Defects in cilia, an antenna-like organelle, result in severe hereditary disorders with overlapping phenotypes, often including nephronophthisis and retinal degeneration. Various studies have shown that the exocyst complex, that regulates polarized exocytosis, also plays a role in the cilium: knock-down of exoc5 in zebrafish causes cystic pronephros, and recently, mutations in EXOC4 and EXOC8 have been found in ciliopathy patients.

Objective: As the molecular relationship between the exocyst and the cilium remains unexplored, our aim is to elucidate the ciliary involvement of the exocyst complex with a proteomics approach.

Methods: To analyze the interactome of the exocyst complex, individual exocyst components were overexpressed in renal cells (HEK293) after which Tandem Affinity Purification (TAP) was performed followed by mass spectrometry. Yeast two-hybrid screening (Y2H) was used to screen for interactors of EXOC4 and EXOC8 using human kidney-, brain-, and retinal cDNA libraries. Finally, recombinant exocyst components fused to fluorescent markers were overexpressed in ciliated retinal cells (hTERT-RPE1) to analyze the ciliary localization of each component.

Results: Affinity proteomics of (individual) exocyst components revealed interactions with >200 proteins and pulled down the complete exocyst complex. Ciliary proteins are enriched in the exocyst interactome, and >60% of these unite with multiple exocyst components. Screening for direct interactors of EXOC4 and EXOC8 identified 57 unique proteins, of which several have a ciliary function. Localization studies showed that recombinant exocyst components are enriched at centrioles of cultured retinal cells.

Conclusion: Our data indicate that the exocyst indeed plays a role in the cilium. The interactome of the exocyst complex in cultured kidney cells is enriched for ciliary proteins, and individual exocyst complex members localize to centrioles in ciliated retinal cells. We also show that exocyst members are directly interacting with ciliary proteins. Future studies will focus on further dissecting the role of the exocyst in the cilium.
P8.  
The use of screening MR angiography in ADPKD  
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Division of Nephrology, University Hospitals Leuven, Belgium  

Objective: Autosomal dominant polycystic kidney disease (ADPKD) is associated with the development of intracranial aneurysms and an elevated risk of hemorrhagic stroke. It is suggested that screening MR angiography reduces the incidence of hemorrhagic stroke. Current screening criteria include positive aneurysmal history in relatives, neurological symptoms and planned major surgery. The accuracy of these indications for detecting patients at risk for hemorrhagic has not been fully explored.

Methods: We performed a single-center retrospective analysis of all ADPKD patients followed at the University Hospitals Leuven, between January 1990 (date of the first MR angiography) and August 2014. Baseline demographics and occurrence of screening criteria, screening MR angiography, intracranial aneurysm, hemorrhagic stroke were evaluated.

Results: We identified 865 patients with ADPKD. Those who were seen at least three times (n=627, median age 51y, 49% males) were included for analysis. Mean duration of follow-up was 11.6 years. In this cohort, current screening criteria were met in 183 ADPKD patients (29.2%). Of these, 136 (74.3%) had screening MR angiography. In patients with no MR angiography, presence of screening criteria was associated with an elevated risk of hemorrhagic stroke (12.8% vs. 4.0%, P 0.02). Use of MR angiography in patients with screening criteria was related with a lower risk of hemorrhagic stroke during follow-up (2.2% vs. 12.8%, P 0.01).

Conclusion: In patients with ADPKD, current screening criteria for intracranial aneurysm are associated with a 3-fold elevated risk for future hemorrhagic stroke and performing MR angiography seems effective in reducing this risk.
**P9. Diagnostic Management of Suspected Acute Cyst Complication in Patients with Autosomal Dominant Polycystic Kidney Disease**

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(2) Division of Nuclear Medicine, University of Liège Hospital (ULg CHU), Liège, Belgium  
(3) Groupe Interdisciplinaire de Génoprotéomique Appliquée (GIGA), Cardiovascular Sciences, University of Liège, Liège, Belgium

**Objectives.** Acute cyst complications, namely infection (CyI) and hemorrhage (CyH), represent a diagnostic challenge in patients with autosomal dominant polycystic kidney disease (ADPKD). Recent reports highlighted common criteria for these conditions. Here, we retrospectively compare clinical, biological and radiological findings in ADPKD patients presenting with suspected acute cyst complications.

**Methods.** ADPKD patients presenting with abdominal pain and/or fever between January 2005 and June 2015 were identified in a prospective computerized database. Medical files were individually reviewed. CyH was defined as spontaneous intracystic density above 25 Hounsfield units on computed tomography (CT). CyI was definite if confirmed by cyst fluid analysis showing bacteria or neutrophils, and was probable if all four of the following criteria were met: temperature >38°C, loin or liver tenderness, C-reactive protein (CRP) plasma level >50 mg/L and no CT evidence for CyH. Episodes out of criteria were grouped as fever of unknown origin (FUO).

**Results.** One hundred and one ADPKD patients experienced 205 episodes of acute abdominal pain (n=172) and/or fever (n=33). In 48 patients (117 events, 57%), a non-cystic disease was positively diagnosed. Concerning the remaining 88 events, 20 patients experienced 30 CyH, whereas 16 patients presented with 23 episodes of CyI. Eleven cases were renal (n=7) or hepatic (n=4) definite CyI, and 12 cases were renal (n=10) or hepatic (n=2) probable CyI. Thirty-five episodes in 31 patients were categorized as FUO. Clinically, abdominal pain was reported in both CyH and definite CyI, whereas fever was mostly observed in CyI (7% vs. 100%, p<0.05). Biologically, no difference was found between CyH vs. CyI regarding hematuria, whereas leucocyturia was detected less frequently in CyH (10% vs. 35%, p<0.05). CRP levels at admission was lower in CyH than in definite CyI (13.3±14.2 vs. 187.3±95.7, p<0.001). By contrast, analysis of variance showed similar levels of white blood cell counts in all groups. Bacteriologically, urine or blood cultures remained sterile in >90% of CyH, but were contributive in 53.4% of 58 suspected infections, with a prevalence of 70.1% for E. coli. Radiologically, ultrasounds, CT and magnetic resonance imaging diagnosed CyI in 2.6% (1/38), 20.6% (7/34) and 16.7% (1/6) of cases, respectively. After excluding CyH using CT, 27 18FDG positron-emission tomography (PET)/CT were done within a median period of 11 days following admission: 10 (37.1%) diagnosed CyI, 5 (18.5%) identified non-cystic diseases, and 12 (44.4%) showed no pathological 18FDG uptake.

**Conclusions.** This retrospective series underscores the usefulness of clinical, i.e. fever, and biological, i.e. CRP levels, criteria, but emphasizes the limitations of bacteriological and radiological investigations in the diagnostic management of ADPKD patients presenting with suspected acute cyst complication. 18FDG-PET/CT may help distinguish CyI from non-cystic pathologies, thereby dictating therapeutic choices.
P10.

URINARY BIOMARKERS AND PREDICTION OF DISEASE PROGRESSION IN AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE

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Objective: The variable disease course of ADPKD underlines the importance of predicting disease progression especially since therapeutic options are now available. Conventional risk markers (age, sex, GFR, total kidney volume (TKV)) lack sensitivity, are expensive or time consuming to measure. We therefore investigated whether easy to measure urinary markers can predict disease progression and have additional value to conventional risk markers.

Methods: At baseline tubular damage and inflammatory markers were measured in 24-hr urine; albumin, IgG, KIM-1, NAG, β2MG, H-FABP, MIF, NGAL and MCP-1. Kidney function was estimated (eGFR) by CKD-EPI, and measured (mGFR) by (125)I-iothalamate, and TKV by MRI. Disease progression was expressed as annual change in eGFR, mGFR, and height-adjusted (ht)TKV). Multivariable linear regression was used to assess the predictive ability of the markers above conventional risk markers.

Results: Included were 104 ADPKD patients, 40±11yrs, 39% female, eGFR 77±30, mGFR 79±29 ml/min/1.73m² and htTKV 852 (510-1243) mL/m. During a follow-up of 3.8±1.2 yrs, annual change in eGFR was -3.2±3.0, in mGFR -3.0±3.0 mL/min/1.73m² and in htTKV 6.2±5.9%. β2MG and MCP-1 were associated with annual change in eGFR (St. β=0.23, p=0.02; St. β=-0.38, p<0.001 resp.) and mGFR (St. β=-0.24, p=0.03; St. β=-0.24, p=0.03 resp.), even when adjusted for conventional risk markers, but not with annual change in htTKV. Combined β2MG and MCP-1 had an added predictive ability for annual change in eGFR (R²=0.222 vs. 0.374, p<0.001) and mGFR (R²=0.186 vs. 0.283, p=0.008) (model 3). When performing a stepwise backward analysis the best model consisted of only β2MG and MCP-1 for both annual change in eGFR as mGFR (R²=0.306 and 0.221 resp.) (model 4). Although there was no significant difference in R² with model 3 (p=0.104 and 0.26 resp.), model 3 was the preferable model in terms of weighted Aikaike Information Criterion w(AIC) (normalized probability=0.95 and 0.82 compared with model 4). Similar results were obtained when patients with an eGFR≥60 mL/min/1.73m² were selected.

Conclusions: β2MG and MCP-1 both predict disease progression, and had an added predictive value on top of conventional risk markers. These markers have therefore potential to serve as a predictive tool for clinical practice.
P11.
A nonsense mutation in the WNT11 gene causes a human ciliopathy-like phenotype

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Introduction Ciliopathies form a class of heterogeneous, sometimes overlapping, disorders associated with genetic mutations encoding defective proteins, which result in either abnormal formation or function of motile or immotile cilia. As cilia are a component of almost all cells in the body, including embryonic nodal cells, their dysfunction can manifest syndromic disorders that might be associated with heart and lung asymmetry defects, like situs inversus. Despite the recent progress in identification of ciliopathy-associated genes, cases with atypical ciliopathies remain unsolved.

Results We performed whole exome sequencing (WES) in boy form Indian descent presenting with a complex congenital heart defect, complete situs inversus and bilateral renal hypoplasia resulting in end stage renal disease during the first year of life. The child further suffered from severe neonatal respiratory distress and we suspected an atypical ciliopathy due to the clinical presentation. However, cilia motility was normal in high-speed video microscopy, electron microscopy did not reveal any ultrastructural ciliary defects and WES showed a homozygous nonsense mutation in the WNT11 gene. The patient phenotype correlates well with a previously published knockout-mouse model except for the situs inversus, which is also found in the patient’s mother who carries the nonsense mutation in a heterozygous state.

Conclusion We show for the first time that a loss of function mutation in the WNT11 gene can cause complex heart and kidney defects in human. If the situs inversus in the proband and mother are due to this variant or may result from a variation in another gene missed by WES is currently still under investigation and we currently screen a large CAKUT and VACTERL cohort in order to identify more cases.
P12.
Genetic defects in a patient with a new congenital disorder of glycosylation presenting with renal- and liver cysts, skeletal dysplasia, and severe combined immunodeficiency

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Objective Glycosylation is essential for human development. More than 100 clinically diverse inherited disorders are known to result from glycosylation defects. One subtype of the congenital disorders of glycosylation (CDG) is caused by defects in glycosaminoglycan (GAG) synthesis, which can result in renal anomalies and other features. Here, we describe an infant from a consanguineous family with a new GAG-CDG. Phenotypic features include renal insufficiency (hyperechoic small-sized cysts and increased serum creatinine and urea levels), liver cysts, respiratory insufficiency, discrete skeletal anomalies, and T-cell depletion.

Methods We performed whole-exome sequencing (WES) on patient leucocyte DNA to identify the causative mutation. Immunocytochemistry was conducted to characterize the detected mutations functionally in patient-derived and control fibroblasts. Different GAG levels were measured in fibroblasts, serum, and urine from the patient and several controls. Mutated genes were screened with next-generation sequencing in a cohort of 96 patients with nephronophthisis and skeletal involvement.

Results By using WES we detected two homozygous missense mutations in highly conserved regions of two glycosylation genes, i.e. EXTL3 and CHST9, regulating GAG synthesis and modification. It remains unclear whether mutations in one or both of the genes contribute to the clinical phenotype. We found that one of the mutated proteins, EXTL, that normally localizes to the Golgi apparatus was absent in this organelle in patient cells. Immunocytochemical analysis of the other mutated protein failed. Finally, heparan sulphate levels were significantly reduced in patient-derived fibroblasts, though within the normal range in urine and serum. Mutation screening of both mutated genes in a cohort of 96 patients with renal and skeletal defects did not identify additional mutations.

Conclusion We describe an infant with renal insufficiency and other congenital anomalies. WES analysis revealed mutations in EXTL3 and CHST9, which are both involved in glycosylation. EXTL3, one of the mutated proteins, mislocalizes in patient-derived fibroblasts and these cells show aberrant heparan sulphate levels. Our data indicate that this patient has a rare GAG-CDG.

Abstracts – BENELUX Kidney Meeting 2015
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3) Children’s Hospital Boston, Harvard University, Boston, USA

Objective
The von Hippel-Lindau (VHL) gene is known to play an important role in suppressing the development of hereditary and sporadic clear cell renal cell carcinoma (ccRCC), the most common type of kidney cancer. To potentially model ccRCC, we aim to characterize the role of the vhl gene in zebrafish pronephros development.

Results
Here, we report that loss of vhl in zebrafish embryos results in severe pronephric abnormalities. In vhl mutant (vhl⁻⁻) zebrafish the glomerulus is enlarged, the Bowman’s space is widened and dilated cxcr4a-positive capillary loops are observed. While wild-type siblings exhibit a single layer of cuboidal cells comprising the proximal tubule, vhl⁻⁻ tubular cells are irregular with a grape-like or alveolar appearance. Further ultrastructural analysis revealed vhl⁻⁻ renal cells, which accumulate excessive amounts of vesicles that are variable in size and electron density. Since glomerular filtration and endocytosis in vhl⁻⁻ proximal tubule cells are not impaired, this might reflect a defect in exocytosis. VEGF receptor inhibition revealed that neovascularization of the vhl⁻⁻ proximal tubule does not obviously contribute to the aberrant cell morphology.

Conclusion
Our preliminary data indicates that vhl is required to maintain pronephric tubule and glomerulus integrity during zebrafish development.
P14. Indoleamine 2,3-dioxygenase Activity and Late Graft Failure after Kidney Transplantation

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Introduction: Long-term graft survival after kidney transplantation remains an important clinical problem. Therefore, markers that allow for early identification of patients at risk for late graft failure (LGF) are urgently needed. The immune-regulating enzyme indoleamine 2,3-dioxygenase (IDO) catabolizes tryptophan along the kynurenine pathway. Recent studies found IDO activity associated with occurrence of acute rejection and renal function decline shortly post-transplant.

Objective: To study whether IDO activity is associated with long-term graft survival in a large cohort of stable RTR.

Methods: We prospectively included outpatient renal transplant recipients (RTR) with a functioning graft >1 yr, between 2001-2003. Follow-up was recorded until May 2009. Death-censored GF was defined as return to dialysis or re-transplantation. Serum kynurenine (KYN) and tryptophan (TRP) were measured with LC-MS/MS; KYN/TRP ratio is a widely accepted measure of IDO activity.

Results: We studied 562 RTR (age 51±12 yrs, 56% men, 6.0 [2.6-11.6] yrs post-transplant). Baseline concentration of serum KYN was 1.8 [1.4-2.2] µmol/l, that of TRP was 40.0 [34.5-46.0] µmol/l, and KYN/TRP was 44.3 [35.0-57.9] µmol/mmol. In multivariable linear regression analyses, KYN/TRP was positively associated with proteinuria (β=0.17, P<0.001) and waist circumference (β=0.12, P<0.001), and inversely with eGFR (β=−0.54, P<0.001) and HDL-c (β=−0.14, P<0.001). During follow-up for 6.9 [6.1-7.4] years, 51 (9.2%) RTR developed GF. In multivariable Cox-regression analyses, KYN/TRP was positively associated with GF (age, sex, eGFR, and proteinuria adjusted HR 3.6 [95% CI 1.3-9.9], P=0.01). Further adjustment for waist circumference and HDL-c did not materially change this association (HR 3.1 [95% CI 1.3-8.9], P=0.02).

Discussion: IDO activity, as measured by KYN/TRP, is cross-sectionally associated with waist circumference, HDL-c, eGFR and proteinuria. Prospectively, it is associated with increased risk for LGF after kidney transplantation. Increased IDO activity may not only be a marker for LGF, but also an interesting target for intervention to prevent decline of renal transplant function leading to LGF.
P15.
Abatacept Treatment and B7-1 Immunostaining in Patients with Primary and Post-Transplant FSGS

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Objective: podocyte B7-1 expression has been implicated in the pathogenesis of idiopathic nephrotic syndrome for many years. Recently, podocyte B7-1 was described as a potential therapeutic target by demonstrating efficacy of B7-1 blocking agent abatacept in five patients with primary and post-transplant FSGS (Yu et al. New Engl J Med 2013). The authors speculated that patients who will respond to abatacept can be identified by positive B7-1 immunostaining on kidney biopsy specimens. Here, we report our experience with abatacept and B7-1 staining in patients with FSGS.

Methods: patients with no or partial proteinuria remission after plasmapheresis for treatment of FSGS received abatacept (1-3 doses of 10 mg/kg). After several trials of unsuccessful immunofluorescence staining, we performed B7-1 immunohistochemistry on paraffin embedded tissue with a primary antibody mouse anti CD80 (clone #37711, R&D systems MAB140). Tonsil and colon tissue were used as a positive controls.

Results: three patients with post-transplant FSGS, and one patient with FSGS in the native kidney were treated with abatacept (Table). All transplant patients had developed nephrotic proteinuria immediately after transplantation. One of the transplant patients was previously unresponsive to B7-1 blocking agent belatacept. None of the patients had proteinuria remissions after abatacept, nor did we observe positive podocyte B7-1 immunostaining.

Conclusion: In our hands B7-1 staining was absent in patients with FSGS. Our data caution against too much optimism regarding the efficacy of abatacept.

Clinical Characteristics of FSGS patients treated with abatacept

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patient 1</th>
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<th>Patient 4</th>
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<td>Age</td>
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<td>PP</td>
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<td>17 days</td>
<td>4 years</td>
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<td>Urinary PCR before / after treatment (g/10 mmol)</td>
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<td>7.2 / anuric</td>
<td>20.1 / 8.7</td>
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<tr>
<td>Screat before / after treatment (μmol/L)</td>
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<td>dialysis</td>
<td>218 / 204</td>
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</table>

FSGS = focal segmental glomerulosclerosis
MMF = mycophenolate mofetil
PCR = protein creatinine ratio
PP = plasmapheresis
Screat = serum creatinine
P16. Tubulointerstitial expression of connective tissue growth factor in renal allograft protocol biopsies at 3 months predicts interstitial fibrosis at 5 years.

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Objective. Connective tissue growth factor (CTGF) is an important mediator of renal allograft fibrosis and urinary CTGF levels correlate with the development of human allograft interstitial fibrosis. We set out to evaluate the predictive value of CTGF protein expression in early protocol biopsies for the subsequent development of allograft fibrosis.

Methods. Single center observational cohort study of 171 kidney transplant recipients with protocol biopsies at 3 months and repeat protocol biopsies 5 years after transplantation. Biopsies were scored according to the revised 1997 Banff criteria. Three month biopsies were immunohistochemically stained for CTGF. Tubulointerstitial CTGF positive surface area was scored visually and categorized as absent (<1%), minimal (1-10%), moderate (10-25%) or extensive (>25%).

Results. In multivariate multinomial regression analysis, the only predictors of interstitial fibrosis at 5 years were donor age (OR 1.04 [1.01-1.07], OR 1.06 [1.03-1.11], OR 1.05 [1.01-1.08] for ci score 1, 2 and 3 vs. 0, respectively; p<0.01 for all) and moderate to extensive CTGF positivity (OR 2.48 [1.09-5.61], p=0.03; OR 4.57 [1.33-16.63], p=0.016; OR 2.12 [0.77-5.81], p=0.15 for ci score 1, 2 and 3 vs. 0, respectively). Independent predictors of renal function at 5 years were donor age (p<0.001), renal function at 3 months (p<0.001) and occurrence of biopsy-proven acute rejection in the first 3 months (p=0.019).

Conclusion. CTGF expression in protocol biopsies 3 months after transplantation predicts interstitial fibrosis in protocol biopsies at 5 years, independent of donor age. CTGF staining might contribute to early detection of patients at high risk for chronic allograft injury.
P17.
Ischemia-induced DNA methylation changes in kidney transplantation.

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Objective: Ischemia-reperfusion injury is one of the main factors adversely impacting transplant outcome, and yet the mechanisms by which short-lived ischemia influences long-term allograft function remains unknown. Recent progress in different fields demonstrated epigenetic changes in cells following ischemia. In this study, we investigated differences in DNA methylation in kidney transplants from living donors and deceased donors with short and long ischemia times.

Methods: Whole-genome array-based methylation analysis using the Infinium HumanMethylation 450K Beadchips was performed on 29 kidney allograft biopsies obtained during transplantation surgery after reperfusion: 6 from living donors (LD), 12 from brain-dead donor (DBD) transplants with cold ischemia time ≥ 18h and 11 from DBD transplants with cold ischemia time ≤ 9h. Inadequate samples were filtered and correction for batch effect was performed. Samples were clustered through hierarchical clustering. The methylation state at each CpG dinucleotide was compared between the 3 groups of kidney transplants with Benjamini-Hochberg correction for multiple testing using the Champ package in R. The genes mapped to these significant CpGs were selected for gene ontology analyses.

Results: Hierarchical clustering of samples based on their DNA methylation profile revealed two clusters that differed only in regard to cold ischemia time. DNA methylation of DBD transplants with long ischemia differed most from LD transplants (696 CpGs), followed by DBD transplants with short ischemia versus LD transplants (86 CpGs) and DBD transplants with long versus short ischemia time (1 CpG). At these 696 CpGs, mostly hypomethylation was observed in DBD transplants compared to LD (691 hypomethylated, 5 hypermethylated). When we entered these significant genes in the functional annotation platform DAVID, several pathways were enriched, including the Wnt signalling pathway, which is involved in kidney injury, repair and fibrosis, and pathways involved in hypertrophic and dilated cardiomyopathy.

Conclusion: Kidney transplants differed in DNA methylation profile according to ischemia time. Whether these changes are involved in the worse allograft outcome of transplants from deceased donors with long ischemia times needs further investigation.
P18.  
The intrarenal resistive index measured after transplantation is determined by the upstream vascular system of the recipient.

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Objective: We recently demonstrated that the renal resistive index, measured after renal transplantation, reflects recipient characteristics, like recipient age and central hemodynamics, and not so much allograft function nor histology. In this study, we provide further evidence.

Methods: Aortic calcifications were measured by lateral lumbar X-ray in 262 recipients at the time of admission for renal transplantation. In a subgroup of patients, also pulse wave velocity (PWV) (n=119) and intima media thickness (IMT) (n=74) were measured. After transplantation, the renal resistive index, systolic (SBP) and diastolic blood pressure (DBP), pulse pressure, allograft function (eGFR), proteinuria, hematocrit and lipid blood tests were routinely measured at 3 months, 1 year and 2 years.

Results: There was a highly significant correlation between the severity of aortic calcifications of the recipient at transplantation and the resistive index measurements after transplantation (P<0.0001 at all time points). Also the IMT and PWV correlated with the resistive index (P<0.01 at all time points for all correlations). In the univariate repeated measurements model, recipients with higher resistive indices after transplantation were older, more likely diabetic and had higher body mass index. They had higher SBP, lower DBP, higher pulse pressures and lower hematocrit during follow-up and allograft function was also lower. When all significant variables were included in a multivariate mixed model, aortic calcifications, recipient age, diabetes, pulse pressure, DBP and hematocrit remained independently and significantly associated with the resistive index measurements after transplantation, whereas allograft function, donor age and recipient body mass index did not. In the subgroup of patients in whom also PWV and IMT was measured, PWV and IMT were not associated with the resistive index independently from the other covariates.

Conclusion: The renal resistive index measured regularly after renal transplantation depends on characteristics of the upstream vasculature of the recipient.
P19. 
The Impact of Renal Transplantation on Microbiota Derived Uremic Retention Solutes

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Objective: The gut microbial metabolism contributes substantially to uremic retention solutes accumulating in CKD. Both p-cresyl sulfate and indoxyl sulfate are representatives of this group of solutes and associate with adverse outcome in patients with renal dysfunction. Although it can be expected that serum levels of these microbial metabolites will decrease following renal transplantation, this has not been studied to date. In addition, whether serum levels of p-cresyl sulfate and indoxyl sulfate in renal transplant recipients are quantitatively different when compared to regular CKD patients is unknown.

Methods: A cohort of 51 CKD patients was prospectively followed from time of transplantation to 12 months post renal transplantation. Serum levels of p-cresyl sulfate and indoxyl sulfate were determined at time of transplantation, day 7, month 3 and month 12. At each time point, serum levels of both solutes were compared with an unrelated group of CKD patients matched for age, gender, BMI, diabetes, and dialysis modality/vintage at time of transplantation or renal function (serum creatinine, eGFR and measured creatinine clearance) at other time points.

Results: Serum levels of p-cresyl sulfate and indoxyl sulfate substantially decreased after renal transplantation (P<0.0001 for both solutes at each time point vs. time of transplantation). When compared to CKD control patients, serum levels of both solutes were still significantly lower in renal allograft recipients at each time point (see figure). Additional analyses demonstrated lower urinary excretion rates of microbial metabolites in renal transplant patients (P<0.0001).

Conclusion: Microbiota derived uremic retention solutes substantially decrease following renal transplantation. In addition, serum levels of these solutes are significantly lower when compared to regular CKD patients, suggesting an independent influence of renal transplantation or immunosuppressive drugs on gut microbial metabolism. Whether these microbial metabolites are also associated with allograft and patient outcome needs further investigation.
P20.
A heparin-grafted membrane plus citrate containing dialysate vs. regional citrate anticoagulation: results of the CiTED study

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Objective: Heparin is the mainstay anticoagulant during dialysis. Alternative anticoagulant strategies include regional citrate anticoagulation (RCA), heparin-grafted dialyzers or saline flushes. Of these, RCA is the most efficacious, although technical complexity and labor intensiveness preclude widespread use. Heparin-grafted membranes are easy to use, but dialyzer patency is inferior to RCA. Whether the combination of citrate-containing dialysate plus heparin-grafted membranes is non-inferior to RCA is not known.

Methods: The CiTrate plus EVodial in Dialysis (CiTED) study is a prospective, open-label randomized cross-over study comparing citrate-containing dialysate plus heparin-grafted membranes vs. RCA. In the study arm, we scheduled 750 dialysis sessions using the combination of a heparin-grafted AN69ST dialyzer (Evodial 180®, BAXTER) and 1 mmol/L citrate-containing dialysate (Selectbag Citrate®, BAXTER). In the control arm, 750 sessions of RCA were scheduled using Polyflux 170 (BAXTER) dialyzers and calcium-containing dialysate. In all sessions, scheduled treatment duration was 4 hours. No systemic heparin was used. Primary endpoint was non-inferiority for clotting events of the combination of a heparin-grafted membrane plus citrate-containing dialysate vs. RCA, with a prespecified non-inferiority margin of 10%.

Results: We included 25 patients, receiving 1285 study dialysis sessions in total, 636 in the study arm and 649 in the control arm. Both anticoagulation strategies were safe. Overall, clotting rates were low: 37/636 (5.82%) in the study arm and 42/649 (6.47%) in the control arm. The primary endpoint of non-inferiority was met ($P < 0.0001$). In secondary analysis, using Cox proportional hazard analysis, time to clotting did not differ between study arms ($P = 0.62$).

Conclusion: The combination of a heparin-grafted dialyzer with a citrate-containing dialysate is non-inferior to conventional RCA. The procedure is easy to perform, without additional pumps or measurements of calcium. The combination of a heparin-grafted dialyzer and citrate-containing dialysate is a valid alternative to RCA in patients requiring heparin-free dialysis.
P21.
Pre-dialysis decline of mGFR but not eGFR is a risk factor for mortality on dialysis

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**Objective.** Monitoring of renal function is important in patients with Chronic Kidney Disease (CKD) progressing toward end-stage renal failure, both for timing the start of renal replacement therapy, and for determining prognosis on dialysis. Thus far, studies on associations between estimated (e)GFR measurements in the pre-dialysis stage and mortality on dialysis have shown no or even inverse relations, which may result from the poor validity of serum creatinine-based estimation equations for renal function in pre-dialysis patients. We argued that the decline in renal function may be better reflected by GFR measured by 24 hour urine collection (mGFR). The objective of this study is to explore the effect of mGFR pre-dialysis decline rate on mortality during dialysis, and to compare this with the possible effect of eGFR decline.

**Method.** A subset of 208 individuals were included from the prospective, multicenter NECOSAD cohort, for whom a minimum of 2 mGFR pre-dialysis values was available. For these patients annual decline of mGFR and eGFR was estimated with linear regression, and classified according to KDOQI as fast (>4 mL/min/1.73m\(^2\) per year) or slow (≤ 4 mL/min/1.73m\(^2\) per year). Cox regression was used to adjust for potential confounders in survival analysis.

**Results.** Patients with a fast mGFR decline had an increased risk of mortality on dialysis: crude HR 1.697 (95% CI: 1.069-2.692). Adjusting for the confounders age, gender, primary kidney disease, cardiovascular disease, diabetes, cancer, ethnicity, smoking and mean GFR slightly increased this association: HR 1.740 (1.029-2.944). In contrast, no association was found between a fast eGFR decline in the pre-dialysis phase and mortality on dialysis: crude HR 1.113 (0.720-1.720) and adjusted HR 0.976 (0.601-1.587).

**Conclusions.** This study found that a fast mGFR decline during pre-dialysis is an independent risk factor for survival on dialysis in contrast to eGFR decline. In conclusion, this study gives incentive for repeated mGFR measurements in patients on pre-dialysis care, as it is of importance for the follow up and prognosis of dialysis patients.
P22.
ABDOMINAL ARTERIAL CALCIFICATION IS HIGHLY PREVALENT AMONG PERITONEAL DIALYSIS PATIENTS AND PROGRESSES AFTER KIDNEY TRANSPLANTATION

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Background: Cardiovascular disease is the leading cause of death in patients with end stage kidney disease, and this remains so after kidney transplantation. Vascular calcification is an important contributor to this. In contrast to coronary artery calcification, prevalence and risk factors of abdominal vascular calcification and its progression after kidney transplantation are less well documented, especially in patients on peritoneal dialysis.

Methods: In this prospective observational study consecutive patients on peritoneal dialysis who underwent kidney transplantation in a single center from 2009 to 2013 were included. Unenhanced computed tomography of the abdomen was performed at baseline (median 3 weeks post-transplantation) and after a median period of 19 months (IQR 18-20) of follow up. Total calcification score was calculated by adding calcification scores (Agatston-like) of the abdominal aorta, superior mesenteric artery, splenic artery, renal arteries and iliac arteries. Uni- and multivariate linear regression analyses were performed to identify risk factors for calcification score progression.

Results: 47 patients were included (age 50±14 yrs, 64% males, 15% diabetics, 92% hypertensive, peritoneal dialysis duration 30±21 months). Prevalence of vascular calcification at baseline was 74% in any artery (range: 21% superior mesenteric artery, 30% splenic artery, 30% renal arteries, 53% external iliac arteries, 57% internal iliac arteries, 64% common iliac arteries, 68% abdominal aorta). Total median calcification score increased significantly after kidney transplantation from 5192 to 5532 (p<0.001). Independent risk factors for an increase in total calcification score were age at transplantation (β=0.831, p<0.001) and a history of cardiovascular disease (β=0.199, p=0.005).

Conclusion: Abdominal vascular calcification is highly prevalent in patients on peritoneal dialysis and progresses after kidney transplantation. Age, and to a lesser extent a history of cardiovascular disease are independent risk factors for abdominal vascular calcification progression.
P23.
EXTRACELLULAR VESICLES IN PERITONEAL EFFLUENT

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Objective: Continuous and long-term exposure to peritoneal dialysis (PD) solutions induces constant low-grade inflammation and remodeling of peritoneal membrane morphology. Although the biochemical composition of the peritoneal effluent offers the opportunity to explore the peritoneal membrane status in a simple and non-invasive manner, still no clinically useful effluent biomarker has been identified that reflects the peritoneal membrane integrity sufficiently. As human body fluids contain extracellular vesicles (EVs), which are now believed to provide novel biomarkers for diseases, we investigated the presence of such EVs in peritoneal effluent.

Methods: Peritoneal effluent of a short-term continuous ambulatory PD patient was collected from a regular dialysis dwell. After centrifugation, aliquots of the cell-free effluent were frozen in liquid nitrogen and stored at -80°C until analysis. Primarily, EVs were isolated by size exclusion chromatography. Transmission electron microscopy (TEM) and flow cytometry (comprising markers for epithelial and mesothelial cells, leucocytes, platelets and erythrocytes) were used to detect the presence of single EVs.

Results: EVs and liposomes were identified by TEM and flow cytometry. EVs exhibited their characteristic cup shape. The majority of EVs had a mean diameter <100 nm, and were present in similar quantities as in human plasma. Most peritoneal effluent EVs originate from epithelial and mesothelial cells as determined by flow cytometry. Moreover, the vesicles stained positive for leucocyte antigens, mesothelin and cancer antigen 125.

Conclusion: This is the first study to demonstrate the presence of EVs in human peritoneal effluent. Furthermore, the cellular origin of most peritoneal EVs is established. We postulate that the presence and composition of such EVs in peritoneal effluent could closely mirror the morphology of the peritoneal membrane. Future studies will be necessary to investigate the clinical relevance for the detection of peritoneal membrane damage and potential use of these peritoneal EVs in PD patient care.

Figure 1. TEM image of extracellular vesicles in cell-free human peritoneal effluent. The arrow indicates a si
P24.
Age and Gender Specific Lifetime Risk of Renal Replacement Therapy

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**Objective:** Kidney transplantation is the preferred treatment of end stage renal disease (ESRD). Graft and patient survival are highest after transplantation with a graft from a living donor. However, persons who donate a kidney are themselves at risk of ESRD. Personalized risk prediction requires age and gender specific risk estimates. Here, we report lifetime risk of renal replacement therapy (RRT) for ESRD by age and gender across Europe.

**Methods:** We defined ESRD as chronic RRT and age 80 as the lifetime horizon. Death was considered a competing event. We obtained RRT incidence rates by age and gender from the ERA-EDTA Registry. Mortality rates were calculated from census data provided by EuroStat. We used these rates to estimate cumulative incidence of RRT by age and gender for countries providing individual patient data to the ERA-EDTA Registry. We pooled lifetime RRT risks using inverse variance weighted means.

**Results:** At index age 20, lifetime RRT risk for females ranged between 0.40\% and 0.87\% across countries, and for males between 0.77\% and 1.59\%. At age 60, lifetime RRT risk ranged between 0.26\% and 0.68\% for females and 0.56\% and 1.32\% for males. Pooled lifetime RRT risk in Europe was 0.62\%, 0.58\% and 0.43\% in 20, 40 and 60 year old females. In males the respective risks were 1.16\%, 1.10\% and 0.87\% for index ages 20, 40 and 60. The figure shows pooled lifetime RRT risk estimates by index age.

**Conclusion:** Lifetime RRT risk differs across Europe. Women are at lower risk compared to men. These data offer a basis to provide personalized prediction of lifetime ESRD risk when evaluating a potential kidney donor. The estimates presented here are population averages. We expect that lifetime risk is lower in persons with normal eGFR and no albuminuria.
P25.
Intravoxel Incoherent Motion analysis of Diffusion Weighted Imaging to measure glomerular filtration fraction: proof of concept

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Glomerular filtration fraction (FF) can be calculated from the $^{125}$I-thalamate clearance (glomerular filtration rate (GFR)) and $^{131}$I-hippuran clearance (effective renal plasma flow (ERPF)). This technique is costly and time consuming. Intravoxel Incoherent Motion (IVIM) analysis provides an assessment of diffusion weighted imaging (DWI) for fractions of blood and (pre-)urine within kidney tissue. This could serve as a surrogate for filtration fraction. With this study we a proof of concept for an MRI derived kidney function measurement.

After a baseline phase, 8 healthy volunteers (age 18-24 yrs) were subjected to continuous Angiotensin II (Ang-II) infusion at 3.0 ng/kg/min. Blood and urine fractions and renal blood flow (RBF) were assessed using DWI and phase contrast MRI (Ingenia 3.0T, Philips Healthcare). Fractions were calculated via a tri-exponential IVIM model fit to the DWI data (TE 45ms; TR 1344 ms; matrix: 112x68, FOV 336x204 mm²; voxel size 3.0x3.0x3.0 mm, b-values: 0, 2, 4, 8, 12, 18, 24, 32, 40, 50, 70, 110, 200, 300, 450, 600 s/mm²; gradient directions: 15). RBF was calculated in the proximal renal artery after manual vessel segmentation. During a second visit, gold standard GFR, ERPF and FF were measured during a similar infusion protocol.

Ang-II decreased both GFR and ERPF (8±7%, p=0.14; 22±6%, p<0.001), resulting in an increase in FF of 18±2% (p<0.001). RBF decreased from 11±2 to 8±1 ml/s (p=0.001). Renal IVIM imaging showed an increase in the urine fraction of 16±10% (p=0.002) and a decrease of the perfusion fraction by 7±16% (p=0.1). Delta renal IVIM measures of urine fractions and FF were correlated (R=0.57, p=0.001).

These data suggest that a combination of IVIM and Phase contrast kidney imaging may provide a reliable and fast noninvasive kidney function measurement.
P26.
The succinate receptor 1 is a physiological regulator of the renin-angiotensin aldosterone system

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Background: It has been shown that oxidative cell stress in diabetes type I (T1DM) induces tubular release of mitochondrial succinate and that subsequent activation of the SUCNR1 in the juxta-glomerular apparatus is needed for renin release and T1DM-induced hypertension. Here, we tested whether the SUCNR1 also has a physiological role in renal water and electrolyte handling.

Methods: wild-type (wt) and SUCNR1−/− mice 10 weeks old were placed in metabolic cages and 24h clinical parameters were analyzed in order to assess physiologically relevant differences. After sacrifice, kidneys were weighed and collected to perform immunoblotting and immunohistochemical analysis.

Results: Blood and urine analysis of wt and SUCNR1−/− mice showed that loss of SUCNR1 increased sodium and urea excretion, reduced renal renin and plasma angiotensin II (AngII) and aldosterone levels. Immunoblotting revealed a downregulation of the most crucial sodium transporters (NHE3, NCC and ENAC) in SUCNR1−/− mice. Fractional excretion of urea and water were increased in absence of the receptor, coinciding with reduced AQP2 abundance and weaker apical membrane staining of AQP2 and UT-A1 in the inner medulla. With an unchanged overall morphology, wet, but not dry, weights of kidneys of SUCNR1−/− mice were significantly increased than of wild-type littermates.

Conclusions: Our data reveal that the SUCNR1 is essential for the physiological maintenance of renin and AngII levels, and proper proximal tubule and collecting duct sodium, water and urea reabsorption. The increased wet kidney mass is likely due to tubular dilation due to increased tubular pressure because of life-long diuresis. Our data thus indicate that the mammalian SUCNR1 is a physiological regulator of water and volume homeostasis.
P27.
Magnetic Resonance Imaging Derived renal oxygenation and perfusion during continuous, steady-state angiotensin-II infusion in healthy humans

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Kidney hypoxia is considered pivotal in the progression of chronic kidney disease. A widely used method to assess kidney oxygenation is blood oxygen level dependent (BOLD)-MRI; but its interpretation remains problematic. The BOLD MRI signal is the result of kidney oxygen consumption (a proxy of glomerular filtration) and supply (i.e. glomerular perfusion). Therefore, we hypothesized that during pharmacological modulation of kidney blood flow, renal oxygenation, as assessed by BOLD-MRI, correlates to filtration fraction (i.e. glomerular filtration/effective renal plasma flow) in healthy humans.

Eight healthy volunteers were subjected to continuous angiotensin-II infusion at 0.3, 0.9 and 3.0 ng/kg/min. At each dose, renal oxygenation and blood flow was assessed using BOLD and phase contrast MRI. Subsequently, gold standard GFR/ERPF measurements were performed under the same conditions.

Renal plasma flow decreased dose dependently from 1231±249 to 863±167 mL/min/1.73m² (p=0.02). GFR did not change (121±23 to 110±18 ml/min/1.73m² p=0.14). There was a cortical R2* increase of 7.2 ± 3.76% (p=0.041); medullar R2* did not change. Cortical R2* correlated to filtration fraction (R² 0.46, p<0.001) and the product of cortical R2* and renal blood flow correlated to GFR (R² 0.54, p<0.001).

These data question the role of angiotensin-II in the progression of CKD via medullar hypoxia. The data also indicate that cortical oxygenation measured by BOLD MRI associates with filtration fraction. For correct interpretation of renal BOLD-MRI in future studies, there may be a need to include renal plasma flow measurements.
P28.
The succinate receptor contributes to obesity-induced type II diabetes and chronic kidney disease

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Background: Cell stress leads to cellular release of mitochondrial succinate and activation of its receptor, the SUCNR1, in the renal macula densa is essential for type I diabetes mellitus (T1DM)-related renin release and hypertension. Obesity-induced T2DM and Chronic Kidney Disease (CKD) development is aggravated by hypertension and goes with cell stress in adipose/kidney tissue and macrophages, which express SUCNR1. Here we tested the role of SUCNR1 in obesity-induced T2DM and CKD.

Methods: Wild-type and SUCNR1-/− mice were fed with a low fat diet (10%, LFD) or high fat diet (60%, HFD) for 16 weeks. At weeks 1, 2, 4, 8, 12 and 16 mice were weighted, subjected to metabolic cages and then to a glucose tolerance test (GTT) in order to assess the diabetes type-II onset. Tissues were collected for further analysis.

Results: after 16 weeks of HFD, similar gain in body weight, adipose tissue and kidneys was observed in both HFD groups compared to the LFD controls, but the gain was higher for liver and heart in wt than SUCNR1-/− HFD mice. Starving glucose levels were similarly increased in both HFD groups, but SUCNR1-/− mice had a better glucose tolerance test response, indicating less insulin resistance in SUCNR1-/− mice. For both HFD groups, blood sodium and urine volumes were similarly reduced, whereas GFR was similarly increased. Importantly, HFD wt mice showed albuminuria and glomerular/interstitial expression of collagen IV, indicative of fibrosis, which was not observed in HFD SUCNR1-/− mice, nor LFD groups. RT-PCR analysis on gene involved in macrophages infiltration revealed that SUCNR1-/− HFD mice tended to have less kidney inflammation compared to the HFD wt mice.

Conclusion: Together, our data reveal that SUCNR1 activation contributes to obesity-induced T2DM and CKD development and, if similar in humans, that SUCNR1 blockers may form novel therapeutics for the treatment of these common disorders.
P29.
The influence of chronic kidney disease on gut microbial metabolism

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Objective: The gut microbial metabolism contributes substantially to the human metabolome and is a well-known source of so-called uremic retention solutes, e.g., indoxyl sulfate and p-cresyl sulfate. Mounting evidence indicates that the gut microbial metabolism can be disease-specific. Whether chronic kidney disease is associated with a distinct gut microbial metabolism has not been studied to date.

Methods: Fecal samples of patients on maintenance hemodialysis were collected. We included 2 control groups composed of unrelated healthy controls, as well as household contacts on the same diet. Untargeted fecal metabolic fingerprinting with characterization of individual volatile organic compounds was performed with a dedicated gas chromatography mass spectrometry method. Differences in fecal metabolite profiles were examined with partial least square discriminant analysis. Discriminating volatile organic compounds and chemical classes were identified using correlation loading plot and Wilcoxon rank-sum test.

Results: Fecal samples of 20 hemodialysis patients, 20 unrelated healthy controls and 20 household contacts on the same diet were included for analysis. A total of 286 different metabolites were identified. Partial least square discriminant analysis demonstrated a clear distinction between fecal metabolite profiles of hemodialysis patients and healthy controls (see figure). A total of 81 volatile organic compounds were significantly different between these 2 groups with, among others, an increased generation of indole and p-cresol in hemodialysis patients. According to chemical classes, there was an upregulation of alcohols, aldehydes, benzenes, BCFA, furans, indoles and SCFA, while alkenes and ketones were downregulated in hemodialysis patients. In contrast, discrimination between hemodialysis patients and their household contacts on the same diet was less pronounced (see figure) with an increased generation of aldehydes and furans in hemodialysis patients.

Conclusion: The renal phenotype is associated with a distinct gut microbial metabolism. While there is a clear impact of dietary and other chronic kidney disease related factors on gut microbial metabolism, possibly aggravated in the presence of renal dysfunction, the influence of renal function loss per se is less pronounced. The potential beneficial effect of therapeutics targeting gut microbiota in patients with renal disease has to be awaited.
P30. Hypertension induces progression of renal damage in LDLr-/- mice on high fat diet.

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Objective: Diabetic nephropathy (DN) is a major complication in metabolic syndrome and diabetes. Previously, we developed a metabolic syndrome mouse model which shows multiple diabetic complications including non-alcoholic steatohepatitis and atherosclerosis upon high fat diet feeding. Although mild renal changes are observed in this model, pathology does not progress to moderate DN. Therefore, we investigated whether inducing hypertension on top of metabolic syndrome leads to progression of DN.

Methods: Male LDLr-/- mice (8 wk old) received high fat (45%) + high salt (6%) diet for 6 wk. One group underwent uninephrectomy prior to start of the diet (UNX). To induce hypertension, combinations of several pro-hypertensive components were used (angiotensin II infusion (ANGII; 0.7 mg/kg/day), DOCA pellet (2.2 mg/day) and a vasoconstrictor (L-NNA; 20 mg/L)) for additional 10 wk. At regular intervals, blood and 24h urine were sampled and systolic blood pressure (SBP) and albuminuria were assessed. 17 wk after start of the diet, mice were terminated and renal injury was scored. Age-matched chow fed animals were used as controls.

Results: Combining high fat, high salt and ANGII resulted in a significant increase of hypertension (SBP: 127±21 mmHg) and albuminuria (79±24 µg/24h) vs chow (SBP: 96±8 mmHg, albuminuria: 27±9 µg/24h). Renal damage was indicated by mild to moderate mesangial matrix expansion, glomerulosclerosis, glomerular micro-aneurisms and protein casts (fig 1a). Removal of one kidney did not further increase hypertension (SBP: 129±23 mmHg) but induced progression of albuminuria (174±173 µg/24h) and of renal pathology, characterized by moderate mesangial matrix expansion, nodular sclerosis, micro-aneurisms, protein casts and hyalinosis (fig 1b). Addition of the pro-hypertensive components DOCA and L-NNA induced further progression of renal failure and damage, but was complicated by fatal thoracic bleedings, thereby precluding further studies. Interestingly, high salt intake resulted in a reduction of body weight increase and metabolic parameters including liver cholesterol and triglyceride levels.

Conclusions: Induction of hypertension in LDLr-/- mice on high fat diet resulted in a progression of albuminuria and kidney pathology, consistent with hypertension being an important risk factor for development of DN. Remarkably, high salt feeding resulted in a decrease in metabolic syndrome development, possibly as a result of decreased fatty acid uptake.

Figure 1. A: mesangium expansion and glomerulosclerosis in high fat, high salt and ANGII treated mice. B: glomerulosclerosis and micro-aneurisms in uninephrectomized mice on high fat, high salt and ANGII treatment.
P31.
Attenuation of renal fibrosis after unilateral ischemia reperfusion may require a multi-target approach.

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BLR Bio (blrbio.com) is a biotechnology company dedicated to the development of antifibrosis and anticancer therapies, and has a commercial interest in CCN-based drugs.

Objective Acute kidney injury (AKI) is an important risk factor of chronic kidney disease (CKD). We optimized a mouse model of AKI to CKD by unilateral ischemia-reperfusion (UIRI) with development of renal fibrosis. To validate this model for use in therapeutic intervention studies, 3 experimental treatments were tested: administration of 1) recombinant human CCN3 (CCN2/CTGF antagonist), 2) TGFβ1 neutralizing antibody (1D11) or 3) dexamethasone (corticosteroid).

Methods Male C57Bl/6 mice underwent 21 min of unilateral ischemia-reperfusion (UIRI) at 36°C body temperature. 8 treatment groups (n>4/group, ip) were included: dexamethasone (10 mg/kg, daily), vehicle (PBS, daily), rhCCN3 (5 µg/kg, daily), vehicle (PBS, daily), antibody to TGFβ (0.5 mg/kg, every other day), vehicle (PBS, every other day), an untreated UIRI group and a sham group. Three weeks after UIRI renal fibrotic outcome was determined by gene expression analysis (qPCR) of collagen I, TGFβ, CTGF, CCN3, PAI-1 and TNFα.

Results UIRI induced a ~40% reduction in renal mass. Treatment with rhCCN3, anti-TGFβ or dexamethasone did not attenuate this reduction. However, dexamethasone treatment prevented the subsequent hypertrophy of the contralateral kidney, suggesting higher residual renal function of the ischemic kidney. UIRI induces significant upregulation of the fibrosis-related genes. rhCCN3 treatment had no effect on the gene expression. Anti-TGFβ antibody treatment induced significantly less upregulation of TGFβ and CCN3 gene expression, however, vehicle also reduced TGFβ expression. Dexamethasone treatment induced significantly less upregulation of collagen I and CCN2/CTGF gene expression and a trend towards higher CCN3 upregulation.

Conclusion Despite the earlier proven benefits of TGFβ antagonism and CCN3 treatment on the development of fibrosis, neither treatment (at doses demonstrated to be effective in more mild injury models) showed effect in the UIRI model. Broad anti-inflammatory suppression by dexamethasone attenuated fibrotic gene expression. We speculate that the natural course of renal demise after UIRI is very robust and is highly likely to require a multi-target therapeutic approach. Whether some combination of the therapies tested here could have efficacy in this model remains to be determined.
P32.
Targeting cardiorenal connectors reduces renal fibrosis in rats with subtotal nephrectomy followed by coronary ligation

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Objective: Multiple strongly interacting neuro-endocrine and paracrine pathways contribute to progression of renal and cardiac damage in chronic kidney disease followed by chronic heart failure (renocardiac failure). We aimed to determine whether simultaneous pharmacological modulation of critical systems implicated in renocardiac failure would be effective in reducing fibrosis and preserving function in heart and kidney.

Methods: Rats were subjected to subtotal nephrectomy followed 9 weeks later by coronary artery ligation. From wk 11 until wk 16 we administered vehicle or an angiotensin receptor blocker (ARB, losartan), or a combination of the NFkB inhibitor PDTC, the tolerance-free NO donor molsidomine and the superoxide dismutase mimetic tempol, or all four of these plus the beta-blocker metoprolol together. Renal and cardiac structure and function were assessed at the end of the experiment, as well as mRNA expression of various genes.

Results: Individual and combined treatments similarly prevented a further decline in cardiac systolic function and reduced cardiac fibrosis in comparison to vehicle. Combined treatment with all five drugs reduced renal tubulo-interstitial fibrosis and CTGF gene expression more than both other strategies. Combining all five drugs reduced heart rate, inotropy and mean arterial pressure, but did not affect diastolic function.

Conclusion: In our model of chronic renocardiac failure, combined treatments similarly decreased cardiac fibrosis and stabilized systolic function as ARB alone, suggesting a key role for AT1 receptor activation in the network of aberrant systems in chronic renocardiac failure regarding the heart. Kidney fibrosis, however, was most effectively reduced by a five drug regimen, pointing to additive effects of multiple distinct pathophysiological pathways.
P33.
Pathogenesis of Reduced GFR in Children with Nephrotic Syndrome

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Objective:
Acute renal failure (ARF) as a complication of idiopathic nephrotic syndrome is uncommon. Many studies have highlighted the extra-renal causes like renal vein thrombosis, drug induced nephritis, septicaemia either related to immunosuppressant use or disease itself, whereas minimal focus on intra-renal causes like influence of hemodynamic and glomerular permeability. The aim of the study is to provide evidence that a decrease of GFR in a subgroup of nephrotic children likely to be secondary to hypovolaemia.

Methods:
45 nephrotic children with mean age 6.9±4.1 years old were included in the study. Renal perfusion and glomerular permeability were assessed by measuring clearance of para-aminohippurate (PAH) and inulin clearance during a continuous perfusion technique. Filtration fraction (FF) was then calculated to indicate glomerular permeability (Kf). Vasoactive hormones (plasma renin activity (PRA), aldosterone) and urinary sodium and potassium were also measured, as surrogate parameters for functional hyper/hypovolaemia.

Results:
Subjects were grouped into low, normal, and high GFR based on reference value obtained from remission group. The low GFR group coincides with high PRA (log 2.2±0.2), aldosterone (log 2.4±0.5), low RPF (346±107) and elevated quotient $U_K/U_{K+U_{Na}}$ (73±31), all parameters indicating functional hypovolaemia. Large heterogeneity pattern was seen in the normal GFR group with high and low vasoactive hormone level implying a complex pathogenic process. GFR (inulin), RPF, and FF value were also significantly low (p<0.05) indicating very low Kf in the low GFR group.

Conclusion:
Our data showed data there is subgroup of patients in nephrotic syndrome who have a decrease in glomerular filtration, apparently related to functional hypovolaemia. In clinical setting, this group might benefit from albumin administration which will help to prevent them going into hypovolaemia.
P34.
Gut microbiota derived trimethylamine-N-oxide is not a biomarker for mortality and cardiovascular disease in European CKD patients

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Objective: Trimethylamine-N-oxide (TMAO) is a gut microbiota derived metabolite of dietary choline, lecithin and l-carnitine with recent evidence suggesting involvement of TMAO in development of atherosclerosis. Recently, TMAO has been associated with mortality and cardiovascular disease in a general US population as well as in US CKD patients not yet on dialysis. As there may be population-specific differences in diet and/or microbial metabolism, we questioned whether TMAO also relates with adverse outcome in European CKD patients.

Methods: We performed a single-center prospective study in patients with CKD stage 1-5. Baseline serum levels of TMAO were determined using LC-MS. Correlation between eGFR and serum TMAO was explored using Spearman’s rank correlation analysis. The relationship between TMAO, survival and cardiovascular disease was examined using Cox proportional hazard analysis.

Results: 488 CKD patients were followed from November 2005 until December 2010. Median serum level of TMAO was 11.6 µM (IQR 5.7–21.8). We observed a highly significant inverse correlation between eGFR and serum TMAO (rho 0.71, P < 0.0001). During follow-up, we noted a total of 51 deaths and 75 cardiovascular events. In univariate cox proportional hazard analysis, TMAO was a significant predictor of mortality (HR 1.521 (1.183–1.956), P 0.001) and cardiovascular disease (HR 1.570 (1.283–1.921), P < 0.0001). However, significance was lost after adjustment for eGFR for overall mortality (HR 1.126 (0.795–1.595), P 0.50), as well as for cardiovascular events (HR 1.256 (0.958–1.647), P 0.10).

Conclusion: Serum levels of TMAO rise in parallel to a declining renal function. In this European cohort of CKD patients, we were not able to find an association between TMAO and adverse outcome that is beyond renal function, which is in contrast to previous observations in US populations, both general and CKD. This may question the validity of TMAO as a universal biomarker for cardiovascular disease, possibly due to population-specific differences in diet and/or microbial metabolism.
P35.  
Conceptualisation and validation of a paradigm based on uraemic toxins for management of chronic kidney disease in paediatric patients

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Background
Children with chronic kidney disease (CKD) suffer from one of the most devastating diseases in childhood resulting in a lifelong need for health care, and a 3 times decreased life expectancy. In addition, they have important comorbidities that negatively impact on their quality of life and integration in society, jeopardizing their future even after a potential transplantation. Retention of uraemic toxins is accepted to play a major role in the pathogenesis of the comorbid conditions, but studies in children are lacking. Furthermore, there are currently no good tools to evaluate severity and monitor adequacy of treatment, resulting in suboptimal management.

Objective
The scientific objective of this four years project (funded by IWT and starting on the 1st of October 2015), is to provide the clinician with new diagnostic and therapeutic tools for the management of children with CKD, based on the improved understanding of uraemic toxicity.

Methods
In CKD children, we will associate concentrations of a wide variety of uraemic toxins with different comorbidities, i.e. growth, protein-energy wasting, quality of life, cardiovascular risk factors, circadian rhythm, sleep quality, and psychosocial and neurocognitive functioning (cross-sectional and longitudinal). The toxins of which concentrations are best correlated with comorbidities during the progress of CKD will be selected as markers, and will be, together with the comorbidities, further tracked after intervention in the PD and HD strategy. Based on intradialytic uraemic toxin concentrations, kinetics are described during HD, and simulations are performed to find the optimal dialysis strategy to decrease uraemic toxin concentrations, and with it, their toxicity. The kinetic models are finally validated by quantifying uraemic toxin marker concentrations and comorbidities in individual patients after switching to different strategies as well as to the individualised optimal dialysis strategy based on the model. Finally, an open access user-friendly prediction simulator (PAEDSIM) based on patient characteristics and marker concentrations will be developed to optimise and individualise the dialysis therapy. Herewith, a 'CKD Academy' will be organised, intended for nephrologists and laboratory staff with workshops about the simulator and the related laboratory techniques.

Conclusions
By providing clinicians, dealing with children with CKD, with more advanced and appropriate tools to improve management of all children with CKD, i.e. better assessment of the degree of renal dysfunction, better determination of the ideal time to start renal replacement therapy, and more accurate monitoring of the quality of that renal replacement therapy, we aim to improve neurocognitive and psychosocial functioning (short term), growth, maturation into puberty, and social integration (median term) and survival (long term). The developed diagnostic and therapeutic tools can be further used to set up a randomised controlled trial on a large scale, forming the basis for new practice guidelines.
P36.

Alternative pathway complement activation in C3 glomerulopathy

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Objective: Glomerular pathologies characterized by isolated deposition of C3 are nowadays called C3 glomerulopathies (C3G) and include dense deposit disease and C3 glomerulonephritis. It is thought that C3G can be caused by systemic dysregulation of the alternative complement pathway, however, the exact mechanism of complement pathology in C3G patients is poorly understood. To get more insight in complement regulation in C3G, we performed a thorough analysis of alternative pathway activation in nine patients in acute phase of C3G.

Methods: In nine biopsy-proven C3G patients, background levels of complement activation markers C3b/c and C3bBbP (alternative pathway), and TCC (terminal pathway) were measured in EDTA plasma using ELISA and compared to the results of 19 healthy controls. Moreover, the patients were screened for DNA aberrations in alternative complement pathway genes CFH, CFI, C3, CFB and MCP. Presence of anti-FH autoantibodies was analyzed by ELISA.

Results: Patients with acute phase C3G showed elevated plasma levels of C3b/c (P<0.05), C3bBbP (P<0.001) and TCC (P<0.05), indicating alternative complement pathway activation in C3G.

Conclusion: We demonstrated significantly elevated complement activation biomarkers in acute phase C3G. These data indicate that alternative complement pathway activation is involved in the pathogenesis of C3 glomerulopathy and can be detected in blood samples. The assays to detect complement activation biomarkers may be important to monitor complement activation in C3G patients receiving complement inhibition therapy.
P37.
Differences Between Patients With Definite And Suspected ANCA-Associated Vasculitis in a Secondary Care Hospital

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Objective: ANCA-associated vasculitis (AAV) is a rare disease with a broad spectrum of symptoms. Therefore, diagnosing AAV is often challenging. We aimed to identify differences between ANCA positive patients with a definite and suspected diagnosis of AAV.

Methods: In this retrospective study, all patients that tested positive for MPO and/or PR3 ANCA between 2005 and 2015 in a secondary care hospital in the Netherlands were analyzed. Patients with a clinical diagnosis of AAV were compared with patients without AAV. Possible predictors for AAV, such as patient characteristics, clinical symptoms and ANCA titers were identified. The Birmingham Vasculitis Activity Score (BVAS/WG) was calculated in all patients, to evaluate its potential value as a diagnostic marker of AAV.

Results: We included 234 consecutive patients with a positive MPO and/or PR3 ANCA, of which 119 were clinically diagnosed with AAV. Of the 115 ANCA positive patients without AAV, 87 patients had an alternative diagnosis, including other rheumatic diseases (n=23), malignancy (n=4), infection (n=11) and inflammatory bowel disease (n=24), other diagnoses (n=25). These patients presented with multiple symptoms, such as arthritis/arthralgia (in 32%), pulmonary symptoms (in 24%) and renal symptoms (in 35%). BVAS at presentation was higher in patients with AAV (5 versus 2 p<0.001). In a multivariable linear regression model higher ANCA titers (OR 26.54, 95% CI 8.66 to 81.34), higher BVAS (OR 2.04, 95% CI 1.48 to 2.83) and ear-nose-throat (ENT) symptoms (OR 15.48, 95% CI 4.28 to 55.33) were predictive of AAV.

Conclusions: MPO and PR3 ANCA can be positive in a variety of diseases that may mimic AAV. Higher ANCA titers, BVAS and ENT symptoms were predictive of AAV in ANCA positive patients.
P38.
TACROLIMUS-BASED REGIMENS IN THE TREATMENT FOR LUPUS NEPHRITIS: A SYSTEMATIC REVIEW

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Objective:
Recent clinical trials have reported positive effects of tacrolimus-based regimens for the treatment of lupus nephritis (LN). The majority of studies have been conducted in Asian LN population. Thus far, current guidelines do not mention tacrolimus as a treatment option and no consensus has been reported on the role of tacrolimus as therapy for active LN patients. To address this issue, we performed a systematic review on tacrolimus-based regimens for the treatment of LN.

Methods:
From various databases relevant controlled clinical studies investigating tacrolimus-based regimens in LN were selected. Renal response rates and safety profile data were extracted and analysed in the context of induction and maintenance treatment.

Results:
From 24 studies, 9 controlled trials were selected for analysis: 6 randomized controlled trials and 3 case-control studies. Seven studies used tacrolimus in combination with steroids and 2 studies used tacrolimus in combination with mycophenolate and steroids. Five involved induction therapy, 2 maintenance therapy and 2 combined induction and maintenance treatment. Overall, these studies encompassed 888 patients. As induction treatment, tacrolimus treated arms resulted in 83% responders of which 50% complete responders (CR). In the control arms, 68% responders of which 34% CR were observed. As maintenance treatment, tacrolimus treated arms resulted in 100% responders of which 56% CR. In the control arm, 95% responders of which 64% CR were observed. With respect to safety, reported adverse events were comparable between tacrolimus treated arms and control arms.

Discussion:
Currently, controlled studies on tacrolimus treatment in LN are limited and exclusively in patients of Asian ethnicity. Our analysis suggests that tacrolimus-based treatments can achieve comparable renal responses in LN as conventional regimens as induction and maintenance treatment. Main limitations are the heterogeneity of tacrolimus-based regimens, lack of studies in non-Asian LN patients and lack of long-term safety data. Altogether, the efficacy of tacrolimus-based regimens needs further confirmation in multi-ethnic, randomized trials. Until then, tacrolimus can be considered as rescue therapy in selected patients, such as therapy-refractory or patients that want to become pregnant.
P39.
Eculizumab treatment efficiently prevents C5 cleavage without C5a generation in vivo

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Objective: The C5 inhibitor eculizumab has been successfully used to treat atypical hemolytic uremic syndrome (aHUS), however, available data on pharmacodynamics of this medication are limited. Recently, increased generation of C5a in a single patient with HELLP syndrome treated with eculizumab has been reported. Since this observation was unexpected, we aimed to reproduce these data and search for possible explanations for the findings.

Methods: Levels of C5a were analyzed in EDTA plasma samples of aHUS (n=3) patients using three commercial ELISA kits, one of the kits was also used in the HELLP study and other two kits were included as controls. The samples were collected before the first eculizumab dose and then at various time points during treatment.

Results: In line with the report on HELLP patient, the C5a values, measured by one of the commercial ELISA kits, increased significantly in all aHUS patients after the first eculizumab dose as compared to the values obtained before treatment (p=0.016). C5a remained elevated throughout the treatment period. Such increase could not be reproduced by using another two commonly available commercial kits. When eculizumab was added in vitro to normal human serum prior to activation, no generation of C5a was measured by all used kits.

Conclusion: Our data indicate that existing commercial assays require validation for specificity before being used to monitor effect of eculizumab, especially in clinical laboratory practice. This example illustrates how false conclusions can be drawn when based on results from one single commercial assay not satisfactorily validated for the purpose it is used.
P40. Sensitive, reliable and easy-performed laboratory monitoring of eculizumab therapy in atypical hemolytic uremic syndrome

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Objective: Atypical hemolytic uremic syndrome is a severe renal illness caused by complement dysregulation. Treatment with the complement C5 inhibitor eculizumab is effective, but associated with high costs. Laboratory monitoring of these patients with respect to complement function has not been standardized. The aim of this study was to evaluate novel complement functional assays for their application in routine follow-up of eculizumab-treated patients.

Methods: Complement activity in serum samples was analyzed using Wieslab® complement screen assay. The presence of eculizumab-C5 complexes in serum, EDTA plasma samples and in urine was measured using ELISA. Levels of sC5b-9 in urine were measured using electroluminescent epitope assay.

Results: First, we documented that the Wieslab® complement screen assay showed a sensitivity of 1-2% of C5 activity by adding purified C5 or normal human serum to a C5 deficient serum. Second, we found that all the patient samples obtained during the standard treatment course, were completely blocked for terminal complement pathway activity. Moreover, complement remained fully blocked when intervals between the eculizumab infusions were extended to four weeks. Levels of complexes between eculizumab and C5 were inversely correlated to the complement activity (p=0.01). Third, titrating serum from eculizumab-treated patients into normal serum, revealed that eculizumab was present in excess up to four weeks after infusion. Finally, we showed that increased urine sC5b-9 disappeared after eculizumab treatment.

Conclusion: We demonstrate sensitive, reliable and easy-performed assays to monitor eculizumab-treated patients, which can be used to design individual dosage regimens.
P41.
Desmopressin melt improves sleep and neuropsychological functioning in monosymptomatic nocturnal enuresis

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Objective: A comorbidity and a possible causality between nocturnal enuresis, sleep disorders and attention deficit-hyperactivity disorder (ADHD) has been suggested in literature. This prospective study in children with monosymptomatic nocturnal enuresis (MNE) aims to evaluate the influence of desmopressin melt on sleep and psychological functioning of the child.

Methods: Thirty patients (23 boys) aged 6-16 years (mean 10.43y, SD +/-3.08)) with MNE based on nocturnal polypus (NP), in this study defined as nocturnal diuresis >100% bladder volume for age, are included. Patients are tested before the start of desmopressin melt and 6 months later. It is a multi-informant multi-method study, using overnight standardized video-polysomnographic study (PSG), questionnaires, clinical interviews and neuropsychological testing.

Results: According to the ICCS definition, 10 patients were full responders, 2 patients were responders, 11 patients were partial responders and 6 patients were non-responders to desmopressin melt. The response status was unknown in 2 patients due to missing values. 87% (26 of 30) patients have a disrupted sleep at the first PSG. They experienced greater than 5 periodic limb movements per sleep hour (PLMS-index). 60% (18 of 30) patients have a disrupted sleep at the second PSG. All except 3 patients had a decrease in PLMS-index. The amelioration of the nocturnal enuresis coincides with a significant reduction of the PLMS-index 6 months later. Moreover, psychological functioning was improved. After 6 months of desmopressin, children experienced significant less internalizing and externalizing problems, a higher quality of life and higher executive functioning.

Conclusion: Desmopressin melt not only improves enuresis but also sleep and psychological functioning in children with MNE based on NP.

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P42.
An enzyme immunoassay for urinary extracellular vesicles

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Objective
Urinary extracellular vesicles (uEVs) are derived from epithelial cells of the kidney and urinary tract. uEVs (also called exosomes) contain disease-related proteins and also transfer information to target cells. As such uEVs offer exciting opportunities for nephrology, but current isolation techniques rely on time-consuming ultracentrifugation hindering high-throughput clinical application.

Methods
To navigate this problem, we designed an enzyme-linked immunosorbent assay (ELISA) that isolates uEVs using a biotinylated CD9 antibody. uEVs are then lysed with a detergent and treated with an antibody targeting the protein of interest. The use of two conjugated antibodies allows quantification of the protein of interest and CD9. We tested the set-up using aquaporin-2 (AQP2) and the sodium chloride cotransporter (NCC).

Results
CD9 but not CD63 coated immunobeads isolated AQP2+ and NCC+ uEVs; CD9 was therefore used as capture antibody. Urinary creatinine and CD9 correlated strongly (n=20, r=0.9, P<0.001); thus CD9 can also be used for normalization in spot urines. Our uEV-ELISA sensitively detected AQP2 and NCC (coefficients of variance 5.6 and 3.3%). To verify whether the expected physiological response in vasopressin on AQP2 and NCC were captured by our uEV-ELISA, we performed overnight thirsting followed by water loading in 4 healthy volunteers. After water loading, similar 2-3 fold decreases in AQP2 and NCC were observed using either uEV-ELISA or the traditional approach (isolation of uEVs by ultracentrifugation followed by immunoblotting). The results by uEV-ELISA showed good correlations with immunoblot (r=0.8 for AQP2, r=0.6 for NCC, both P<0.001). Finally, the uEV-ELISA reliably detected lower and higher AQP2 or NCC levels in uEVs from patients with pathological water or salt reabsorption, including diabetes insipidus, syndrome of inappropriate antiuresis, Gitelman and Gordon syndrome.

Conclusion
We successfully developed an ELISA to capture and quantify uEV-proteins and validated this technique for AQP2 and NCC in physiological and clinical settings. Our uEV-ELISA set-up does not require ultracentrifugation or measurement of urinary creatinine and may be used as a platform to examine other uEV proteins of interest in nephrology.
P43. DYSREGULATION OF THE CHOLESTEROL PATHWAY ASSOCIATES WITH ARTERIOSCLEROSIS AND INTRARENAL TELOMERE ATTRITION.


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Background
We demonstrated that arteriosclerosis in the smaller intrarenal arteries is associated with shorter telomere length, independent of cardiovascular risk factors and calendar age. The underlying mechanisms of this association remain unclear.

Methods
A test cohort of 40 consecutive kidney donors, with pre-implantation renal allograft biopsies, was included in this study. All biopsies were rescored according to the Banff classification. Intrarenal donor telomere length content was assessed using quantitative RT-PCR. In these same samples, whole genome microarray mRNA expression analysis was performed using Affymetrix Gene 2.0 ST arrays. The associations between mRNA gene expression, telomere length as marker of replicative senescence, and intrarenal arteriosclerosis were investigated using multiple regression models, adjusted for calendar age, gender and batch number. For biological interpretation and pathway overrepresentation analysis, Ingenuity Pathway Analysis software was used. An second cohort of 160 implantation biopsies was used for independent validation.

Results
Shorter intrarenal telomere length associated significantly with the presence of renal arteriosclerosis (p=0.007). Pathway analysis revealed enrichment of transcripts coding for proteins of the superpathway of cholesterol biosynthesis as the most significant in the telomere attrition- arteriosclerosis model (q= 0.0003 ; q = 2.69.10⁻⁸ ). The 10 most significant pathways in the model are all involved in cholesterol metabolism. These pathways are upregulated in the presence of arteriosclerosis and in case of shorter telomere length.

Conclusion
Arteriosclerosis in smaller intrarenal arteries is independently associated with shorter telomere length. Our unbiased suggests that the pathways involved in the cholesterol metabolism are the missing link between the appearance of arteriosclerosis and telomere attrition. Validation of these findings is underway, in a cohort of 160 independent implantation biopsies.
P44.
Aerobic glycolysis in lithium-induced Nephrogenic Diabetes Insipidus

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Objective: Lithium is the first-choice medication for treatment of bipolar disorders and is used by 0.1% of the western population. Lithium causes a urinary concentrating defect, which develops in ~20% of patients into symptomatic Nephrogenic Diabetes Insipidus (Li-NDI). This disorder is characterized by polyuria and polydipsia and is caused by downregulation of AQP2 water channels in principal cells of the renal collecting duct. Recently, others and we have shown that lithium induces proliferation of these cells in vitro and in vivo. By inhibiting glycogen synthase kinase 3-beta (GSK-3β), lithium has been suggested to stabilize β-catenin and hypoxia-inducible factor-1 alpha (HIF-1α) mediated transcription. This causes a metabolic switch from oxidative phosphorylation to aerobic glycolysis (Warburg effect). The Na-H exchanger (NHE1) is major player in the pH-regulating system that is required to prevent acidification caused by the metabolic switch. Here, we investigated whether lithium may induce Warburg-like aerobic glycolysis and whether inhibition of this Warburg effect may rescue Li-NDI.

Methods: Polarized mouse cortical collecting duct (mpkCCD) cells were cultured in 2D transwell model and exposed to lithium chloride and aerobic glycolysis inhibitors (2-deoxyglucose or Zoniporide). C57BL6/J mice were fed with 40 mmol lithium chloride/kg food and the aerobic glycolysis inhibitors. Mice were housed in metabolic cages in order to determine water intake and urine output during the last 24 hours.

Results: Lithium induced proliferation of mpkCCD cells as shown by increased levels of the proliferation markers PCNA and cyclin D1. In addition, lactate and succinate, main products of the Warburg effect, were both increased in lithium-treated mpkCCD cells and mice. This was accompanied by a decrease in the ratio of phospho-pyruvate dehydrogenase (pPDH/PDH) and an increase in HIF-1α, confirming the induction of aerobic glycolysis by lithium in mice. Interestingly, inhibition of lithium-induced glycolysis with 2-deoxyglucose attenuated lithium-induced AQP2 downregulation in mpkCCD cells. Moreover, zoniporide (NHE1 inhibitor) rescued AQP2 in lithium treated mpkCCD cells and attenuated Li-NDI in mice.

Conclusion: Our results reveal that lithium induces aerobic glycolysis in mpkCCD cells and mice. Targeting aerobic glycolysis with zoniporide attenuated lithium-induced AQP2 downregulation and may represent a potential target for the treatment of Li-NDI.
P45. LITHIUM NEPHROPATHY: A LONG-TERM COMPLICATION OF CHRONIC LITHIUM THERAPY

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Objective
Lithium is the treatment of choice for bipolar disorder. Lithium-induced nephropathy is a known complication limiting its use. The aim of this study is to establish the prevalence of renal failure in our population. We sought to quantify the contribution of lithium therapy to the risk of developing renal failure.

Methods
We selected 1751 patients on lithium therapy from the laboratory database of the Delta Center for Mental Health Care, Rotterdam. The database contains measurements of lithium and creatinine concentration over a period from 2000 to 2015. eGFR was calculated using the 4-variable MDRD formula. Renal failure was defined as having GFR<60mL/min on at least 2 measurements 6 weeks apart. A comparison was made between patients with and without renal insufficiency regarding gender, mean lithium concentration in serum, lithium intoxication, duration of therapy, age at initiation of therapy, cardiovascular disease, hypertension and diabetes mellitus.

Results
297 out of 1751 (17.0%) patients were classified as having renal failure. Occurrence of renal failure was positively correlated with female sex, mean serum lithium level, age at initiation of therapy and duration of lithium therapy (p<0.001). Significant correlation was also observed between renal failure and cardiovascular disease, hypertension and diabetes mellitus. In 251 patients follow up data was available for a period of more than 10 years. In these patients history of lithium intoxication did not predict occurrence of renal failure.

Conclusions
Prevalence of renal failure in our cohort is similar to other reports. Longer duration of lithium therapy was found to be associated with an increased risk of renal failure. Contrary to our expectation, lithium intoxication was not correlated with renal failure. Although therapy duration was a significant predictor, one should not forget the importance of cardiovascular risk factors in development of renal failure.
P46.
Rapid Correction of chronic hyponatremia with low sodium increment induces gliotic phenotype in the brain.

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Introduction
Rapid correction of hyponatremia is a risk factor for brain demyelination. It is widely accepted that the risk is proportional to the increment of serum sodium and most authorities will recommend an increment of less than 10mEq/24hr/L. These recommendations are based on animals’ studies showing that there is no demyelination when the sodium gradient is less than 18-25mEq/l/24hrs. we wanted to see if rapid correction of chronic hyponatremia with minimal increment of serum sodium resulted in adverse neuropathological findings apart of demyelination

Material and Methods
We induced chronic hyponatremia in male rat aged 12-18 weeks and weighting 250-300 grs with infusion of desmopressin and water diet. Chronic hyponatremia was then corrected with hypertonic saline and the serum sodium was measured 1 and 2 days after the correction. Animals were divided in 4 groups according to their serum sodium increment. Weight changes, neurological manifestations and mortality were assesses up to 6 days after the initiation of the correction. Later, animals were killed and their brain was processed for immunohistochemistry for marker of gliosis such as GFAP, Vimentin, Nestin and microglial activation as well as demyelination markers.

Results
Rapid correction of chronic hyponatremia was induced in 4 groups. Group 1 (n=6) had serum sodium increased by less than 10 but more than 5 mEq/24hrs /day. Group 2 (n=23) had an increment between 10 and 15, Group 3 (n=26) had an increment between 15 and 20 and group 4 (n=17) between 20 and 23. Mortality was 0% in group 1 and 2 and 26% in group 3 and 25% in group 4. Animals in group 1 and 2 had no evidence of brain demyelination and regained weight with no neurological manifestations. However, 15% of animals showed microglial activation and 40% showed evidence of gliosis with vimentin and /or nestin staining.

Conclusion
Rapid correction of chronic hyponatremia induces gliosis and microglial activation related changes in the brain of a significant proportion of asymptomatic animals who display no signs of demyelination and had a serum sodium increment of less than 15 mEq/l. This support the hypothesis that gliosis is part of the spectrum of osmotic brain injury in presence or not of demyelination. We propose the term of osmotic brain damage should be used in lieu of osmotic demyelination to reflect the wide spectrum of the pathological damage secondary to osmotic stress.
Moreover our finding provides scientific validation of the recent suggestion that during correction of chronic hyponatremia serum sodium increment should be limited to less than 8 mEq/L/24 hrs.
P47.
HYPONATREMIA AS A RISK FACTOR FOR FRACTURES: A META ANALYSIS

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Introduction
Hyponatremia is the most common electrolyte disorder in clinical practice. Recent data have suggested that chronic hyponatremia is associated with attention deficit, falls and bone fractures. Fractures, in particular hip fractures, represent a serious health risk in the elderly with a significant morbidity and mortality. We sought to investigate whether an association between hyponatremia and fractures is present on a meta-analysis of existing studies.

Material and Methods
We searched electronic literature databases (Medline, Scopus and Cochrane) to identify all studies addressing the risk of bone fracture in patients with hyponatremia which were published prior to October 2014. Identified studies were independently reviewed by two researchers. We used a random effects model to calculate pooled odds ratio (POR). Heterogeneity amongst studies was examined using Cochran’s Q and I\textsuperscript{2} tests and the symmetry of the funnel plots was tested using the Begg-Mazumdar rank correlation test and Horbold-Egger’s test.

Results
A total of 9 studies (7 case-control studies and 2 prospective cohort studies) met the inclusion criteria. The endpoint was the occurrence of fractures (vertebral or nonvertebral fracture). Hyponatremia was defined as a natremia < 135mmol/l in 5 studies, < 136mmol/l in 2 studies, < 132mmol/l and < 130mmol/l in one study respectively. The pooled sample consisted of a total of 25,716 patients (56.5% males). Median age of the included participants varied between 61 to 81 years across the studies. Hyponatremia was present in 1,731 (6.7%) patients. Across all 9 studies hyponatremia was significantly associated with an increased risk for fracture OR = 2.12 (95%CI: 1.86-2.43, P<0.0001). There was no significant heterogeneity (Q=12.7; P=0.119; I\textsuperscript{2} = 37.5%) among studies. Both Begg-Mazumdar's and Horbold-Egger's bias test were non significant (P>0.05).

Conclusion
A meta-analysis of the published trials confirms the previously found strong association between hyponatremia and bone fracture. Prospective interventional studies are warranted to determine if intervention aimed to prevent or correct hyponatremia might lead to a decrease in the bone fracture incidence.
P48. Serum Magnesium and the Risk of Death from Coronary Heart Disease and Sudden Cardiac Death: a prospective population based-cohort study

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Objective: Few studies have analyzed the associations between serum magnesium, coronary heart disease (CHD) mortality and sudden cardiac death (SCD). Results are conflicting and the pathway is unclear. We therefore analyzed the association of serum magnesium with CHD mortality and SCD within the prospective population-based Rotterdam Study, with adjudicated endpoints and long-term follow-up.

Methods and Results: 9820 participants (mean age 65.1 years, 56.8% female) were included with a median follow-up of 8.7 years. We used multivariable Cox proportional hazard models and analyzed quartiles of serum magnesium, with the second and third quartile combined as reference group (0.81-0.88 mmol/L). Low serum magnesium (≤ 0.80 mmol/L) was associated with an increased risk of CHD mortality (N=431, HR 1.36, 95%CI 1.09-1.69) and SCD (N=217, HR 1.54, 95%CI 1.12-2.11). Low serum magnesium was also associated with increased subclinical atherosclerosis, as indicated by increased carotid intima-media thickness (cIMT, +0.013 mm, 95%CI 0.005-0.020). Low serum magnesium was also associated with an increased heart-rate corrected QT interval, mainly through an effect on heart rate (RR interval -7.1 ms, 95%CI -13.5 to -0.8). Additional adjustments for cIMT and heart rate did not change the associations with CHD mortality and SCD.

Conclusions: Low serum magnesium is associated with an increased risk of CHD mortality and SCD. Although low magnesium was associated with both cIMT and heart rate, this did not explain the relationship between serum magnesium and CHD mortality or SCD. Future studies should focus on why magnesium associates with CHD mortality and SCD and whether intervention reduces these risks.
P49. Disregulated proteostasis and ER stress underlies brain pathologic response to rapid correction of chronic hyponatremia in rats.

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Introduction
Adequate protein folding is necessary for normal cell function and is a tightly regulated process which requires proper intracellular ionic strength. In many cell types, imbalance between protein synthesis and degradation can induce unfolded protein response (UPR) and endoplasmic reticulum (ER) ER stress which if sustained can in turn lead to DNA damage, autophagy and apoptosis. In nematodes, osmotic stress induce massive protein aggregation coupled with unfolded protein response and ER stress. The consequences of osmotic stress on mammalian brain have never been investigated and we wanted to know if rapid correction of chronic hyponatremia induced brain cell UPR and ER stress.

Material and Methods
We induced hyponatremia and performed rapid correction as previously described in the literature. Brain was collected and processed for immunohistochemistry or western blot to investigate UPR and ER stress in animals’ brain 12 and 24 hrs after rapid correction of chronic hyponatremia. P62, LC3 and ATG5 were used as autophagy marker. KDEL, BIP and PDI were used and UPR marker and EIF2a as ER stress marker.

Results
Our results suggest that rapid correction of hyponatremia induces severe alterations in proteostasis characterized by diffuse protein aggregation and ubiquitynlation. Moreover, we show that brain of hyonatremic animals treated with after rapid correction of hyponatremia displayed vigorous activation of both unfolded protein response and ER stress which eventually culminate into an increased autophagic activity and apoptosis. Interestingly, most of these processes are restricted to astrocytes located in the brain regions that will later be demyelinated. These results identifies osmotic stress as a potent protein aggregation stimuli in mammalian brain and further suggest that osmotic demyelination might be a consequence of protein damage that occurs as a result of proteostasis failure upon severe osmotic stress in astrocytes.

Conclusions
These results identifies osmotic stress as a potent deregulator of proteostasis. They further suggest that osmotic demyelination might be a consequence of protein damage that occurs as a result of proteostasis failure upon severe osmotic stress in astrocytes.
P50. High Salt Affects Toll-Like Receptor-Induced Gene Expression in Macrophages

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Objective
High dietary salt intake is a major risk factor for cardiovascular disease. A high-salt (NaCl) diet increases NaCl concentrations in the skin. In the skin, macrophages respond to high NaCl by the osmosensitive transcription factor NFAT5 and promote NaCl efflux via lymph vessels. Other effects of NaCl on local macrophages have remained largely elusive. Recent evidence suggests that NFAT5 also regulates multiple Toll-like receptor (TLR)-induced genes such as NOS2, TNF and IL6 in macrophages, independently of osmotic stress. We aimed to investigate the effects of NaCl-generated hypertonicity on the expression of these genes in TLR-activated macrophages.

Methods
We simulated the hypertonic skin microenvironment by culturing macrophage-like RAW 264.7 cells in NaCl-induced hypertonic (340-480 mOsm/kg) media and compared this with normotonic media (320 mOsm/kg). After 1 to 24 hours of stimulation with lipopolysaccharide (LPS) or Zymosan A, a TLR2 ligand, we measured nitrite production, and analyzed TLR response genes by quantitative RT-PCR, and p38-MAPK, NFAT5, and NOS2 by immunoblot.

Results
NaCl significantly and dose-dependently increased nitric oxide (NO) production (2-fold), NOS2 mRNA (3-fold) and NOS2 protein expression (2.25-fold) in LPS- and Zymosan A-stimulated cells. In contrast, equiosmolar mannitol or urea did not affect expression levels. NaCl also increased LPS-induced p38 phosphorylation and total NFAT5 protein expression. Similarly, NaCl increased TNF mRNA expression 1.25-fold. Remarkably, NaCl significantly downregulated LPS-induced expression of CCL5 (4-fold), IL6 (2.4-fold) and IL12b mRNA (3.5-fold).

Conclusion
Elevated concentrations of NaCl, comparable with those found in the skin after high dietary salt intake, amplify expression of NOS2 and TNF in TLR-stimulated macrophages. This effect is likely mediated via p38 and NFAT5. Increases in NOS2 and TNF expression were paralleled by a sharp decline in mRNA expression of other proinflammatory genes. Modulation of TLR-mediated macrophage activation by NaCl may be relevant both for the physiological response to high dietary salt and salt-sensitive hypertension.
Abstracts – BENELUX Kidney Meeting 2015

P51. Red hot chili peppers and drinking water: a risky combination?

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**Objective:** Hyponatremia is the most common electrolyte disorder found in clinical practice and is independently associated with increased morbidity and mortality in elderly but also in the general population. The pathophysiology of hyponatremia is incompletely understood. Recently, a non-synonymous single nucleotide polymorphism (SNP) in the transient receptor potential cation channel vanilloid subfamily member 1 (TRPV1) gene has been associated with hyponatremia in elderly acutely admitted to the hospital. TRPV1 is a calcium channel located in central osmosensing neurons in the hypothalamus, which translates the osmosensing signal into vasopressin release. Capsaicin, the active pungent of red hot chili pepper and endogenous ligand of TRPV1, may therefore affect osmoregulation. In this study, we investigated the effects of TRPV1 activation by dietary capsaicin on plasma sodium concentration in healthy subjects.

**Methods:** In a randomized, placebo-controlled, cross-over study, we have investigated the effects of oral capsaicin intake in healthy male subjects. Study participants received either capsaicin (5.2-6.2 mg, 81.250-97.200 SHU) or matching placebo in random order on two different study days, at least one week apart. Both interventions were studied, with and without a concurrent water loading test (20 mL water/kg in 20 minutes). Blood and urine samples were collected at fixed time points. We used a general linear model for repeated measurements, with baseline concentration included as a covariate, to compare placebo and capsaicin during follow-up.

**Results:** We included 12 healthy male subjects aged 23.0 ± 3.8 years. Baseline characteristics were comparable between both study days. Relative to placebo, capsaicin did not affect plasma sodium concentration (p=0.54, Fig 1A). Water loading decreased plasma sodium concentration maximally after 30 minutes in the placebo (3.4 ± 1.6 mmol/L, p<0.001) and capsaicin group (2.7 ± 1.5 mmol/L, p<0.001) to a similar extent (p=0.24). During the 4-hour follow-up, capsaicin decreased plasma sodium concentration (1.7 ± 1.0 mmol/L) to a similar extent as placebo (1.5 ± 1.0 mmol/L) (p=0.67, Fig 1B). Four hours after water loading, the amount of excreted water was equal in the capsaicin (110.6 ± 29.4%) and placebo group (107.7 ± 33.1%) (p=0.82, Fig 1C). There were no differences in total urine sodium content after capsaicin and placebo treatment, both during the follow-up without (31.6 ± 12.6 vs 35.7 ± 18.2 mmol, p=0.44) and with a concurrent water loading test (24.4 ± 10.9 vs 25.9 ± 11.5 mmol, p=0.76).

**Conclusions:** After short-term exposure, capsaicin did not affect the plasma sodium concentration in healthy subjects. However, the long-term effects of capsaicin have yet to be examined. Whether these results can also be applied to individuals at risk for hyponatremia remains to be determined.