to rising SCr (>30% in 1st month), 6% developed hyperkalemia (K+ > 5.3 mmol/l) during drug therapy and 20% patients couldn’t attain full dose due to low BP.

Conclusions: It may be concluded that higher dose of angiotensin converting enzyme inhibitor (ACEI)-Enalapril and angiotensin receptor blocker (ARB)-Losartan alone or in combination can be used safely in majority of the patients with glomerulonephritis and diabetic chronic kidney disease (CKD).

Vascular access and bioengineering

**SO009 ♦ MULTISLICE COMPUTED TOMOGRAPHIC ANGIOGRAPHY IN EVALUATING HEMODIALYSIS VASCULAR ACCESS**

Zhiguo MAO, Chaoyang Ye, Changlin Mei, Shu Rong, Yuqiang Zhang. Department of Nephrology, Changzheng Hospital, Second Military Medical University, Shanghai, China

Introduction and Aims: Maintenance of long term function of vascular access is a challenge in hemodialysis patient management. Early detection of the dysfunction of hemodialysis vascular access (stenosis is a major cause) and prompt intervention seem to be the crucial step. The purpose of this study was to assess multislice computed tomographic angiography (MSCTA) for detecting stenosis of hemodialysis vascular accesses and guiding the revision operation or percutaneous transluminal angioplasty (PTA).

Methods: Contrast-enhanced 16 slice spiral CT was used to examine 22 hemodialysis subjects with various dysfunctions of vascular access. Parameters for the MSCT angiographic acquisition were 1.0mm section thickness, 0.8mm section interval, pitch of 3 (high-quality mode), 120-130kVp, 200-300 mAs. The scanning volume extended from the AV fistula up to the superior caval vein. The transverse source images were reformatted as maximum intensity projection (MIP), volume rendering (VR) and curved planar reconstruction (CPR) images. The whole procedure was usually accomplished in 20-30 minutes in outpatient setting and no hospitalization was needed for this examination.

Results: High-spatial-resolution images of vascular accesses were obtained with MSCTA in all patients. MIP, VR and CPR images displayed whole spectrum the AV fistula with the feeding artery, anastomoses and outflow tract up to the superior caval vein. On MSCTA, the stenotic segments of vascular access were mostly string-like. The access distal to the stenosis dilated and distorted locally and the borders between the dilated access and the stenoses were usually obvious. Numerous venous collateral branches were seen in 4 patients. According to the results of MSCTA. AV fistula revising surgery was done in 11 patients and PTA under the guide of digital subtraction angiography (DSA) was done in 5 patients. The results of MSCTA coincide with the finding of surgery or DSA in these 16 subjects. Fig. 1 and Fig. 2 showed the MSCTA-VRand DSA images of a patient’s vascular access.

**Conclusions:** MSCTA is a good non-invasive diagnostic technique to detect various hemodialysis vascular access abnormalities. It is more economical than DSA at present medical setting, and could replace DSA in the imaging of hemodialysis vascular access and provide important information for further AVF revising surgery or PTA.

Edema and extracellular water

**SO010 ♦ A NEW CLASS OF MUTATIONS INVOLVED IN GITELMAN'S SYNDROME AFFECTS THE INTRINSIC ACTIVITY OF THE NA-CL COTRANSPORTER NCCT**

Eva Riveira-Munoz 1, Karin Daham 1, Nathalie Godefrid 1, Qing Chang 2, Joost G. Hoenderop 2, René Bindels 2, Olivier Devuyyst 1. Nephrology, UCL Medical School, Brussels, Belgium; 2Phisiology, UMCN, Nijmegen, Netherlands

Introduction and Aims: The SLC12A3 gene codes for the thiazide-sensitive Na-Cl cotransporter (NCCT) which is expressed in the apical membrane of the cells lining the distal convoluted tubule (DCT). Mutations of SLC12A3 lead to Gitelman’s syndrome (GS), a rare, recessive disorder characterized by hypokalemia, hypomagnesemia, metabolic alkalosis and hypocalciuria. Although most patients with GS are either asymptomatic or show very mild symptoms, recent studies suggest that the clinical manifestations of the GS can be severe. Two different classes of mutations have been identified in GS. Class I mutations result in non-functional cotransporters that are not fully glycosylated and fail to reach the cell surface, whereas class II mutations result in functional cotransporters, normally glycosylated but partly impaired in their routing to the plasma membrane. The molecular basis of the phenotype variability in GS has not been established, and there is no evidence for a possible correlation between the clinical manifestations of GS and the position/nature of the mutation in SLC12A3.

Methods: Coding region and flanking intronic sequences of the SLC12A3 gene were amplified by PCR and directly sequenced. Functional analyses were performed in Xenopus laevis oocytes including 22Na+ uptake, plasma membrane and total membrane isolation, and immunocytochemistry.

Results: In this study, 19 unrelated probands from non-consanguineous families with a well-established clinical diagnosis of GS were screened for SLC12A3 mutations. Among them, 13 were identified to be compound heterozygous, whereas a single mutant allele was identified in 6 patients. Twenty-six mutations, distributed throughout the gene, have been identified, including 14 novel mutations (9 missense, 4 splice-site and one insertion): R145C, G316V, S350L, C484W, A523T, N611S, G729A, D839N, R968Q, c.2667+1G>T, c.1925+1G>A, c.2633+1G>C, c.2633+4A>G and c.2667_2668insA. All the mutations were not detected in 200 control chromosomes. A correlation between early-onset (age < 12 years old) and severe clinical manifestations (severe growth and pubertal delay, extra-renal and cardiac complications) in a few GS patients and mutations located in the middle hydrophobic region of NCCT was observed. Based on this, six mutations were studied in Xenopus laevis oocytes. In addition to the previously described Class I and Class II mutants, we described a novel Class III of mutations involved in GS. These mutations result in a cotransporter that is not functional, despite normal glycosylation and expression in the plasma membrane.

Conclusions: In conclusion, we have identified 26 mutations of SLC12A3, including 14 novel. The mutations located in the hydrophobic region of NCCT are associated with an early and clinically severe phenotype. The fact that mutations can affect the intrinsic transport activity of NCCT (Class III mutants) implies that a processing defect of the mutant NCCT is not the only pathogenic mechanism underlying GS.

**SO011 ♦ PROGNOSTIC VALUE OF EXTRACELLULAR WATER, DETERMINED WITH ELECTRIC BIOIMPEDANCE, IN HEMODIALYSIS PATIENTS**

Riccardo Maria Fagugli 1, Giorgio Gentile 1, Gaetano Ferrara 1, Paolo Pasini 2, Franca Pasticci 3, Giuseppe Quintaliani 1. 1Nephrology, Ospedaliera di Perugia, Perugia, PG, Italy; 2Cardiology, Ospedaliera di Perugia, Perugia, PG, Italy; 3Nephrology, ASL 2, Perugia, PG, Italy

Introduction and Aims: Fluid overload, measured as Extracellular Water

**Fig. 1** **Fig. 2**

**Conclusions:** In our opinion, MSCTA is a good non-invasive diagnostic technique to detect various hemodialysis vascular access abnormalities. It is more economical than DSA at present medical setting, and could replace DSA in the imaging of hemodialysis vascular access and provide important information for further AVF revising surgery or PTA.
(ECW) with bioimpedance, is correlated to hypertension and left ventricular hypertrophy in Hemodialysis patients. It is not known if ECW increase represents an independent risk factor of mortality. Therefore we have conducted this study with the aim to investigate the prognostic value of fluid overload measured as ECW on total and cardiovascular mortality.

**Methods:** The study was performed in a cohort of 127 hemodialysis patients, for a period of five years: we have divided the patients in two groups depending on ECW distribution: group 1 was considered to be affected from fluid overload, having and ECW ≤ 46.5%, group 2 was considered normal hydrated, having ECW < 46.5%. We determined at the beginning of the study ECW by using tetrapolar electric bioimpedance, left ventricular mass index, and 24h blood pressure. Comorbidities, as well as blood chemistries, were also analysed.

**Results:** During the follow-up period (47.8 ± 18.9 months) 40 patients were transplanted. 35 out of 87 not transplanted patients died (40.2%); in 25 patients a cardiovascular event was the cause (71.4%). At the end of the study mortality was 55.3% in group 1, in comparison to 22.5% of group 2 (p < 0.01). Cardiovascular mortality was 38.3%, in the group 1, and 17.5% in group 2 (p = 0.01). The Cox analysis, computing ECW and other variables such as age, gender, serum albumin levels, hypertension, left ventricular hypertrophy, ischemic heart disease, and diabetes, confirm ECW as a strong independent risk factor of mortality (HR = 4.00; p < 0.01). Other independent risk factors were the presence of ischemic heart disease (HR = 3.37; p = 0.02), left ventricular hypertrophy (HR = 3.5; p = 0.056), and the age of patients (HR = 1.12; p < 0.01).

**Conclusions:** Fluid overload measured as Extracellular Water by tetrapolar electric bioimpedance represents and important independent risk factor of total and cardiovascular mortality in hemodialysis patients.

---

**SO013 TISSUE KALLIKREIN-DEFICIENT MICE HAVE A DEFECT IN γENaC PROCESSING**

Nicolas Picard1, Carole Planes2, Michel Burnier1, Georges Deschênes4, Pierre Menetton1, Dominique Eladari1, Régine Chambrey1, 1U652, INSERM, Paris, France; 2U426, INSERM, Paris, France; 3Division of Nephrology, University Hospital, Lausanne, Switzerland; 4UMR 7134, CNRS/UPMC, Paris, France

**Introduction and Aims:** Tissue kallikrein (TK) is a serine protease highly expressed in epithelia such as kidney, colon or salivary glands, and forms locally kinins. In the kidneys, TK is secreted in very large amount into the urinary fluid from distal tubules and reach the luminal surface of all the cells present within the aldosterone sensitive nephron, where fine tuning of Na+ balance is achieved. In the present study, a mouse model with genetic disruption of TK gene was used to test the hypothesis that TK is involved in the regulation of Na+ transporters in the kidney.

**Results:** TK deficient mice (TK−/−) were normal with respect to plasma electrolyte concentration and blood pressure. Urinary Na+, K+ and Cl− output were similar for both genotypes indicating that all the animals were in steady state with similar NaCl intake. Semiquantitative immunoblotting was used to study changes in all the sodium transporters proteins abundances expressed along the renal tubule in response to gene deletion. We did not find any changes in NHE3, NaPi, NKCC2. However, α or β Na,K-ATPase, αENaC, or βENaC protein abundances. However, the 70kDa form of γENaC, which has been proposed to result from the aldosterone dependent proteolytic cleavage of the 85-kDa γENaC form associated with activation of the channel was not detectable in TK−/− when compared to WT mice. As expected, in WT mice, aldosterone infusion induced a shift in the molecular weight of γENaC from 85kDa to 70kDa. In TK−/− mice, aldosterone infusion induced a shift in molecular weight of γENaC, but not in the 85 kDa form. In TK−/−, the abundance of the 70kDa γENaC was increased in mice lacking kinin-B2 receptor compared with WT mice, indicating that the absence of the active 70kDa form of γENaC in TK−/− mice is not kinase mediated. Moreover, when renal membranes fraction prepared from TK−/− mouse was incubated with desalted urines from WT mice, the 70kDa band of γENaC appeared unlike with urine from TK−/− mice indicating that urinary kallikrein was able to cleave γENaC in vitro.

The distal colon exhibit amiloride-sensitive Na transport where TK is also abundantly expressed. An index of ENaC mediated Na+ transport, the transepithelial amiloride sensitive potential difference, is significantly decreased in TK−/− mice. Unlike kidneys and colon, lungs alveolar epithelium shows an aldosterone-independent amiloride-sensitive sodium uptake and do not express TK. Semiquantitative immunoblots carried out for the ENaC γ subunit on lung membranes fractions showed that γENaC appeared as a 85 kDa band and a broad band at 75-70kDa at the same level in TK−/− and WT mice. Moreover, amiloride sensitive lung alveolar clearance was not different in TK−/− and WT mice.

**Conclusions:** All these results suggest that TK is part of the protease cascade that activates γENaC through proteolytic processing in the kidney but not in the lung. Whether TK can cleaves γENaC directly or through the activation of other proteases like Chanell Activating Protease remains to be determined.