the substrates (such as WNK1/4) for ubiquitination. The consequences of exon 9 skipping on Cul3 activity are still debated. It could result in an increased degradation of KLHL3 and thus decreased recruitment and degradation of the substrates. However, the expression level of KLHL3 was similar in Cul3+/d9 and control mice.

Conclusions: As in humans, the phenotype of Cul3-FHHt mice is more severe than that of the WNK1-FHHt mice we previously described. Two hypotheses have been proposed: a broader dysfunction of the distal nephron or an increased vascular reactivity. Further studies of the Cul3+/d9 mice are required to define the causes of this severity.

OP.LB01.11 MEASUREMENT OF ANGIOTENSIN II AT EQUILIBRIUM IN THE DIAGNOSTIC WORKUP OF PRIMARY ALDOSTERONISM. IMPACT OF PATIENT POSITIONING AND ACE INHIBITOR TREATMENT

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Objective: Primary aldosteronism (PA) is a widely under-diagnosed, potentially curable and specifically treatable cause of hypertension. PA screening involves measuring the aldosterone-to-renin-ratio (ARR), but false negative results can occur in the setting of medications, which block the renin-angiotensin system (RAS). Withdrawing RAS blockers from patients with resistant hypertension is not without cardiovascular risk. A novel diagnostic approach, the aldosterone-to-angiotensin-II-ratio (AA2-Ratio), has the potential for less drug interference and improved reliability in PA screening and confirmation of diagnosis.

Design and method: Serum samples from 80 patients undergoing PA confirmation testing were analyzed. Sampling was performed in a recumbent (7 a.m.) and in an upright (10 a.m.) position before and after 4 days of oral administration of fludrocortisone and salt loading. The concentrations of renin, aldosterone and equilibrium Angiotensin-II were determined and ARR and AA2-Ratios were calculated. The interference of ACE-inhibition with the AA2-Ratio was investigated in healthy volunteers receiving 10 mg enalapril daily for 8 days.

Results: Renin concentration was undetectable in more than 40% of samples, while equilibrium Angiotensin-II was measurable in 98% of all 320 samples analyzed. Angiotensin-II levels were significantly higher in upright collected samples compared to samples collected in a recumbent position. Comparison of the ARR with the AA2-Ratio revealed a significantly larger diagnostic window for the AA2-Ratio. While the ARR was significantly suppressed by ACE-inhibitor treatment, the AA2-Ratio remained unaffected by ACE-inhibition.

Conclusions: The AA2-Ratio may be superior to the ARR in PA screening among hypertensive patients. Equilibrium Angiotensin-II levels show expected responses to posture and appear to outperform renin concentration as a marker for RAS activation in terms of sensitivity, giving a measurable readout even in clinical states characterized by markedly suppressed RAS activity. The stability of the AA2-Ratio in the presence of ACE-inhibition points to a potential use of the AA2-Ratio PA screening in hypertensive patients without ACE-inhibitor discontinuation.

OP.LB01.12 CACNA1H MUTATIONS ARE ASSOCIATED WITH YOUNG ONSET AND FAMILIAL FORMS OF PRIMARY ALDOSTERONISM

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Objective: Primary aldosteronism (PA) is the most common form of secondary hypertension. Mutations in KCNJ5, ATP1A1, ATP2B3 and CACNA1D are found in aldosterone producing adenomas (APA) and familial hyperaldosteronism (FH). Recently, a recurrent germline mutation in CACNA1H (encoding the T-type voltage-dependent calcium channel Cav3.2) was identified in a new familial form of early onset hypertension and PA.

Design and method: To identify new genes responsible for PA, we have performed whole exome sequencing in 23 patients with APA, 10 patients with FH and in two trios with the proband presenting early onset PA.

Results: We identified four germline CACNA1H variations. p.Ser196Leu and p.Pro2083Leu were found in two patients with FH resembling to FH-II. A p.Val1951Glu variant was identified in a patient with APA, and a de novo p.Met1549Ile variant in a patient showing hypokalemia, hypertension and PA at age 3 months.
Electrophysiological analysis of mutant Cav3.2 in HEK293 cells revealed significant changes in the Ca2+ current properties, including slower inactivation (Cav3.21549Ile and Cav3.2196Leu) and a shift in the steady-state inactivation (Cav3.21549Ile, Cav3.2196Leu and Cav3.22083Leu). All mutations (except Cav3.21549Ile) generated an increase in the current facilitation induced by a previous stimulation, very likely increasing the calcium entry via Cav3.2 during sustained activities.
Transient transfections of adrenocortical carcinoma H295R-S2 cells showed that, after K+ stimulation, cells overexpressing mutants Cav3.2196Leu and Cav3.21549Ile showed a 2.5-fold (Cav3.2196Leu, $p < 0.001$) and 4.5-fold (Cav3.21549Ile, $p < 0.001$) increase in aldosterone biosynthesis compared to wild type Cav3.2. This was associated to an increase in CYP11B2 mRNA levels in cells expressing Cav3.2196Leu (7.5 fold; $p < 0.001$) and Cav3.21549Ile (3.2-fold; $p < 0.001$). No difference in aldosterone levels was observed in basal conditions or after Ang2 stimulation.

Conclusions: In summary, we identified 4 germline CACNA1H mutations in PA patients with different phenotypic presentations. These mutations induce significant changes in channel properties and are responsible for increased aldosterone production in adrenocortical cells after K+ stimulation. Identification of new CACNA1H mutations that are associated with early onset PA or familial forms diagnosed as FH-II suggests that CACNA1H might be a susceptibility gene predisposing to PA with different phenotypes.