APA development. Also, APA show important cellular and molecular heterogeneity which may be due to interaction of different signaling pathways involved in adrenal cortex cell differentiation and function. The aim of this study was to investigate the role of Wnt/β-catenin and ACTH signaling as well as elements of paracrine regulation of aldosterone biosynthesis and vascularization in the development of APA and aldosterone producing cell clusters (APCC) and their relationship with intratumoral heterogeneity and mutational status. We performed immunohistochemistry and multiplex immunofluorescence (CYP11B2, CYP17A1, β-catenin, MC2R, pCREB, Tryptase, S100, CD34) multispectral image analysis on 11 adrenals with APA and one with micronodular hyperplasia from patients with PA. CYP11B2 (aldosterone synthase) IHC guided RT-qPCR was performed on RNA extracted from formalin-fixed paraffin-embedded tissues in 7 adrenals. Multiplex immunofluorescence revealed high abundance of tryptase positive mast cells and a dense vascular component in APA, which were independent of the mutational status. Within APA, mast cells were mainly localized in zones expressing CYP11B2, but not in areas expressing CYP17A1, and were rarely colocalized with nerve fibers, suggesting that their activity is not controlled by innervation. In cells expressing aldosterone synthase, β-catenin was activated, i.e. shows nuclear and/or cytoplasmic staining, features suggestive of a zona glomerulosa cell identity; MC2R was found at the cell membrane. Expression of *MC2R* mRNA was observed at different levels in APA, similar to expression of *MRAP* and *VEGFA*; *MRAP2* was not detected. Within heterogeneous APA carrying *KCNJ5* mutations, both *MC2R* and *VEGFA* expression was higher in areas expressing CYP11B2. Remarkably, this pattern was maintained in APCC, where cells show high CYP11B2 expression, together with activated β-catenin, independently of the mutation status. In addition, a high number of mast cells was detected around APCC, with a reorganization of the capillaries around the CYP11B2 positive cells. Our results suggest that aldosterone producing structures in adrenals with APA share common molecular characteristics and cellular environment, despite different mutation status. Mast cells appear to be closely associated with cells expressing aldosterone synthase, both in APA and APCC, and their role in regulating aldosterone biosynthesis in the context of somatic mutations in PA remains to be established.

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Laterality Diagnosis of Adrenal Vein Sampling for Primary Aldosteronism Using Aldosterone Alone

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Background: In adrenal vein sampling (AVS) for primary aldosteronism (PA), cortisol concentration is used to determine successful AVS, and laterality diagnosis is performed using a combination of aldosterone and cortisol concentrations. In this study, we examined the comparison with the conventional method when AVS was determined by aldosterone alone. **Subjects and methods:** We studied the data from 277 patients with PA who underwent AVS in Sapporo City General Hospital from July 2007 to April 2020. The patients with autonomous cortisol production were excluded. Using the blood samples from adrenal veins and inferior vena cava (IVC) after ACTH stimulation, the predicting ability of the left and right ratio of aldosterone concentration (aldosterone ratio, AR) for lateralization Index (LI) was examined by Receiver operating characteristic (ROC) analysis. The predicting abilities of the ratio of aldosterone concentration between adrenal vein and IVC (aldosterone index, AI) and aldosterone concentration for selectivity index (SI) and contralateral ratio (CR) were also examined by ROC analysis. **Results:** Six samples (0.01%) with SI <5 after ACTH stimulation those were determined unsuccessful AVS. The results of the area under the curve (AUC) in ROC analysis of aldosterone concentration and AI for prediction of SI>5 was 0.998, 0.990, respectively, p=0.39. The optimal cut-off values of aldosterone concentration and AI for prediction successful AVS were 1700 pg/ml (sensitivity 99.5%, specificity 100%), 7.44 (sensitivity 94.0%, specificity 100%), respectively. Seventy-two patients (27.3%) had LI >4 who were diagnosed as unilateral aldosterone excess. AR had 0.94 of AUC for prediction of LR >4. The optimal cut-off value of AR was 3.53 (sensitivity 86.1%, specificity 94.8%). Eighty-two patients (31.1%) had unilateral CR<1. The AUC of aldosterone concentration and AI for prediction of CR<1 was 0.96, 0.98, respectively, p=0.07. The optimal cut-off values of aldosterone concentration and AI were 13600 pg/ml, 42, respectively. The sensitivity and the specificity at the optimal cut-off points of aldosterone concentration and AI were 91.5%, 91.5% and 91.5%, 94.8%, respectively. **Conclusions:** The determination of successful AVS and unilateral result in AVS can be predicted using aldosterone alone. It was suggested that AR is useful for tentative interpretation in the cases where the results of aldosterone were previously reported and lateralizing diagnosis of the cases with autonomous cortisol production.

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Lipoprotein Insulin Resistance Score: Validation and Utility in African Ancestry Populations

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Lipoprotein insulin resistance (LPIR) is an emerging biomarker of insulin resistance (IR), and a score of >48 is a strong predictor of incident cardiometabolic disease disease in a predominantly European ancestry population. LPIR is derived from a composite score of nuclear magnetic resonance (NMR) lipoprotein (Lp) parameters: triglyceride-rich (TRLp), low density (LDLp), and high density (HDLp). Yet, there is a paucity of data in African ancestry population, in whom there is low-normal TRLp despite high rates of IR and diabetes. Therefore, we examined Lp profiles and LPIR in a large African ancestry cohort, stratified by sex to determine the relationship of LPIR with established markers

of IR. This is a secondary analysis from 2 studies (The Africans in America and Federal Women's Study) designed to evaluate the genetic, biological and socio-environmental factors of diabetes risk in those of African ancestry. All participants self-identified as healthy and lived in the DC metro area, n= 518: 87.7% African immigrant,12.3% African American; age 39±10 (20-65y); BMI 28.1±4.8 (18.2–45.2 kg/m2); 58% male; 31% with obesity, and 37% with abnormal glucose tolerance; mean±SD (range); median (25^{th}-75^{th} percentile). Fasting measures of IR (LPIR, triglyceride/HDL (TG/HDL) ratio and homeostasis model of insulin resistance (HOMA-IR)) were compared with the whole-body insulin sensitivity index (WBISI) obtained during a multi-sample 75g OGTT, using spearman correlations. Lp particle size and subclass concentrations were measured by the amplitudes of the lipid-methyl group signals (NMR LipoProfile®). Men had lower BMI (27.1±3.9 vs 29.3±5.6 kg/m^2), fat mass (23.5±5.5 vs 37.9±6.8 %), insulin resistance (WBISI: 6.2 (3.7–10.1) vs 4.9 (3.2–8.6), HOMA-IR: 1.3 (0.7–2.0) vs 1.6 (0.9–2.4), TG/HDL: 1.4 (1.0–2.2) vs 1.1 (0.8–1.5)), all P<0.001. LDLp (1226 (959–1531) vs 1239 (981–1553) nmol/L) and HDLp (17.6 (16.2–19) vs 17.5 (15.9–19.7) µmol/L) were similar by sex, P>0.6, while small LDLp 734 (523–1039) vs 541 (370–805) nmol/L and TRLp 80.5 (52.2–116.4) vs 53.6 (28.7 -89.3) nmol/L were higher in men. The total mean LPIR score was 28.9±18.7 and was higher in men (34±19 vs. 23±17), P<0.001. LPIR and TG/HDL ratio correlated with WBISI (r≥-0.40) and HOMA-IR (r≥0.40), P<0.001 with no differences by sex. HOMA-IR correlated with WBISI (r=-0.95, P<0.001). Overall, African ancestry individuals had high rates of abnormal glucose tolerance, obesity and LDLp but LPIR was 20 points lower than the established score for predicting cardiometabolic disease. It's utility for detecting IR was modest but it may be an important adjunct for early cardiometabolic risk stratification in African ancestry populations in whom traditional screening methods have lower sensitivity.

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Long Term Effects of Leptin on Hepatic Fibrosis in Generalized Lipodystrophy

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Lipodystrophy syndromes are caused by deficiency of adipose tissue leading to severe insulin resistance, hypertriglyceridemia, and non-alcoholic fatty liver disease (NAFLD), which may progress to cirrhosis. Advanced fibrosis/cirrhosis was previously thought to be irreversible; however, eradication of hepatitis C or long-term viral suppression of hepatitis B can reverse cirrhosis. Metreleptin treatment in patients with lipodystrophy improves liver transaminases and NAFLD activity score (NAS) after a mean of 2 years, but the latter improvements were due to lower inflammation and steatosis, with no change in fibrosis. The long-term effects of metreleptin in subjects with advanced fibrosis are unknown. We analyzed 24 subjects with advanced fibrosis (NAS stage 3 or 4) prior to metreleptin. Seven had liver biopsies both before and after ≥ 3 years of metreleptin with mean treatment duration 7.8±2.9 years. Five of six subjects with stage 3 fibrosis at baseline had improved fibrosis scores after metreleptin, and 0 of 1 with stage 4 fibrosis improved. 17 patients (8 with stage 3, 9 stage 4) did not have follow up biopsies after ≥3 years. Of these 17, 4 (all stage 4) died from end stage liver disease, and 1 (stage 3) from other causes. There was no clinical indication for repeat biopsy in 6 patients (2 stage 3, 4 stage 4). 6 were lost to follow up. Of the 24 patients with advanced fibrosis, 13 had congenital generalized lipodystrophy (CGL), 7 had acquired generalized lipodystrophy (AGL), 3 had familial partial lipodystrophy (FPL) and 1 had acquired partial lipodystrophy (APL). Of the 5 subjects with improvement in their fibrosis score, 2 had AGL, 2 had CGL, and one had a novel FPL mutation (1). Of the two patients that did not improve, one had FPL and one had CGL. In conclusion, subjects with stage 3 fibrosis due to lipodystrophy may have regression of fibrosis after long-term metreleptin treatment. This improvement may be secondary to near-elimination of the inciting factors (excess nutrient intake leading to ectopic lipid storage in the liver), analogous to clearance of Hepatitis C infection. However, additional data is needed to determine if metreleptin can reverse fibrosis in subjects with stage 4 fibrosis.

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Low Testosterone in Males May Warrant Liver Health Assessment and Intervention

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Low testosterone (T) is a serum marker of hypogonadism. Reportedly, diabetes mellitus, cardiovascular disease, peripheral artery disease, hypertension, hyperlipidemia, depression, obesity, metabolic syndrome, sleep apnea, chronic obstructive pulmonary disease (COPD), and opioid dependency, are associated with low testosterone. However, the association of chronic liver disease with low T is underappreciated. In one study, ~75% of biopsy confirmed Nonalcoholic Steatohepatitis (NASH) male patients had T levels of <372 ng/dL. A study with patients receiving androgen deprivation therapy (ADT), with no pre-therapy evidence of liver disease, increased liver disease risk relative to no ADT suggesting low T can adversely affect liver health. However, prevalence of compromised liver health in hypogonadal males, and any beneficial effects of T therapy intervention on liver health are unclear. The objective of this investigation was to evaluate prevalence of liver disease in hypogonadal males, and to assess for potential beneficial effects of LPCN 1144, a novel oral T therapy candidate, on liver health of hypogonadal males. Investigation was performed through clinical studies in hypogonadal males, and a pre-clinical study. Clinical studies: (1) a one-year treatment, open label, active control, randomized study (NCT02081300); (2) a four-month treatment, open