SaaG E-Poster Discussions

Saag E-Poster Discussion sessions will take place in the Exhibition Hall. In addition, all SaaG e-Posters will be available on the virtual congress platform during the entire congress until 3 months after the congress. The participants will be able to contact the poster presenter through the virtual platform.

SS001 / #348

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.01 Endothelial cell function and biology*

TRANSCRIPTOMIC ANALYSIS OF ENDOTHELIAL DERIVED EXTRACELLULAR VESICLES (LIQUID BIOPSY) FOR THEIDENTIFICATION OF EARLY MARKERS OF ENDOTHELIAL DYSFUNTION

SAAG SESSION 01: MECHANISMS OF ENDOTHELIAL DYSFUNCTION

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Background and Aims: Despite the key role of the endothelium in atherosclerosis, there are no direct techniques for its study. The molecular analysis of circulating endothelial extracellular vesicles (EndEVs) might lead to the identification of early biomarkers of atherosclerosis and potential therapeutic targets.

Methods: We adapted a magnetic immunocapture protocol for EndEVs separation, and an ultra-low input-RNAseq (mcSCRBseq) method for their transcriptomic analysis, and assessed EndEVs of controls (G1, n=5, 60% male, mean age 57), subclinical atherosclerosis (G2, n=5, 60% male, mean age 56), and peripheral artery disease (G3, n=5, 60% male, mean age 66) patients. After the bioinformatic analysis, a candidate was selected and validated in immortalized human aortic endothelial cells (TeloHAECs) upon IL-1β, TNFα, oxLDL and hypoxia.

Results: The transcriptional analysis detected 1667 genes in EndEVs, that were significantly enriched in transcripts expressed by TeloHAECs (NES: 1.93, p adjust= $1.4e^{-73}$). 170 differentially expressed genes (DEG) were identified between G2 vs G1, and 180 between G3 vs G1, from which 17 were similarly expressed in G2 and G3 vs control, including UCP2 that was downregulated in atherosclerosis. In vitro IL-1β and TNFα (10 ng/mL) reduced UCP2 expression in TeloHAECs at 12h (p<0.05), and a similar trend was observed for hypoxia (1% O_2, p=0.05) and oxLDL (100 μg/mL, p=0.055) at 24h.

Conclusions: EndEVs from subjects with atherosclerosis presented reduced levels of UCP2, suggesting an increase in oxidative stress already in subclinical phases. EndEVs could be an alternative for the analysis of endothelial dysfunction in atherosclerosis.

SS002 / #1130

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.01 Endothelial cell function and biology*

LEPTIN RESISTANCE FAVORS VASCULAR INFLAMMATION THROUGH HINDERING PD-L1 EXPRESSION

SAAG SESSION 01: MECHANISMS OF ENDOTHELIAL DYSFUNCTION

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Background and Aims: Endothelial dysfunction is the culprit among the CVDs due to the presence of risk factors including hyperleptinemia. Upon obesity, while most of the studies have highlighted the orexigenic effect of leptin resistance on hypothalamic neuron, how leptin resistance affects vascular functionality remains largely unknown.

Methods: Providing that circulating leptin level is closely associated with body adiposity and feeding behaviors, both hypoleptinemia and hyperleptinemia were generated on C57BL/6 mice by nutritional modulation or genetic knockout on leptin receptor (n=5-10 from independent experiments). Statistical analysis was performed by either t test, one-way or two-way ANOVA or Pearson's correlation test.

Results: Findings across multiple models suggested, the expression of PD-L1, an immune-coinhibitory molecule, was regulated by leptin, the impairment in leptin signaling resulted in vascular PD-L1 downregulation. By employing the mice with obesogenic hyperleptinemia, we showed that the vascular tissue failed to respond to exogenous leptin stimuli, thereby hindering PD-L1 expression in the inflammatory microenvironment. In fact, PD-L1 expression is crucial in maintaining tissue homeostasis by suppressing the activated PD-1+ leukocytes, as such hindered PD-L1 expression may favor the vascular inflammation by failing to inhibit immunogenic attack, especially when other risk factors are present. Beyond acting as a ligand to leukocytes, our findings also revealed PD-L1 reverse signaling in endothelium plays a role in resolving inflammation and rescuing endothelial functionality.

Conclusions: These findings propose a new explanation for why obesity results in vasculopathy in relationship with PD-L1.

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.01 Endothelial cell function and biology*

IRE1A INDUCED SENESCENCE PROMOTE ENDOTHELIAL BARRIER DYSFUNCTION IN DIABETES-INDUCED ATHEROSCLEROSIS

SAAG SESSION 01: MECHANISMS OF ENDOTHELIAL DYSFUNCTION

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Background and Aims: Diabetes mellitus is hallmarked by accelerated atherosclerosis, which is the major cause of mortality in diabetic patients. Efficient therapeutic concepts for diabetes-associated atherosclerosis are lacking. Accelerated atherosclerosis in diabetic patients is associated with reduced endothelial thrombomodulin (TM) expression and impaired activated protein C (aPC) generation.

Methods: To gain insights into pathomechanisms of diabetes induced atherosclerotic plaque development we cultured human coronary artery endothelial cells (HCAECs) under hyperglycemic (HG) or hyperlipidaemic (oxLDL) conditions for 48 h. ApoE-/- mice (age 8 weeks) was made either diabetic by streptozotocin injections (A mouse model of type 1 diabetes) or fed them HFD to induce hyperlipidemia. Mice were analyzed after 20 weeks of treatments.

Results: High glucose induced more pronounced responses in regard to maladaptive unfolded protein response (UPR), senescence, and vascular endothelial cell barrier disruption. *Ex vivo*, diabetic ApoE-/- mice revealed increased expression of senescence and UPR markers within atherosclerotic lesion as compared with nondiabetic ApoE-/- mice. Activated protein C restored barrier integrity and reduced glucose induced expression of senescence and UPR markers *in vitro*. Inhibition of IRE1α (inositol-requiring enzyme 1 alpha), a key UPR activator, prevented glucose induced endothelial barrier disruption and cellular senescence. Conversely, an activator of IRE1α's RNAse domain recapitulated hyperglycaemia-induced effects, suggesting that hyperglycaemia-induced IRE1α RNAse activity is sufficient to induce senescence and vascular dysfunction.

Conclusions: These data suggest that high glucose induced maladaptive UPR and associated senescence promote vascular endothelial cell dysfunction, which —however—can be reversed by aPC. This suggests that reversal of glucose-induced vascular endothelial cell dysfunction is feasible.

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.01 Endothelial cell function and biology*

ROLE OF THE CXCL12/CXCR4 AXIS IN ATHEROSCLEROTIC PLAQUE INSTABILITY

SAAG SESSION 01: MECHANISMS OF ENDOTHELIAL DYSFUNCTION

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Background and Aims: Unstable atherosclerotic plaques show intraplaque angiogenesis and hemorrhage. CXCR4 and its ligand CXCL12 participate in atherosclerosis, however, their role in plaque instability has not yet been evaluated. We aim to unravel the role of CXCL12-CXCR4 axis in intraplaque angiogenesis mediated plaque instability and test if its therapeutic targeting can prevent plaque destabilization and rupture.

Methods: We analyzed a dataset of microarray transcriptional profiling of human carotid atherosclerotic plaques from 43 symptomatic patients undergoing carotid endarterectomy surgery (GSE163154). Mouse aortic endothelial cells (wound-healing assay) and Apoe$^{-/-}$ mice thoracic aortas (aortic ring assay) were treated with FDA-approved CXCR4 antagonist, AMD3100, and/or CXCL12.

Results: CXCR4 showed highest expression among the chemokine receptors and increased in unstable plaques compared to stable plaques. Using immunofluorescence, CXCR4 was expressed by ECs forming intraplaque neovessels. Same expression pattern was observed in murine atherosclerotic vein graft lesions, mouse model that resembles human atherosclerosis, including intraplaque angiogenesis and hemorrhage. AMD3100 reduced CXCL12-induced EC migration by 40%, suggesting that endothelial CXCR4 plays a role in CXCL12-mediated angiogenesis. We next examined CXCR4 inhibition on complex neovessel formation. Rings treated with AMD3100 produced 60% less neovessels compared to control rings treated with CXCL12 alone, confirming that CXCR4-CXCL12 axis directly influences angiogenesis.

Conclusions: Together, our data indicate that CXCR4 is expressed in advanced atherosclerotic lesions and likely contributes to plaque instability by promoting intraplaque neovascularization. In future studies we will use conditional CXCR4-KO mice combined with accelerated-atherosclerosis vein graft to assess the role of endothelial-CXCR4 in intraplaque angiogenesis and hemorrage in *in vivo* atherosclerosis.

SS005 / #1134

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.01 Endothelial cell function and biology*

PPAR-A AGONIST FENOFIBRATE AND 11,12-EPOXYEICOSATRIENOIC ACID SUPPRESSES ENDOPLASMIC RETICULUM STRESS-INDUCED APOPTOSIS AND ENHANCES CELL VIABILITY IN DIABETIC RAT HEARTS

SAAG SESSION 01: MECHANISMS OF ENDOTHELIAL DYSFUNCTION

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Background and Aims: Diabetes mellitus (DM) is associated with an increased risk of cardiovascular diseases, which is related with prolonged endoplasmic reticulum (ER) stress and the activation of apoptotic pathways. Cytochrome P450 (CYP) and its arachidonic acid metabolites 11,12-epoxyeicosatrienoic acid (11,12-EET) have protective roles in heart failure by protecting endothelial cells and myocytes from apoptosis. However, the link between cardioprotective roles of CYP epoxygenase and ER stress has not been well known in diabetic cardiomyopathy. Therefore, we investigate the effects of CYP2J3 and 11,12-EET on ER stress-induced diabetic cardiomyopathy.

Methods: ER stress marker, GRP78, CHOP, pro-apoptotic proteins and autophagy marker LC3-II, beclin-1 were detected by Western blot and RT-PCR in ob-/ob- rats heart and H2O2 treated H9C2 cells. Plasma cholesterol, triglycerides, glycerol, and hemodynamic parameters were measured in ob-/ob- rats.

Results: The body weight, plasma glucose, cholesterol and triglyceride were increased in ob-/ob- rats. The expression of CYP2J3 and PPAR-α were markedly down-regulated in ob-/ob- rats hearts. ER stress markers GRP78, CHOP and apoptosis marker cleaved caspase-3 levels were increased in ob-/ob- rats and H2O2 treated H9C2 cells. In contrast, expression of autophagy markers Beclin-1 and LC3-II were decreased. Administration of fenofibrate and 11,12-EET induced the expression of autophagy markers and simultaneously decreased ER stress markers and apoptosis marker cleaved caspase-3 levels. In hemodynamic evaluation, left ventricular diastolic pressure (LVDP) was also recovered by administration of fenofibrate.

Conclusions: PPAR-α agonist fenofibrate and CYP2J3-derived 11,12-EET attenuates ER stress-induced apoptosis and induces autophagy and cell viability partly via the AMPK/mTOR signaling pathway in ob-/ob- rat hearts.

SS006 / #1102

Topic: *AS04 Clinical Vascular Disease / AS04.02 Endothelial dysfunction; Clinical assessment*

TARGETED PROTEOMICS AND THE PRESENCE OF CAROTID PLAQUES IN SUBJECTS AT LOW CARDIOVASCULAR DISEASE RISK

SAAG SESSION 01: MECHANISMS OF ENDOTHELIAL DYSFUNCTION

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Background and Aims: We demonstrated that targeted proteomics predicts the occurrence of Subclinical Carotid Atherosclerosis (SCA) in apparently healthy subjects in primary prevention for cardiovascular disease (CVD). SCA can be present, as multifocal and/or stable/vulnerable, also in primary prevention. We now aim at addressing whether proteomics can help to characterize the presence of multifocal, stable/vulnerable SCA in low CVD risk subjects.

Methods: A machine learning (ML) model, trained on the ultrasound frames from 317 subjects (118 women) at low CVD risk (SCORE) but with SCA (i) counted the number of plaques (1, 2, 3+) in both carotids and (ii) characterized the vulnerability of SCA ("grayscale", where lower=vulnerable and higher=stable). The same model classified the best set of plasmatic proteins (368 measured with Olink[TM]) associated with number of plaques and the vulnerability of SCA.

Results: The higher number of plaques associated with factors included in clinical algorithms (age, hypertension, low cholesterol in High Density Lipoproteins). By contrast, lower grayscale did not associate with any factor. The ML classified 40 proteins, outperforming the identification of subject with 3+ plaques as compared to those with lower number (AUC=0.659 (0.525-0.777), p=0.035). Also, the same model found 16 proteins, chemokines and inflammatory markers (none in common with those identifying number of plaques) outperforming the identification of subject with more vulnerable vs those with more stable plaque (Area Under the Curve, AUC=0.647 (0.514-0.765), p=0.042).

Conclusions: We provide a first-in-class evidence that combining targeted proteomics with ML can identify subjects with advanced atherosclerosis that cannot be captured by clinical algorithms.

SS007 / #1232

Topic: *AS04 Clinical Vascular Disease / AS04.02 Endothelial dysfunction; Clinical assessment*

THE IMPACT OF STATIN THERAPY ON IN-HOSPITAL PROGNOSIS AND ENDOTHELIAL FUNCTION OF PATIENTS AT HIGH-TO-VERY HIGH CARDIOVASCULAR RISK ADMITTED FOR COVID-19

SAAG SESSION 01: MECHANISMS OF ENDOTHELIAL DYSFUNCTION

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Background and Aims: Compelling evidence suggests that statins may protect against COVID-19-related complications. This study assessed the impact of preadmission statin therapy and its continuation upon hospitalization on clinical outcomes of COVID-19 patients at high-to-very high cardiovascular (CV) risk. Also, it evaluated the possible interaction between preadmission statin therapy and brachial artery flow-mediated dilation (FMD) at hospital admission in the prediction of COVID-19 prognosis.

Methods: A cohort of hospitalized COVID-19 patients at high-to-very high CV risk was retrospectively analyzed.

Results: Among 342 patients (mean age 79±11 years, males 60%), 119 (35%) were on preadmission statin therapy. During hospitalization, 91 (27%) patients continued statin therapy and 92 (27%) met the composite endpoint of ICU admission/in-hospital death. Preadmission statin therapy was associated with up to a 75% risk reduction of ICU admission/in-hospital death (HR 0.252, 95% CI 0.122-0.521, $p<0.001$). Also, it was positively associated with FMD at hospital admission (b=0.150, $p=0.020$). In addition, low FMD (*i.e.*, FMD <4.2%, the median value) was associated with a higher risk of ICU admission/in-hospital death ($p=0.006$). However, no significant interaction emerged between preadmission statin therapy and FMD in the prediction of ICU admission/in-hospital death (F=0.002, $p_{interaction}=0.960$). Statin continuation upon hospitalization was not associated with the risk of ICU admission/in-hospital death ($p=0.674$).

Conclusions: Preadmission statin therapy is associated with better in-hospital outcomes in COVID-19 patients at high-to-very high CV risk, regardless of its endothelium-protective effects. Extensive statin therapy prescription and adherence in patients at higher CV risk may provide a significant health benefit during COVID-19 pandemic.

Topic: *AS02 Lipids and Lipoproteins / AS02.03 HDL*

GENETIC VARIANTS IN THE ADENOSINE TRIPHOSPHATE-BINDING CASSETTE TRANSPORTER A1 AND RISK OF AGE-RELATED MACULAR DEGENERATION

SAAG SESSION 02: TG-RICH LIPOPROTEINS AND HDL

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Background and Aims: Genetic variants in the adenosine triphosphate-binding cassette transporter A1(*ABCA1*) is associated with higher concentrations of high-density lipoprotein (HDL) cholesterol. Higher HDL cholesterol concentrations are observationally and genetically associated with higher risk of age-related macular degeneration (AMD). However, whether amino acid changing genetic variants in *ABCA1* associated with high HDL cholesterol concentrations confer a higher risk of AMD in the general population is currently unknown.

Methods: We genotyped all amino acid changing *ABCA1* variants with a minor allele frequency above 0.002, measured plasma HDL cholesterol, and used Cox regression to assess risk of AMD. We created an HDL cholesterol weighted allele score and tested the association with risk of AMD on a continuous scale and in tertiles. Further, we performed mediation analyses.

Results: We included 90,344 study participants. On a continuous scale higher concentrations of genetically determined HDL cholesterol were associated with higher risk of all-cause AMD, dry AMD, and wet AMD both in a multivariable adjusted model. The *ABCA1* allele score for the third versus the first tertile was associated with HRs (95% confidence intervals (CIs)) of 1.30 (1.14-1.49) for all-cause AMD, 1.26 (1.06-1.50) for dry AMD, and 1.31 (1.12-1.53) for wet AMD. 6-8% of the effect was mediated through HDL cholesterol. There was no interaction between weighted allele score tertiles and confounding factors on risk of AMD.

Conclusions: Amino acid changing genetic variants in *ABCA1* which were associated with higher HDL cholesterol concentrations, were also associated with higher risk of AMD, both on a weighted allele score continuously and when divided into tertiles.

SS009 / #524

Topic: *AS02 Lipids and Lipoproteins / AS02.03 HDL*

GPR146 AND HDL METABOLISM

SAAG SESSION 02: TG-RICH LIPOPROTEINS AND HDL

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Background and Aims: Epidemiological studies revealed that G-coupled protein receptor 146 (GPR146) regulates LDL-c together with HDL-c levels. While studies in mice have shown that GPR146 affects LDL-c through modulating VLDL secretion, it remains unknown how GPR146 affects HDL metabolisms, for which we hypothesize a role for scavenger receptor class B type 1 (SR-B1).

Methods: For our *in vivo* studies, we used *Gpr146-/-* and transgenic mice expressing Cas9 specifically in the liver. The latter were injected with adeno-associated virus harbouring sgRNAs targeting *Gpr146*. Mice were fed a chow diet. Plasma lipids and lipoproteins were assessed using FPLC and after PEG6000 precipitation. SR-B1 protein levels were measured by Western blotting. Primary mouse hepatocytes were used for HDL uptake and SR-B1 localization (biotinylation) studies.

Results: Compared to controls, *Gpr146-/-* mice have 20% reduced plasma HDL-c and increased hepatic SR-B1 protein levels without changes at the mRNA level. In AlbCas9 mice, loss of hepatic *Gpr146* was associated with dose-dependent increased levels of hepatic SR-B1. Primary hepatocytes of *Gpr146-/-* mice were enriched with cell-membrane SR-B1 protein and show increased uptake of HDL compared to wild-type mice.

Conclusions: This study suggests that genetic ablation of *Gpr146* affects HDL metabolism through changing post-translational modification of SR-B1. Whether these effects are mediated through changes in SR-B1 stability, or changes in proteasomal or lysosomal degradation of SR-B1 is under investigation.

SS010 / #222

Topic: *AS02 Lipids and Lipoproteins / AS02.03 HDL*

ISCHEMIC STROKE PATIENTS WITH CAROTID ATHEROSCLEROSIS SHOW DYSFUNCTIONAL HIGH-DENSITY LIPOPROTEIN PARTICLES

SAAG SESSION 02: TG-RICH LIPOPROTEINS AND HDL

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Background and Aims: Twenty percent of ischemic strokes are caused by atherosclerosis from the internal carotid artery. HDL plays a protective role against the development of atherosclerosis. We aimed to compare the composition and the function of the HDL from ischemic stroke patients with carotid atherosclerosis and from healthy controls.

Methods: Plasma was obtained from the healthy controls (n=27) and from the ischemic stroke patients with carotid atherosclerosis (n=64) at 7 days (7d) and 1 year (1y) from the stroke onset. The HDL was isolated by ultracentrifugation. Its content in lipids and apolipoproteins was assessed. The protective effects of HDL on the susceptibility of LDL to oxidation (absorbance at 280 nm) and to aggregation (450 nm) were analyzed. Finally, the anti-inflammatory effect of HDL against electronegative LDL-induced cytokine release in macrophages was evaluated.

Results: The HDL from the patients presented a lower proportion of free cholesterol and apoA-I, and a higher proportion of apoA-II and apoJ than the HDL from controls. The HDL from patients inhibited the oxidation and aggregation of LDL less than the HDL from controls and it also exerted a lesser anti-inflammatory effect. At 1y, HDL increased the free cholesterol content and the apoA-I/apoA-II ratio and decreased the apoJ content. The anti-inflammatory effect of HDL on macrophages also increased at 1y.

Conclusions: In conclusion, patients with ischemic stroke and carotid atherosclerosis showed an altered HDL composition that may lead to a less protection against the oxidation, aggregation, and inflammatory effects of LDL. However, some of these alterations are partly reverted at 1y.

SS011 / #1249

Topic: *AS02 Lipids and Lipoproteins / AS02.09 Lipid and lipoprotein metabolism*

LONG-TERM DIETARY FATS REDUCTION ATTENUATES THE INFLAMMATORY POTENTIAL OF POSTPRANDIAL LIPOPROTEINS

SAAG SESSION 02: TG-RICH LIPOPROTEINS AND HDL

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Background and Aims: Fat-enriched meals are daily consumed and are supposed to iteratively exert known acute pro-inflammatory effects. However, no data clearly affirmed if and how long-term decrease of dietary fats abrogates the inflammatory potential.

Methods: The intake of fats was assessed in the dietary habits of ten subjects (58±7y-old, 6 men) at basal visit (T0) and four weeks later (T4), following a dietary intervention to reduce fats intake. The effectiveness of the dietary intervention was checked with the Dietary Inflammation Index (DII). At T0 and at T4, the inflammatory potential of fats was tested by four-hours stimulating heterologous monocytes (from healthy donors) with the autologous Very Low-Density Lipoprotein (VLDL) of the subjects isolated by ultracentrifugation in fasting and after an oral fat load challenge (OFL) (640 Kcal/body surface; 82% from fats). Then, after six days' culture, monocytes were re-stimulated in acute with LPS or oxLDL.

Results: The daily intake of fats reduced over follow-up (61.00±4.48 to 48.75±3.09 g/day; p=0.020), together with DII (by 1.45±0.46 to 0.32±0.44; p=0.07). Out of the different leukocytes, only the increase (area under the curve AUC) of monocytes during OFL decreased from T0 to T4 (0.16±0.05 vs 0.08±0.10). Then, the over-expression of MCP1, CD11b, NFkβ, TNFα and NRLP3 in monocytes, induced by postprandial VLDL at T0, was attenuated after exposure with VLDL isolated from T4 upon re-stimulation with LPS/oxLDL.

Conclusions: Long-term dietary intervention impacts on inflammatory potential of the iterative postprandial response. We need to study the molecular aspects of these long-term effects.

Topic: *AS02 Lipids and Lipoproteins / AS02.02 TG rich lipoproteins metabolism and lipases*

NATURAL HISTORY OF FAMILIAL CHYLOMICRONAEMIA SYNDROME (FCS) COMPARED TO MULTIFACTORIAL CHYLOMICRONAEMIA SYNDROME (MCS) - INITIAL ANALYSIS FROM UNITED KINGDOM FCS REGISTRY

SAAG SESSION 02: TG-RICH LIPOPROTEINS AND HDL

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Background and Aims: FCS is a rare autosomal recessive disorder. Its natural history is not fully understood. The aim of the study is to investigate the natural history of FCS, genotype-phenotype correlation and to explore the differences between FCS and MCS.

Methods: While data collection is ongoing, we performed an initial analysis of baseline characteristics of 103 patients (FCS: 50, MCS:53) from the UK FCS database.

Results: FCS was prevalent in individuals of Asian ethnicity with history of parental consanguinity (p=<0.001 for both). *LPL* mutations were most prevalent, however its prevalence varied depending on ethnic background (Caucasians 78.9%, Asians: 51.6%). FCS patients had higher prevalence of acute pancreatitis (80% vs 54.7%, p=0.006), recurrent pancreatitis (70% vs 37.7%, p=0.001), unexplained abdominal pain (84% vs 43.4%, p=<0.001), earlier age (years) of symptom onset [19.6 (6.9 – 30.2) vs 34.5 (26.0 – 42.0), p=<0.001] and acute pancreatitis [24.8 (12) vs 32.2 (13.8), p=0.02]. BMI (kg/m^2) of FCS cohort was lower [24.6 (20.3 – 27.0) vs 28.1 (25.4 - 31.9), p= <0.001]. Obesity, clustering of metabolic features (diabetes, hypertension, obesity), and ASCVD was less prevalent in FCS (p=0.002,

0.001 & 0.09 respectively). No difference in the disease burden between LPL mutation-FCS and non-LPL mutations-FCS. Phenotype of patients with one pathogenic variant was intermediate between homozygous and no pathogenic variants.

Conclusions: FCS patients have significantly higher risk of pancreatic complications. Prevalence of diabetes, hypertension, obesity is less compared to MCS. LPL FCS and non-LPL FCS are phenotypically similar. Carriers of one pathogenic variant have an intermediate phenotype.

SS013 / #1521

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.05 Inherited dyslipidemias*

LONG-TERM EFFICACY AND SAFETY OF LOMITAPIDE IN PATIENTS WITH FAMILIAL CHYLOMICRONEMIA SYNDROME (FCS): DATA FROM THE LOCHNES STUDY

SAAG SESSION 02: TG-RICH LIPOPROTEINS AND HDL

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Background and Aims: Background Familial chylomicronemia syndrome (FCS) is a rare, severe, monogenic, recessive disorder mainly characterized by very high TG levels and high risk of acute and/or recurrent pancreatitis. Lomitapide is a microsomal triglyceride transfer protein (MTP) inhibitor approved for the treatment of homozygous familial hypercholesterolaemia. The open-label, single-arm 'LOCHNES' study of lomitapide in adult patients genetically confirmed FCS with a history of pancreatitis (EudraCT 2018-002911-80), showed that lomitapide is effective and well tolerated.

Methods: Methods Fourteen FCS patients, previously enrolled in the 'LOCHNES' study, were admitted to the Lomitapide Expanded Access Program, 2 months after the study termination and were evaluated every three months over a 2-years follow-up (median 28 months). Each patient continued lomitapide at the maximum tolerated dose as determined during the trial. Lipid profile, liver function tests, fatty liver and hepatic stiffness were evaluated.

Results: Results At the beginning of the follow-up, after 2 months discontinuation of study drug, median TG levels were 1899.5 mg/dL (237–4398 mg/dL). Median fasting TGs at the last observation were 383 mg/dL (47–1678 mg/dL; 76.9% reduction); 9 patients achieved TGs ≤750mg/dL. Adverse events were mild-to-moderate and mainly related to gastrointestinal tolerability (n=11). Over the follow up period 2 patients experienced an acute pancreatitis episode. Liver function tests ≥3x ULN were recorded in 2 subjects. Hepatic fat increased in three patients while median hepatic stiffness remained normal.

Conclusions: Conclusions Lomitapide is effective and well tolerated in reducing TGs in FCS patients with a history of pancreatitis over a 2-years follow-up.

SS014 / #513

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.02 Smooth muscle cell biology*

FOXC1 CONTROLS SMOOTH MUSCLE CELL ACTIVATION IN VASCULAR DISEASE

SAAG SESSION 03: SMOOTH MUSCLE CELLS IN ATHEROSCLEROSIS

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Background and Aims: The role of smooth muscle cells (SMCs) in atherosclerosis has been viewed mostly as plaque-stabilizing, but a more complex understanding of their plasticity has recently opened for investigation of SMCs as novel therapeutic targets. SMC loci have been identified in coronary artery disease GWAS studies and we sought to further expand on major transcription factors (TFs) that control SMC processes in human atherosclerosis.

Methods: Biobank of Karolinska Endarterectomies (BiKE) was employed, comprising plaque transcriptomics and proteomics. Genetic investigations were done in the IMPROVE cohort of high-CVD-risk individuals. Mechanistic studies were done using rat carotid artery balloon injury model and primary SMCs in vitro.

Results: Bioinformatic mapping of TF binding motifs in promotors of molecules dysregulated in plaques vs normal arteries and symptomatic vs asymptomatic plaques, identified FOXC1 as key upstream regulator. FOXC1 was downregulated in plaques and functionally linked to actin cytoskeleton binding and thyroid hormone response. By scRNAseq and immunohistochemistry, FOXC1 was expressed in SMA+ cells and co-localized with THRB. In rat intimal hyperplasia, FOXC1 was repressed during early remodelling associated with SMC activation, and negatively correlated with inflammation, extracellular matrix and cell cycle. FOXC1 again positively correlated with THRB and both were downregulated during primary rat SMC dedifferentiation in vitro. FOXC1 ChIP-seq and LC-MS/MS immunoprecipitation data confirmed its involvement in SMC cycle regulation, T3 response and cell adhesion. FOXC1 silencing led to downregulation of SMC markers and increased activation, but T3 stimulation could restore their contractile phenotype.

Conclusions: FOXC1 is a master transcriptional regulator in plaques, controlling SMC quiescence vs. activation in response to T3 hormone.

SS015 / #8

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.02 Smooth muscle cell biology*

INTERMEDIN ALLEVIATES DIABETIC VASCULAR CALCIFICATION BY INHIBITING GLUT1 THROUGH ACTIVATING THE CAMP/PKA SIGNALING PATHWAY

SAAG SESSION 03: SMOOTH MUSCLE CELLS IN ATHEROSCLEROSIS

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Background and Aims: Vascular calcification (VC) is regarded as an independent risk factor for cardiovascular events in type 2 diabetic patients. Glucose transporter 1 (GLUT1) involves VC. Intermedin/Adrenomedullin-2 (IMD/ADM2) is a cardiovascular protective peptide that can inhibit multiple disease-associated VC. However, the role and mechanism of IMD in diabetic VC remains unclear. Here, we investigated whether IMD inhibits diabetic VC by inhibiting GLUT1.

Methods: diabetic VC was induced and related index were determined

Results: It was found that plasma IMD concentration was significantly decreased in type 2 diabetic patients and in fructose-induced diabetic rats compared with that in controls. Plasma IMD content was inversely correlated with fasting blood glucose level and VC severity. IMD alleviated VC in fructose-induced diabetic rats. Deficiency of *Adm2* aggravated and *Adm2* overexpression attenuated VC in high-fat diet-induced diabetic mice. *In vitro,* IMD mitigated the high glucose-induced calcification of vascular smooth muscle cells (VSMCs). Mechanistically, IMD reduced advanced glycation end products (AGEs) content and the level of receptor for AGEs (RAGE). IMD decreased the glucose transporter 1 (GLUT1) level. The inhibitory effect of IMD on RAGE protein level was blocked by GLUT1 knockdown. GLUT1 knockdown abolished the effect of IMD on alleviating VSMC calcification. IMD receptor antagonist IMD_{17-47} and cyclic adenosine monophosphate/protein kinase A (cAMP/PKA) inhibitor H89 abolished the inhibitory effects of IMD on GLUT1 and VSMC calcification.

Conclusions: These findings revealed that IMD exerted its anti-calcification effect by inhibiting GLUT1, providing a novel therapeutic target for diabetic VC

SS016 / #313

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.02 Smooth muscle cell biology*

THE ROLE OF MIR-127-3P, MIR-367-3P, AND MIR-148A-3P FOR VASCULAR SMOOTH MUSCLE CELL SENESCENCE IN THE CONTEXT OF VASCULAR REMODELING

SAAG SESSION 03: SMOOTH MUSCLE CELLS IN ATHEROSCLEROSIS

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Background and Aims: The accumulation of senescent vascular smooth muscle cells (VSMC) in the vasculature over the lifespan contributes significantly to vascular remodeling. Severely impaired functional properties characterize senescent cells. MicroRNAs are key regulators in various pathological processes. Therefore, identifying microRNAs that contribute to vascular remodeling processes during the development of cellular senescence can result in new therapeutic approaches.

Methods: Screenings of primary vascular cells *in vitro* and *in vivo* were performed to reveal regulated microRNAs. Utilizing qRT-PCR expression levels of microRNAs were determined in human and murine VSMC. Functional effects of the microRNAs on human VSMC were assessed via transfection of pre-miR and antagomir. We verified potential targets with established effects on cellular aging on the mRNA level and the protein level.

Results: The initial screenings revealed miR-127-3p, miR-367-3p, and miR-148a-3p as highly regulated microRNAs in the setting of replicative senescence *in vitro* and in aged (20 months) C57BL6 mice *in vivo.* Senescent cells showed compared to non-senescent human and murine VSMCs alterations in expression levels of the microRNAs. Overexpression of the microRNAs *in vitro* led to an increased VSMC proliferation and total cell count. A reverse effect was shown after the knockdown. Data revealed respective alterations in target mRNA levels and protein levels of the identified targets after overexpression and downregulation of the microRNAs.

Conclusions: MiR-127-3p, miR-367-3p, and miR-148a-3p are contributing factors in VSMC function during the development of cellular senescence. Consequently, future studies should elicit the potential of the microRNAs as targets in vascular aging and remodeling *in vivo*.

SS017 / #816

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.02 Smooth muscle cell biology*

SMOOTH MUSCLE CELL-SPECIFIC TRANSLATOME PROFILING OF MOUSE ATHEROSCLEROSIS UNCOVERS SMC-DERIVED MICROENVIRONMENTAL FACTOR IN ATHEROSCLEROTIC PLAQUES

SAAG SESSION 03: SMOOTH MUSCLE CELLS IN ATHEROSCLEROSIS

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Background and Aims: Vascular smooth muscle cells (SMCs) and SMC-derived cells are a major source of plaque cells and extracellular matrix at all stages of atherosclerosis. Recent single cell sequencing studies have identified new subsets of SMCs and demonstrated disease-associated changes in gene expression. Still, single cell studies are limited by the high cost and tissue dissociation artefacts. To address these limitations, we developed a mouse model that allows cost-effective analysis of SMC-specific translatome from any tissue using Translating Ribosome Affinity Purification (TRAP-Seq).

Methods: We developed a transgenic mouse model, that expresses EGFP-tagged ribosomal protein L10a under smooth muscle cell specific aSMA promoter. The aortas of 15-month-old control and atherosclerotic Ldlr -/- ApoB100/100 mice were extracted, and further affinity purified using EGFP antibodies conjugated to magnetic beads. The extracted RNA was prepared into RNA-Seq libraries and analyzed for atherosclerosis-associated changes in the expression profiles of input RNA vs pulldown RNA.

Results: Our results demonstrate high enrichment of SMC-specific genes in the pulldown fraction, supporting the success of TRAP-Seq approach. Further composite analysis of SMC-specific genes (pulldown) and atherosclerosis-induced genes (input) highlighted the contribution of SMCs to the expression several known disease genes (e.g., Serpina3, Cemip and Lum) while allowing identification of novel genes that warrant further studies.

Conclusions: To this end, we demonstrated the association of a novel gene, Itih4, with phenotypic switching of SMCs in atherosclerosis. TRAP-Seq is a promising approach to gain in depth knowledge of the translational dynamics of SMCs in any given tissue.

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.02 Smooth muscle cell biology*

HIF PATHWAY ACTIVATION BY DAPRODUSTAT PROMOTES AORTA AND VALVE CALCIFICATION IN MICE WITH CHRONIC KIDNEY DISEASE

SAAG SESSION 03: SMOOTH MUSCLE CELLS IN ATHEROSCLEROSIS

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Background and Aims: Vascular calcification, valvular heart disease and anemia are highly prevalent in patients with chronic kidney disease (CKD). Anemia treatment with erythropoiesis stimulating agents increases the risk of major cardiovascular events in CKD patients. Activation of the hypoxia-inducible factor (HIF) pathway with prolyl hydroxylase inhibitors (PHI) has emerged as an alternative therapeutic strategy to increase erythropoiesis. Recent studies revealed that HIF pathway activation by hypoxia promotes vascular calcification. Here we investigated the effect of Daprodustat (DPD) on osteogenic transition of human aorta vascular smooth muscle cells (VSMCs) and human valve interstitial cells (VICs) *in vitro*, and on CKD-associated aortic and valve calcification *in vivo*.

Methods: We triggered calcification of VSMCs and VICs with high phosphate (Pi) and DPD. VSMCs calcification was assessed by alizarin red (AR) staining. In vivo we induced CKD in mice by adenine+high Pi diet with or without DPD treatment. Aorta and valve calcification was evaluated by OsteoSense[TM] staining. Osteogenic (alkaline phosphatase (ALP), Runx2) and hypoxia markers were evaluated by western blot.

Results: DPD induced activation of the HIF pathway and promoted high phosphate-induced osteogenic transition and calcification of both VSMCs and VICs. DPD (15 mg/kg/day) corrected hematological parameters of anemia in CKD mice, but increased aorta and valve calcification compared to the vehicle-treated CKD mice.

Conclusions: Further studies are needed to investigate whether this mechanism contributes to the occurrence of major cardiovascular events which was reported to happen in 19.5 and 25.2% of non-dialyzed and hemodialysis-dependent CKD patients on DPD treatment during the 2.5-year follow-up period.

SS019 / #773

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.05 Extracellular matrix and calcification*

INHIBITION OF PHOSPHOINOSITIDE KINASES PIKFYVE AND VPS34 REDUCES EXTRACELLULAR VESICLE-MEDIATED VASCULAR CALCIFICATION.

SAAG SESSION 03: SMOOTH MUSCLE CELLS IN ATHEROSCLEROSIS

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Background and Aims: Cardiovascular calcification is a contributor to cardiovascular disease. Recent work indicated that cellular-derived extracellular vesicles (EVs) are critical for microcalcification nucleation that might cause atherosclerotic plaque rupture. EVs originate from endomembrane organelles which are characterized by phosphoinositides. Here, we targeted phosphoinositide kinases to study the role of phosphoinositide metabolism in vascular smooth muscle cell (SMC) calcification and EV biology.

Methods: In SMCs, PIKfyve was inhibited using Apilimod or YM201636 and VPS34 using SAR405. Transcriptome and kinome analyses were performed. EVs were assessed using NTA, TEM, turbidity assay, and tissue non-specific alkaline phosphatase (TNAP) activity. Apilimod was applied in Ldlr-deficient mice fed a high-fat, high-cholesterol diet.

Results: Inhibition of PIKfyve and VPS34 reduced TNAP protein expression and activity, as well as matrix mineralization and collagen. EVs released from calcifying SMCs under PIKfyve and VPS34 inhibition exhibited reduced mineralization and aggregation potential and accumulation of the autophagosomal marker Lc3b-II. PIKfyve but not VPS34 inhibition promoted EV release. Omics data revealed a link to adipocyte-like differentiation and SMC phenotype specifying pathways. Validation supported increased expression of adipogenic markers and enhanced fatty acid uptake by PIKfyve and VPS34 inhibition. Apilimod reduced ROS and ATP production. In vivo, Apilimod increased PI3P levels and decreased vascular calcification but did not alter plaque size or collagen in atherosclerotic plaques.
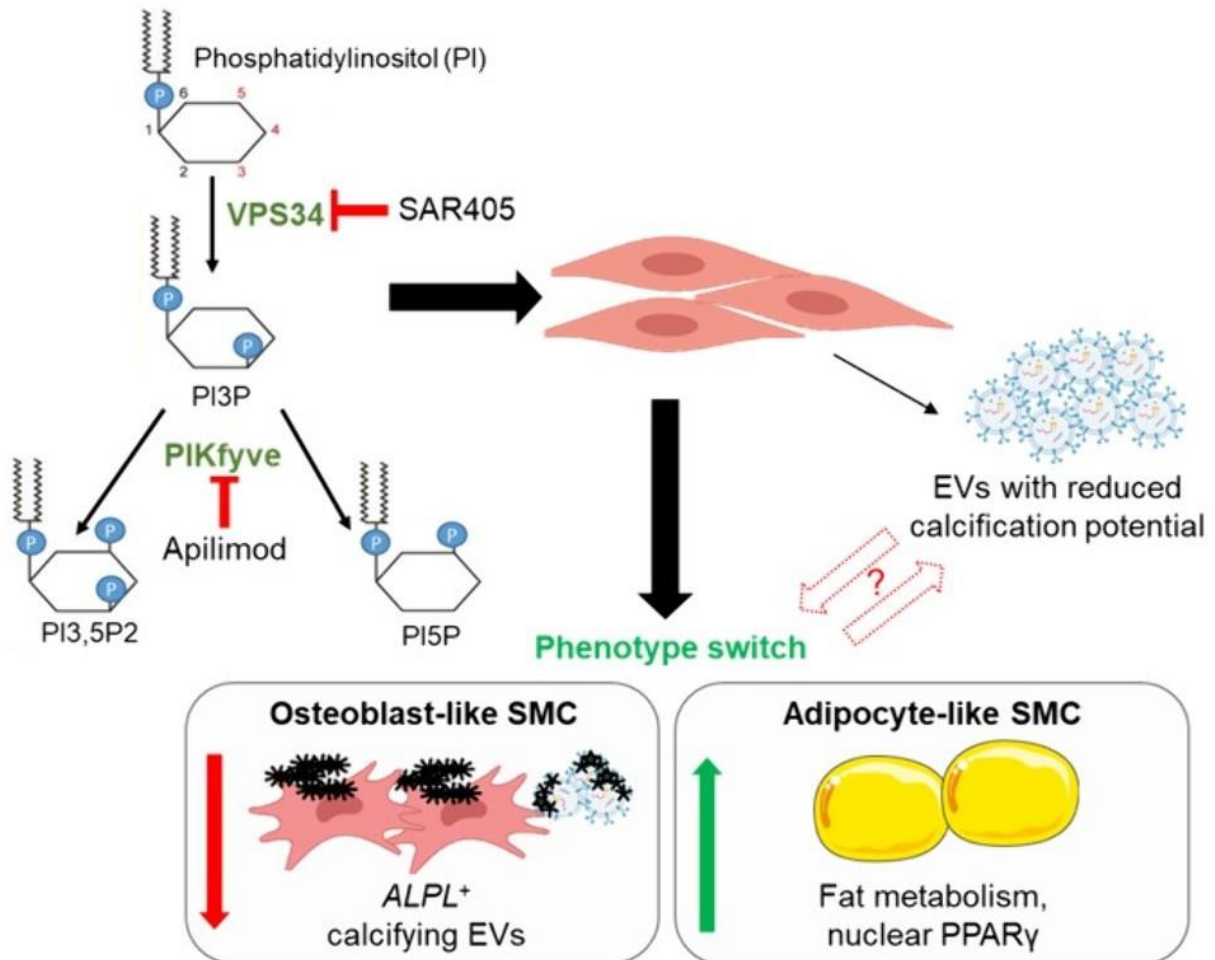
Conclusions: Targeting phosphoinositide metabolism by inhibiting PIKfyve or VPS34 promotes the release of EVs with reduced calcification potential and induces a phenotypic adaption towards adipocyte-like SMCs, causing reduced SMC calcification. Anti-calcific effects of Apilimod in vivo remain to be further

investigated.

SS020 / #635

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.06 Vascular biology of the arterial wall: Miscellaneous*

TRANSIENT EXPRESSION OF REG3B BY SMOOTH MUSCLE CELLS AFFECTS ATHEROSCLEROSIS IN MICE

SAAG SESSION 03: SMOOTH MUSCLE CELLS IN ATHEROSCLEROSIS

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Background and Aims: Inflammatory reactions within arterial walls are major hallmarks of atherosclerosis and contribute to the initiation and progression of the disease. Recent studies by our group uncovered regenerating islet-derived proteins (Reg) as central hubs for orchestrating immune-inflammatory reactions during cardiac remodeling. The present work aimed to study a potential contribution of the paralogue Reg3β for progression of atherosclerosis.

Methods: Kinetic and cell-type specific expression analysis of Reg3β was monitored in ApoE$^{-/-}$ mice at 4, 6, 8, 10, 12 and 16 weeks upon feeding a high-fat diet by means of western blotting and immunohistochemistry. Furthermore, plaque formation was evaluated in male and female mice deficient for Reg3β in SMCs with an ApoE$^{-/-}$ background by light sheet fluorescence microscopy. Identification of factors mediating enhanced Reg3β expression was monitored in *in vitro* studies.

Results: Reg3β was found to be transiently upregulated in aortic arch and serum of ApoE$^{-/-}$ mice. SMC allocated within the media layer of the aortic arch specifically expressed Reg3β. SMC-restricted *Reg3b* deficient female but not male mice displayed more pronounced plaque formation. The growth factors FGF-2 and PDGF-BB were identified as major inducers of Reg3β, whereas the Il-6 class cytokine Oncostatin M was detected to inhibit its expression in cultured SMCs.

Conclusions: Local expression and secretion of Reg3β by SMCs affects plaque formation and contributes to the progression of atherosclerosis.

SS021 / #1330

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.04 Inflammation, immunity and infection in atherosclerosis*

INJECTABLE LIPOSOMAL DHA ALLEVIATES ATHEROSCLEROSIS PROGRESSION AND ENHANCES PLAQUE STABILITY

SAAG SESSION 04: THE ROLE OF LIPIDS AND LIPOPROTEINS IN INFLAMMATION

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Background and Aims: The anti-atherosclerosis effects of docosahexaenoic acid (DHA) have been controversial likely due to variations in bioavailability after oral intake. Hereby we aim to fully exploit the potential therapeutic effects of DHA on atherosclerosis by intravenous administration of liposomal DHA.

Methods: ApoE$^{-/-}$ and LDLr$^{-/-}$ mice were fed on athero-inducing high fat diet for 12 weeks and started at week 5 receiving either control or DHA-containing liposomes via intravenous injection, twice a week for 8 weeks. Plaque area was quantified by Oil Red O staining.

Results: The liposomal formulation protected DHA against chemical degradation and increased its local concentration within atherosclerotic lesions. Mechanistically, *in vitro*, DHA liposomes were readily phagocytosed by activated macrophages, exerted potent anti-inflammatory and antioxidant effects, and inhibited foam cell formation. Upon intravenous administration *in vivo*, DHA liposomes accumulated preferentially in atherosclerotic lesional macrophages and promoted polarization of macrophages towards an anti-inflammatory M2 phenotype, resulting in attenuation of atherosclerosis in both ApoE$^{-/-}$ and Ldlr$^{-/-}$ mouse models of experimental atherosclerosis (total plaque area decreased by 35.8% in ApoE$^{-/-}$ mice, *p<0.001*; and by 22.4% in LDLr$^{-/-}$ mice, *p<0.05*). Plaque composition analysis demonstrated that liposomal DHA inhibited macrophage infiltration, reduced lipid deposition, and increased collagen content, thus improving the stability of atherosclerotic plaques against rupture. Moreover, matrix-assisted laser desorption/ionisation mass spectrometry imaging revealed that DHA liposomes partly restored the lipid composition profile of the vascular wall.

Conclusions: Intravenous administration of DHA liposomes offers a promising approach for applying DHA to stabilize atherosclerotic plaques and attenuate atherosclerosis progression, thereby preventing cardiovascular events.

SS022 / #62

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.04 Inflammation, immunity and infection in atherosclerosis*

EFFECTS OF COVID-19 SEVERITY ON THE ATHEROSCLEROTIC RISK POS-RECOVERY: RELATION BETWEEN INFLAMMATION AND LIPOPROTEINS

SAAG SESSION 04: THE ROLE OF LIPIDS AND LIPOPROTEINS IN INFLAMMATION

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Background and Aims: COVID-19 is associated with a high degree of inflammation, and an increased atherosclerotic risk. This study aims to evaluate the relationship between inflammatory markers (cytokines and chemokines) with a wide range of metabolites and lipoproteins during the acute phase of COVID-19 and its recovery period depending on the disease severity.

Methods: Lipoproteins from 226 blood serum samples were analyzed by nuclear magnetic resonance (NMR) spectroscopy. The samples were divided into five groups based on the need and length of hospitalization. Furthermore, cytokine and chemokine content of these samples was quantified by flow cytometry. Univariate analysis was performed to assess metabolome changes and cytokines and chemokines levels throughout COVID-19 infection and its recovery. Moreover, Spearman's correlation was applied to assess the relationship between cytokines and chemokines, and lipoproteins.

Results: During the period of infection, liporpteins and cytokines and chemokines levels were found significantly altered in severe and in intensive unit care (ICU) patients. Cytokines and chemokines involved in the innate immune response were impaired in ICU cases in comparison with severe cases. During recovery phase, small dende LDL and VLDL were found decreased in non-hospitalized cases , and increased in hospitalized patients. As for small dense LDL and other lipoproteins, they were negatively correlated with cytokines and chemokines in severe cases during the infection and the recovery phase, but not in ICU cases. In non-hospitalized patients, innate immune response markers were negatively linked with VLDL.

Conclusions: COVID-19 severity affects the atherosclerotic risk after infection and the relation of immune markers with lipoproteins.

SS023 / #361

AGING ACCELERATES ATHEROSCLEROSIS DEVELOPMENT IN C57BL/6 MICE UPON PCSK9 OVEREXPRESSION

SAAG SESSION 04: THE ROLE OF LIPIDS AND LIPOPROTEINS IN INFLAMMATION

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Background and Aims: Aging is a dominant risk factor for cardiovascular disease (CVD) and is associated with compositional and functional changes in our adaptive immunity, called immunosenescence. It is, however, unknown whether age-associated immune alterations promote susceptibility to develop atherosclerosis, the main underlying pathology of CVD. In this study, we aimed to investigate the impact of aging and immunosenescence on atherosclerosis development.

Methods: To this extent, young (3 months) and aged (20 months) C57Bl/6 mice received an i.v. injection of an adeno-associated virus encoding murine PCSK9 (rAAV8-D377Y-mPCSK9) and were fed a Western-type diet for 10 weeks to induce atherosclerosis.

Results: At sacrifice, cholesterol and plasma PCSK9 levels did not differ. Atherosclerosis development in the aortic root was significantly enhanced with 38% in aged compared to young mice. Concomitantly, we found increased numbers of leukocytes within the aortic arch of aged mice. Although the percentage of circulating Ly6C^{hi} monocytes was increased in aged mice, plaque macrophage content was reduced with 13% compared to young mice. While T-cell percentages were reduced in the periphery and lymphoid organs upon aging, we observed a shift towards effector (memory) subsets, including Th1-cells and Tregs, with elevated cytokine production, within the CD4$^+$ T-cell compartment. The percentage of regulatory and age-associated B-cells was increased in lymphoid organs upon aging.

Conclusions: Collectively, we show that atherosclerosis development is accelerated in aged mice upon PCSK9 overexpression, accompanied by pro-inflammatory immune alterations, including increased monocytes, effector T-cells and age-associated B-cells. Further research must elucidate if these immune changes are detrimental for increased atherosclerosis development upon aging.

SS024 / #760

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.04 Inflammation, immunity and infection in atherosclerosis*

APOB-SPECIFIC CD4+ MEMORY T CELLS CIRCULATE IN HUMAN BLOOD AND ASSOCIATE WITH CORONARY DISEASE AND THE CARDIOVASCULAR RISK PROFILE

SAAG SESSION 04: THE ROLE OF LIPIDS AND LIPOPROTEINS IN INFLAMMATION

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Background and Aims: Atherosclerosis involves auto-antibodies and auto-reactive $CD4^+$ T cells recognizing peptides from ApolipoproteinB (ApoB), the core protein of low-density lipoprotein (LDL) cholesterol. Here, we characterized ApoB-specific T cells in humans.

Methods: Peripheral blood mononuclear cells (PBMCs) from 230 patients that underwent coronary angiography were co-incubated with a pool of immunodominant peptides from human Apolipoprotein B. Reactive ApoB-specific T cells ($ApoB^+$) were defined in flow cytometry by expression of the activation marker CD40L.

Results: We found that a median of 0.69±0.1% of circulating $CD4^+$ T cells were reactive to Apolipoprotein B. We found detectable concentrations of auto-reactive $ApoB^+$ T cells in 86,44% of all healthy individuals. Compared to $ApoB^{neg.}$ T cells, we detected a higher fraction of memory T cells and a more frequent polarization into T_H1 and T_{reg} cells among $ApoB^+$ T cells. While we did not detect an association of all $ApoB^+$ T cells with clinically apparent atherosclerosis, the presence of coronary artery disease (CAD) or a high cardiovascular risk score (SCORE>5%) was associated with elevated fractions of central-memory T cells among $ApoB^+$. These effects largely grounded on strong associations with hypertension, obesity, and higher levels of lipoprotein (a). In addition, autoantibodies directed against the same peptide epitopes as used in the re-stimulation assay correlated with $ApoB^+$ $CD4^+$ memory T cells (p=0.0023).

Conclusions: Our data show for the first time that ApoB-reactive T cells exist even in healthy individuals. Cardiovascular risk factors and clinically relevant atherosclerotic disease drive memory formation and activation of $ApoB^+$ T cells.

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.04 Inflammation, immunity and infection in atherosclerosis*

IMMUNOPEPTIDOMIC ANALYSIS OF HUMAN ATHEROSCLEROSIS IDENTIFIES NOVEL APOB100-DERIVED ANTIGENIC DRIVERS OF ATHEROSCLEROSIS

SAAG SESSION 04: THE ROLE OF LIPIDS AND LIPOPROTEINS IN INFLAMMATION

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Background and Aims: Recent work suggests atherosclerosis has an auto-immune component, however the main antigenic drivers of T cell responses in this disease are still not well defined. ApoB100 might have an important role but previous attempts to identify immunogenic epitopes of ApoB100 have been based on the screening of the protein sequence and *in silico* prediction of strong HLA binders.

Methods: We used an immunopeptidomic approach to study the peptide repertoire presented by HLA-DR in human atherosclerosis plaques. Selected peptides were used to stimulate patients PBMCs, followed by analysis of CD40L expression in CD4 T cells using flow cytometry and cytokine production using multiplex ELISA.

Results: We identified ApoB100 as one of the main sources of peptides presented by HLA-DR in the plaque and selected 20 epitopes from this protein based on their predicted binding affinity for a wide range of HLA-DR isotypes. Next, we studied the presence of antigen-specific CD4 T cells against these epitopes in PBMCs of patients. Results revealed a subgroup of patients (25%) that presented significant CD4 T cell activation in response to these ApoB100 peptides. Furthermore, this T cell response correlated positively with plaque vulnerability, a parameter histologically determined by taking into account neovascularization and necrotic core, calcified, foam cell, cholesterol crystal and inflammatory cell content. Finally, the analysis of the cytokines produced by the stimulated PBMCs showed increased production of IL10 and IL17A but not IFNγ.

Conclusions: In conclusion, we identified 20 plaque derived epitopes from ApoB100 which could be used as biomarkers of disease progression and potential targets for therapeutic manipulation.

SS026 / #984

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.04 Inflammation, immunity and infection in atherosclerosis*

CHOLESTEROL CRYSTALS UPTAKE IN VASCULAR SMOOTH MUSCLE CELLS MODULATES LOCAL IMMUNE RESPONSES

SAAG SESSION 04: THE ROLE OF LIPIDS AND LIPOPROTEINS IN INFLAMMATION

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Background and Aims: Cholesterol crystals (CCs) have been identified as major contributor of atherosclerotic plaque vulnerability. The formation and accumulation of CCs at the lesion site is a hallmark of atherosclerosis. Even though studies have shown the importance of vascular smooth muscle cells (VSMCs) in the contribution towards atherosclerosis, little known about the molecular mechanism behind the uptake of CCs in VSMCs and their role in alterations of immune response.

Methods: Human aortic smooth muscle cells were cultured and treated with CC. CC uptake, CC mediated signalling pathway and protein induction were studied using flowcytometry, confocal microscopy, westernblot and Olink proteomics. Conditioned medium from CC treated smooth muscle cells was used to study neutrophil adhesion, reactive oxygen species (ROS) production and phagocytosis. Neutrophil extracellular traps formation were visualized using confocal microscopy.

Results: VSMCs and macrophages are found in the vicinity of CCs in human atherosclerotic lesions. CC uptake in VSMCs are largely through macropinocytosis and phagocytosis via PI3K-AKT dependant pathway. The uptake of CCs in VSMCs significantly altered the release of inflammatory cytokines, neutrophil adhesion, ROS production and NET formation.

Conclusions: The present study suggests that CCs uptake in VSMCs can possibly impart an inflammatory mileu in the atherosclerotic microenvironment promoting neutrophil adhesion and NET formation.

SS027 / #305

Topic: *AS02 Lipids and Lipoproteins / AS02.03 HDL*

THE PREVENTION AGAINST THE DELETERIOUS EFFECTS OF ELECTRONEGATIVE LDL ON CARDIOMYOCYTES BY HDL IS PARTLY IMPAIRED IN TYPE 2 DIABETIC PATIENTS

SAAG SESSION 04: THE ROLE OF LIPIDS AND LIPOPROTEINS IN INFLAMMATION

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Background and Aims: Type 2 diabetic (T2DM) patients have high incidence of heart failure and cardiovascular disease, which is associated with alterations in the lipid profile. Besides low levels of HDLc, these patients show dysfunctional HDL. We aimed to study the functionality of HDL from T2DM patients against the deleterious effect of electronegative LDL (LDL(-)) on cardiomyocytes.

Methods: LDL(-) was isolated by anion-exchange chromatography from a pool of plasma from healthy subjects. HDL was isolated by ultracentrifugation from T2DM patients (n=32) in poor and good glycaemic control (PC-T2DM and GC-T2DM), and healthy controls (n=32). LDL(-) was incubated with AC16 cardiomyocytes, in the presence or absence of HDL, for 24 hours. Inflammation (IL6 and MCP1 quantified by ELISA) and cytotoxicity (LDH measurement) were assessed in the supernatant. The expression of genes related to lipid metabolism and inflammation was evaluated by real-time PCR.

Results: LDL(-) induced the release of MCP1 and IL6 by AC16 cells, which was counteracted by HDL, being HDL from PC-T2DM the least protective (PC-T2DM<GC-T2DM<Controls). LDL(-) also induced the expression of *DGAT2*, *PLIN2*, *MCP1*, and *IL6*. HDL from DM2 patients inhibited this induction less than HDL from controls. The loss of the protective effect of HDL from T2DM patients could be related with its altered composition, including less apoA-I and platelet-activating factor acetylhydrolase activity, and more apoCIII and apoJ compared with HDL from controls.

Conclusions: In conclusion, LDL(-) promotes a deleterious effect on cardiomyocytes that is counteracted by HDL. HDL from patients, and particularly from PC-T2DM, partly loses this protective action.

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.05 Inherited dyslipidemias*

A GENOME-FIRST APPROACH TO IDENTIFY CARRIERS OF FAMILIAL HYPERCHOLESTEROLEMIA-CAUSING VARIANTS

SAAG SESSION 05: CLINICAL AND GENETIC IDENTIFICATION OF FH

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Background and Aims: Familial hypercholesterolemia (FH) is a highly penetrant monogenic condition affecting ~1:300 individuals. FH is associated with lifelong LDL-C elevation and increases the risk of premature ASCVD and mortality when not adequately treated. The aim of this study was to evaluate the extent to which FH is underdiagnosed in patients of a large academic medical center, using a genome-first approach.

Methods: Carriers of known FH-causing variants were identified among European or African Ancestry individuals participating in a large biobank. Genomic data were matched with data extrapolated from patients' electronic health records to determine the extent to which these patients were being diagnosed.

Results: Exome sequencing was available for 41,579 individuals. Of these, 135 were carriers of a pathogenic or likely pathogenic variant in one of the FH-causing genes. Their median (IQR) age of enrollment into the biobank was 58 years (47-66) and 51.4% were male. Only 5.9% of them were diagnosed with FH, 10.4% with hypercholesterolemia, 56.3% with other dyslipidemias, and the remaining 27.4% had no lipid-related diagnosis mentioned in their record. Carriers with a diagnosis of other dyslipidemias had higher triglycerides and lower HDL-C than carriers diagnosed with FH or hypercholesterolemia (p<0.02 for both).

Conclusions: Our findings strongly support the statement that FH is grossly underdiagnosed and highlight the need for increased awareness and earlier universal screening for a timely diagnosis, when the presence of other risk factors masking the phenotype is minimized. Appropriate strategies are needed for the communication of actionable genetic results to biobank participants.

SS029 / #191

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.05 Inherited dyslipidemias*

DIAGNOSIS OF FAMILIAL HYPERCHOLESTEROLEMIA WITH CLINICAL CRITERIA AND GENETIC TESTING IN PATIENTS SCHEDULED FOR CORONARY ANGIOGRAPHY

SAAG SESSION 05: CLINICAL AND GENETIC IDENTIFICATION OF FH

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Background and Aims: To investigate the prevalence of familial hypercholesterolemia (FH) and the diagnostic performance of clinical criteria to detect an FH-causing mutation in individuals undergoing coronary angiography.

Methods: The prevalence of FH was estimated using the Dutch Lipid Clinical Network (DLCN), US-MEDPED, Simon Broome (SB) criteria, and the "Familial Hypercholesterolaemia Case Ascertainment Tool" (FAMCAT). A custom array from Affymetrix (CARRENAL array) featuring 43,094 SNPs including 944 SNPs classified as FH mutations was used for genetic diagnostics.

Results: Among the 3267 patients (78.6% with coronary artery disease), FH was diagnosed in 2.8%, 2.2%, 3.9%, and 7.9% using the DLCN, US-MEDPED, SB criteria, and the FAMCAT. FH was genetically confirmed in 1.2% of the patients. With genetically proven FH as reference, the clinical criteria achieved an area under the curve (AUC) in the range of 0.56–0.68 (**Table; Figure**). Using only LDL cholesterol (LDL-C) corrected for statin intake, the highest AUC of 0.68 was achieved. The AUC for LDL-C was not significantly higher than those of the other criteria except for the SB criteria.
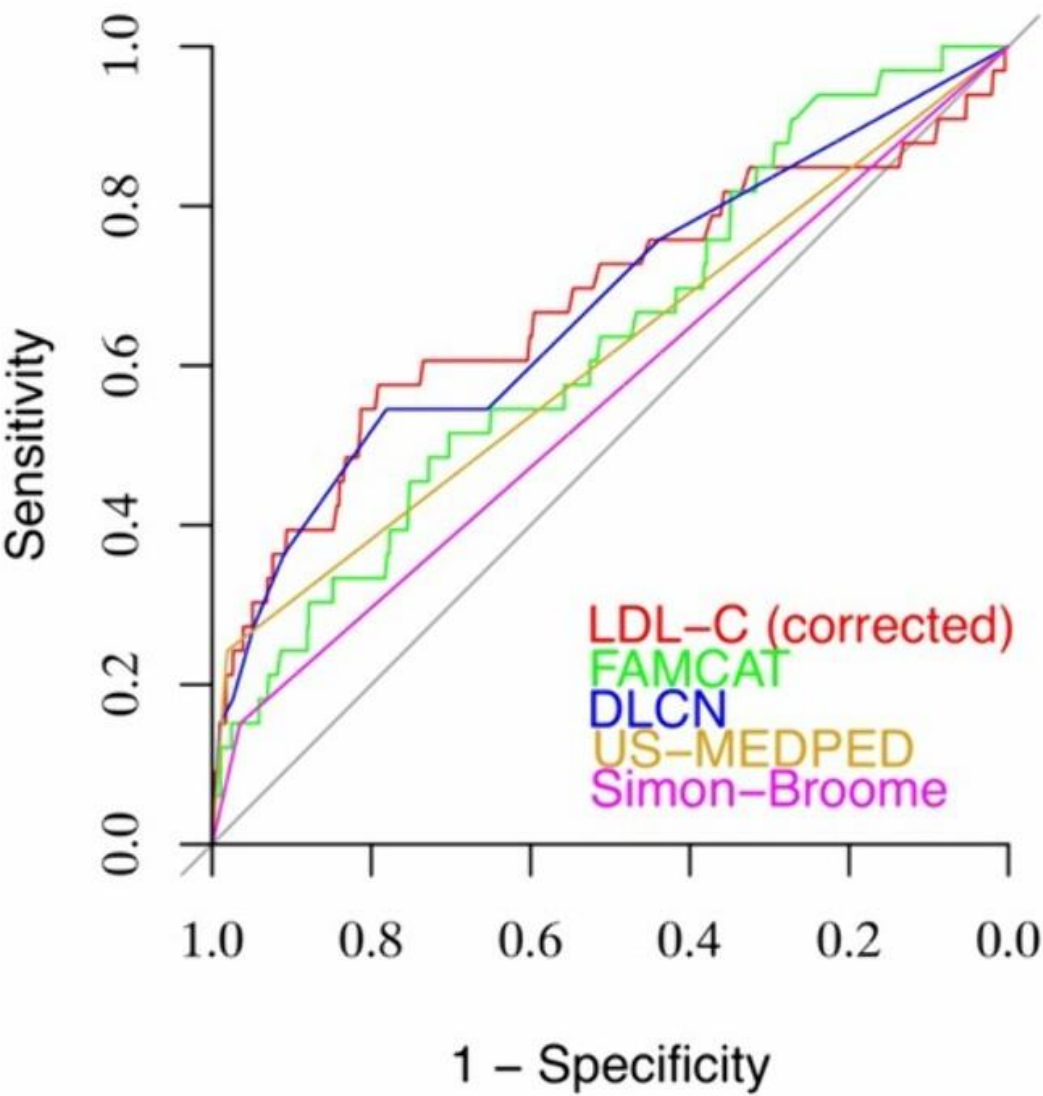
Table

	AUC	95% CI	p (vs LDL-C)
LDL-C	0.678	0.563–0.792	–
DLCN	0.675	0.571–0.780	0.952
US-MEDPED	0.612	0.538–0.686	0.186
Simon Broome	0.558	0.496–0.620	0.028
FAMCAT	0.631	0.534-0.728	0.348

Conclusions: Genetically confirmed FH is approximately 12fold more prevalent in patients undergoing coronary angiography than in the general population. The application of different clinical diagnostic scores results in substantially different rates of diagnosed FH, whereas LDL-C corrected for statin intake alone showed a similar, moderate performance to detect an FH-causing mutation. For clinical purposes, LDL-C and family history may be sufficient to establish a clinical FH diagnosis, which can be confirmed by genetic testing.

SS030 / #920

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.05 Inherited dyslipidemias*

GENETIC SPECTRUM IN LATVIAN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA: FIRST DATA FROM A WHOLE GENOME SEQUENCING STUDY

SAAG SESSION 05: CLINICAL AND GENETIC IDENTIFICATION OF FH

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Background and Aims: Familial hypercholesterolemia (FH) is an autosomal semi-dominant disease associated with pathogenic (P) or likely pathogenic (LP) variants in LDLR, APOB and PCSK9 genes. Here we report the first results of the detection rate of monogenic variants in FH patients in Latvia.

Methods: Whole genome sequencing (WGS) with 30x coverage was performed in index cases selected from the Latvian Registry of FH. The diagnosis was defined according to the Dutch Lipid Clinic Network criteria. LDLR, APOB, PCSK9, LDLRAP1, ABCG5, ABCG8, LIPA, LPA, CYP27A1, APOE genes were analyzed. Here only variants annotated as P/LP using the FH Variant Curation Expert Panel (VCEP) guidelines for LDLR and adaptations for APOB and PCSK9 are reported. The statistical analysis was performed with IBM SPSS, version 22.

Results: Among 164 patients, 66.7% were women, mean age was 52.9+/-11.4 years and mean highest documented LDL-cholesterol level was 7.5+/-1.7 mmol/L. The diagnosis was definite, probable and possible FH in 57 (34.8%), 105 (64.0%) and 2 (1.2%) patients, respectively. A total 15 P/LP variants were found in 34 patients (diagnostic yield 20.7%), 14 in LDLR and 1 in APOB gene. Additionally, 10 VUS were also detected in

LDLR.

Gene	Variant	Classification (Chora et al., 2022)	Heterozygous or homozygous	Number of cases
LDLR	g.11089559G>A c.11G>A (p.Trp4*)	Pathogenic	Heterozygous	1
LDLR	g.11105333T>A c.427T>A (p.Cys143Ser)	Likely pathogenic	Heterozygous	1
LDLR	g.11105436C>T c.530C>T (p.Ser177Leu)	Pathogenic	Heterozygous	2
LDLR	g.11105572C>A c.666C>A (p.Cys222*)	Pathogenic	Heterozygous	1
LDLR	g.11106668T>A c.798T>A (p.Asp266Glu)	Pathogenic	Heterozygous	1
LDLR	g.11107484G>A c.910G>A (p.Asp304Asn)	Pathogenic	Heterozygous	2
LDLR	g.11110697G>A c.986G>A (p.Cys329Tyr)	Pathogenic	Heterozygous	6
LDLR	g.11113313G>A c.1222G>A (p.Glu408Lys)	Likely pathogenic	Heterozygous	1
LDLR	g.11113376G>Ac. 1285G>A (p.Val429Met)	Pathogenic	Heterozygous	2
LDLR	g.11116928G>A c.1775G>A (p.Gly592Glu)	Pathogenic	Heterozygous	1
LDLR	g.11120224C>T c.1978C>T (p.Gln660*)	Likely pathogenic	Heterozygous	1
LDLR	g.11120380G>A c.1998G>A (p.Trp666*)	Pathogenic	Heterozygous	3
LDLR	g.11105531T>G c.625T>G (p.Cys209Gly)	Likely pathogenic	Heterozygous	1
LDLR	g.11113383C>T c.1292C>T (p.Ala431Val)	Likely pathogenic	Heterozygous	2
APOB	g.21006288C>T c.10580G>A (p.Arg3527Gln)	Pathogenic*	Heterozygous	9

* Classified with adaptations of the general ACMG (Richards et al., 2015) guidelines

Conclusions: Despite high clinical likelihood of FH, confirmed P/LP variants were detected only in 20.7% of patients. Future studies will extend to internal analysis of other variants based on VCEP criteria, impact of polygenic mechanisms, and WGS in a larger Latvian sample.

SS031 / #1151

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.05 Inherited dyslipidemias*

DUTCH SCORE RELEVANCE FOR ASSESSING THE PROBABILITY OF FAMILIAL HYPERCHOLESTEROLEMIA DIAGNOSIS

SAAG SESSION 05: CLINICAL AND GENETIC IDENTIFICATION OF FH

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Background and Aims: Familial hypercholesterolemia (FH) is a monogenic genetic disorder caused by mutations altering the clearance of LDL-cholesterol (LDLc) and associated with accelerated atherosclerosis. FH screening in his heterozygous form (hetFH) is based on scores combining clinics and biologicals items. In France, we usually used the « Dutch-score ». The aim of our study was to evaluate the relevance of the Dutch-score to predict the risk of being hetHF and to identify new parameters that could be added in this diagnosis.

Methods: This is a monocentric retrospective study with patients included in one lipid clinic (Marseille) involved in the French registry for HF (REFERCHOL) with a FH genetic result (positive or negative).

Results: We selected 245 patients with a hetFH mutation and 271 patients with no mutation found. There was no difference between the groups about sex, diabetes, and smoking. In contrast, patients with hetFH mutation were younger (45±17years vs 56±13years,p<0.01), with a lower BMI (25±5kg/m² vs 26±5kg/m²,p<0,01), with more extravascular cholesterol depositions (29% vs 12%,p<0,01). We also found a younger onset of personal cardiovascular event (14%vs22%,p=0,02) in the group with mutation. In mutated patients, LDLc and maximal-LDLc concentrations were higher (203±85 mg/dL and 314±16mg/dL vs 186±6mg/dL and 246±59mg/L,p<0,01) and triglyceride concentration was lower (112±75mg/dL vs 16±155mg/dL,p<0,05). About the distribution of Dutch-scores, almost half mutated patients had "definite" score versus 20% in non-mutated patients.

Conclusions: Dutch-score is a helping tool for assessing the probability of FH diagnosis. However, it could be improved by adding new variables such as age at diagnosis, BMI or triglyceride concentration.

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.05 Inherited dyslipidemias*

FAMILIAL HYPERCHOLESTEROLEMIA CASCADE TESTING UPTAKE: RESULTS FROM AN INTERIM ANALYSIS OF A PRAGMATIC TRIAL UTILIZING INNOVATIVE FAMILY COMMUNICATION STRATEGIES

SAAG SESSION 05: CLINICAL AND GENETIC IDENTIFICATION OF FH

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Background and Aims: It is recommended for relatives of individuals diagnosed with FH, probands, to have testing to determine whether they also have FH. Cascade testing can help prevent cardiovascular disease, yet initial estimates from the MyCode® Community Health Initiative (MyCode) showed 3.5% uptake by first-degree relatives (FDRs). The IMPACT-FH study aims to improve cascade testing uptake by offering innovative family communication strategies to probands in MyCode.

Methods: A prospective, pragmatic comparative effectiveness study was conducted to evaluate family communication preferences and cascade testing uptake. Probands chose to share results with relatives via informational packet, chatbot, and/or direct clinician outreach and could choose different strategies for different relatives. Relatives could also request strategies. Cascade testing uptake included completing genetic or lipid testing for FH. Chi-squared testing of interim study results are reported 15 months from trial initiation.

Results: A total of 175 probands received a genetic result for FH. The average age of probands was 56.6 (SD 17.0) and 58.9% were female. There was an average of 4.6 (792/173) living FDRs and 11.9 (2,088/175) "any living relatives" reported per proband. Packets were selected for 437 relatives, chatbots for 193 and direct clinician outreach for 51 (Table 1). To date, 10.2% (214/2,088) of "any living relatives" completed cascade testing and a higher percentage of testing occurred in FDRs (21.1% (167/792)). About 40% of probands had cascade testing completed in their family, representing an overall 21.1% uptake compared to 3.5%

(p<0.001).

Table 1. Utilization of Family Communication Strategies

Strategy[1]	Who Selected the Strategy for the Relative?			Was the Strategy Sent?				
				Yes			No	Unknown
				Who reported?				
	Proband or their Designee	Relative Themselves	Unknown[2]	Proband or their Designee	Relative Themselves	No One[3]		
Informational Packet	377	2	58	252	8	5	49	123
Chatbot	159	13	21	80	5	24	35	49

Strategy[1]	Who Selected the Strategy for the Relative?			What was the Outcome?			
	Proband or their Designee	Relative Themselves	Unknown[2]	Relative Opted Out or Requested No Contact	Relative Successfully Reached	Relative Lost to Follow Up	Relative's Clinician Lost to Follow Up[4]
Direct Clinician Outreach	40	11	0	1	43	6	1

[1]In cases where more than one strategy was selected/used for a particular relative, that relative is counted under each of the strategies
[2]Represents when a strategy was not formally "selected" but was later reported as sent
[3]Represents when a strategy was never officially reported as "sent" but was found out to be used via another mechanism (e.g., data reports)
[4]Represents when a proband selected Direct Clinician Outreach to an at-risk relative's clinician, but the clinician was unable to be reached by the study team after 3 attempts

Conclusions: IMPACT-FH study significantly improves FH cascade testing uptake compared to MyCode.

SS033 / #1258

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.05 Inherited dyslipidemias*

ADULT CASCADE SCREENING VERSUS CHILD REVERSE CASCADE SCREENING IN FAMILIAL HYPERCHOLESTEROLEMIA

SAAG SESSION 05: CLINICAL AND GENETIC IDENTIFICATION OF FH

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Background and Aims: Familial hypercholesterolemia (FH) is a common inherited lipid disorder that predisposes to cardiovascular disease (CVD). Despite most cascade screening programs are initiated by adult index cases, reverse cascade screening pediatric index cases is starting to be described. Therefore, we aimed to assess the outcome of adult cascade screening and child reverse cascade screening strategies in families from the Portuguese FH Study (PFHS).

Methods: The PFHS database was consulted, and 423 index cases genetically identified with FH (224 adults and 199 children) and their 997 relatives referred to the PFHS were analysed.

Results: From 224 adults with FH, 485 relatives were enrolled for cascade screening and 290 were identified with FH. From 199 paediatric cases with FH, 512 relatives were screened and 286 were identified with FH. Child reverse cascade screening presented a slightly higher diagnostic rate than adult cascade screening, 1.44 vs 1.29 new cases with FH *per* index case, and the age of the relatives identified was younger, 29 vs 37 years. For 94% of index children, relatives were referred (2.56 relatives *per* index), in contrast with the adult cohort whereas only 70% were referred with family-members (2.17 relatives *per* index).

Conclusions: Overall, both screening approaches constitute valuable tools to identify new cases with FH, but the child reverse cascade screening creates the opportunity for more relatives to be tested at a younger age. It is crucial to improve relatives' recruitment rate since early identification allows a correct FH diagnosis and treatment to prevent CVD.

SS034 / #1325

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.05 Inherited dyslipidemias*

GENETIC BACKGROUND OF INDIVIDUALS WITH CLINICAL DIAGNOSIS OF FH FROM THE PORTUGUESE FH STUDY COHORT

SAAG SESSION 05: CLINICAL AND GENETIC IDENTIFICATION OF FH

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Background and Aims: Familial Hypercholesterolemia (FH) is a common genetic disorder of lipid metabolism associated to increased CAD risk. Three genes are associated with FH (*LDLR*, *APOB, PCSK9)*. Variants in FH phenocopies genes (*LDLRAP1*, *APOE*, *LIPA*, *ABCG5*, *ABCG8*), LDL-C polygenic risk score (PRS) and hyper-Lp(a) can mimic the FH phenotype. In the present work we intend to unravel the genetic background in individuals with clinical diagnosis of FH.

Methods: A biochemical and genetic study was performed to 1005 patients with clinical diagnosis of FH referred to the Portuguese FH Study until December 2021. Since 2017, genetic diagnosis is performed by an NGS panel with 8 genes and 6-SNPs to determine PRS.

Results: FH was genetically confirmed in 41% of the cases. In the FH-negative cohort (N=590), 33% (N=192) present Lp(a)>50mg/dl, 17% (N=102) have high PRS, 1% (N=7) have other monogenic cause and 1% (N=6) have one pathogenic variant in *ABCG5/ABCG8*. Additionally, 5% (N=32) carry heterozygous VUS in either *LDLR*, *APOB* or *PCSK9* and 5% (N=29) carry heterozygous variants of unknown significance (VUS) in FH phenocopies genes. No identifiable cause of dyslipidemia was found in the remaining 38% patients.

Conclusions: Overall, FH was confirmed genetically in 41% of the cohort. In 50% of the FH negatives the FH phenotype can be caused by Hyper-Lp(a) or high PRS. A small part of patients has pathogenic variants in *ABCG5/8* in heterozygosity and this can be the cause of hypercholesterolemia and should be further investigated. This extended NGS panel is important to identify FH/FH-phenocopies and therefore personalize each patient's treatment.

SS035 / #1273

Topic: *AS02 Lipids and Lipoproteins / AS02.04 Lipoprotein receptors*

RESOLVING CONFLICTING LDLR VARIANTS IN CLINVAR - PROGRESS OF THE CLINGEN FAMILIAL HYPERCHOLESTEROLEMIA VARIANT CURATION EXPERT PANEL

SAAG SESSION 06: NEW PATHWAYS VIA LDL RECEPTOR

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Background and Aims: Familial hypercholesterolemia (FH) is the most common monogenic disorder of lipid metabolism. Genetic testing can confirm the clinical diagnosis, but there are currently over 3300 different variants in *LDLR* deposited in ClinVar and about ~400 had conflicting classifications of pathogenicity. Here, we present the progress of *LDLR* variant classification by the FH Variant Curation Expert Panel (VCEP), composed of 13 reviewers, 17 curators, and 12 associated labs, with our *LDLR* consensus variant classification guidelines.

Methods: Variants with conflicting classifications and other variants in the same codon (required to properly classify conflicting variants) are prioritized. Associated labs send internal variant case-level data, which is uploaded into the Variant Curation Interface (VCI) and supplemented by literature evidence. Each variant is assessed by one (very experienced) or two curators and approved by three reviewers before being officially published to ClinVar.

Results: As of December 2022, we have completed classification of 316 *LDLR* variants. Of those with prior conflicting classifications (n=165), 33% were classified as Pathogenic/Likely pathogenic (P/LP), 9% as Benign/Likely benign (B/LB), 55% as Variant of Uncertain Significance (VUS) by insufficient evidence and only 3% remained conflicting. Of the remaining 135 variants, 53% were classified as P/LP, 2% as B/LB and 45% as VUS. Until May 2023, we will evaluate 451 *LDLR* variants, 247 of them with prior conflicting classifications.

Conclusions: Ultimately, efforts of the FH VCEP hope to improve FH genetic diagnosis, which relies on accurate *LDLR* variant classification. FH VCEP's guidelines significantly decrease conflicting classifications, which will be especially helpful to the FH community.

SS036 / #198

Topic: *AS02 Lipids and Lipoproteins / AS02.04 Lipoprotein receptors*

MAPPING LDL RECEPTOR HOT SPOT PATHOGENIC RESIDUES THROUGH INTEGRATION OF PREDICTIVE SOFTWARE

SAAG SESSION 06: NEW PATHWAYS VIA LDL RECEPTOR

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Background and Aims: Familial hypercholesterolemia (FH) is an inherited metabolic disease causing the malfunction of cholesterol metabolism. In 90% of the cases, FH is caused by mutations in the *LDL receptor* (*LDLr*) gene, being missense mutations the most common. Predictive software has arisen as a powerful tool to predict the pathogenicity of LDLr variants. However, each predictive-software uses different criteria to infer substitution's pathogenicity and therefore, the results often show discrepancies. The aim of this work is integrating the most used software to predict the pathogenicity of LDLr mutations in a new predictive model and to map LDL receptor hot spot pathogenic residues.

Methods: Four predictive-software were selected: Polyphen-2, SIFT and MutationTaster (not specifically devoted to LDLr) and MLb-LDLr (specific for LDLr). Software accuracy was tested with the characterized variants annotated in ClinVar so far and, by bioinformatic and machine learning techniques we integrated all models into a more accurate one.
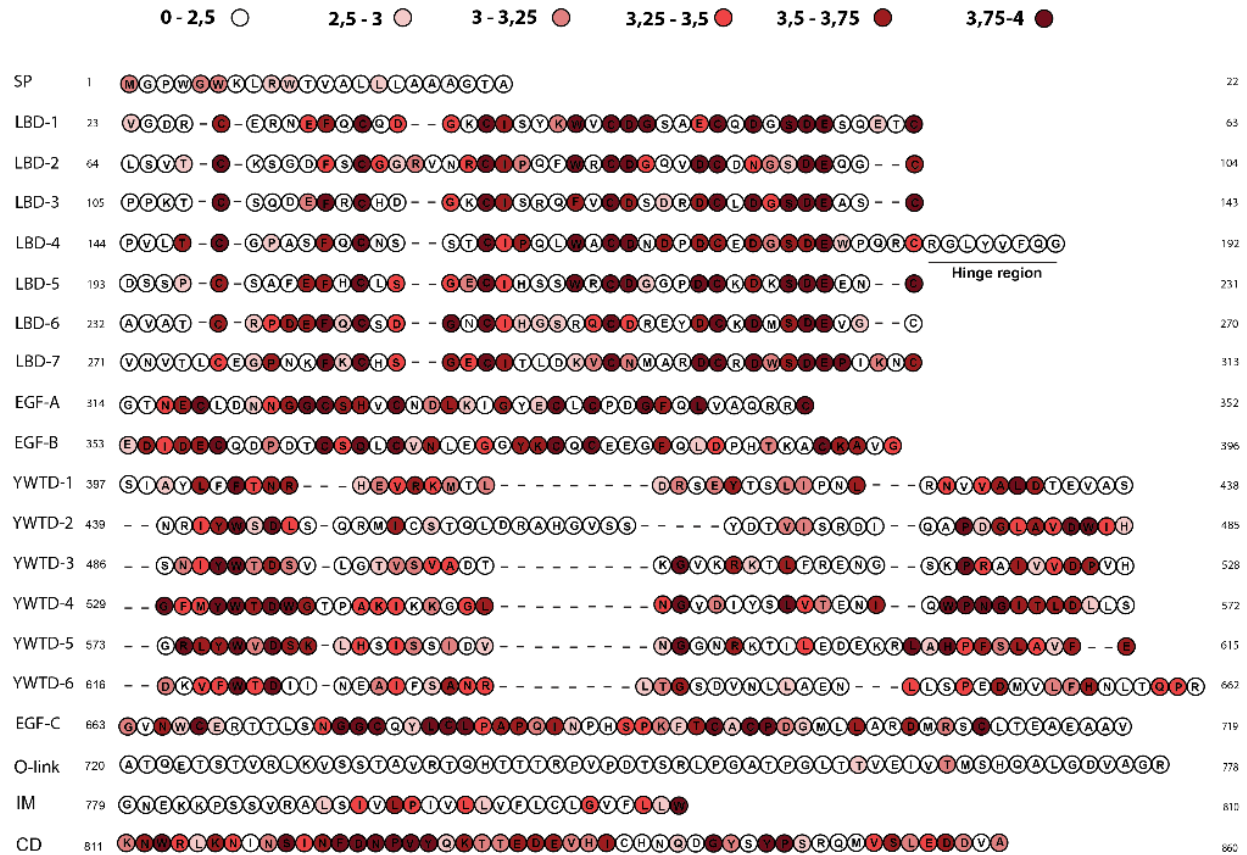
Results: The resulting optimized model presents a specificity of 96.67% and a sensitivity of 95.73%. The hot-spot map is shown in Figure1. Figure 1: Each colour represents a pathogenicity-score; the greater the number, the more potentially damaging a mutation in a residue

is.

0 - 2,5 ○   2,5 - 3 ○   3 - 3,25 ●   3,25 - 3,5 ●   3,5 - 3,75 ●   3,75-4 ●

Region	Start	Sequence	End
SP	1	MGPWGWKLRWTVALLLAAAGTA	22
LBD-1	23	VGDR-C-ERNEFQCQD--GKCISYKWVCDGSAECQDGSDESQETC	63
LBD-2	64	LSVT-C-KSGDFSCGGRVNRCIPQFWRCDGQVDCDNGSDEQG--C	104
LBD-3	105	PPKT-C-SQDEFRCHD--GKCISRQFVCDSDRDCLDGSDEAS--C	143
LBD-4	144	PVLT-C-GPASFQCNS--STCIPQLWACDNDPDCEDGSDEWPQRCRGLYVFQG	192
LBD-5	193	DSSP-C-SAFEFHCLS--GECIHSSWRCDGGPDCKDKSDEEN--C	231
LBD-6	232	AVAT-C-RPDEFQCSD--GNCIHGSRQCDREYPDCKDMSDEVG--C	270
LBD-7	271	VNVTLCEGPNKFKCHS--GECITLDKVCNMARDCRDWSDEPIKNC	313
EGF-A	314	GTNECLDNNGGCSHVCNDLKIGYECLCPDGFQLVAGRRC	352
EGF-B	353	EDIDECQDPDTCSQLCVNLEGGYKCQCEEGFQLDPHTKACKAVG	396
YWTD-1	397	SIAYLFFTNR---HEVRKMTL--------DRSEYTSLIPNL---RNVVALDTEVAS	438
YWTD-2	439	--NRIYWSDLS-QRMICSTQLDRAHGVSS----YDTVISRDI--QAPDGLAVDWIH	485
YWTD-3	486	--SNIYWTDSV-LGTVSVADT--------KCVKRKTLFRENG--SKPRAIVVDPVH	528
YWTD-4	529	--CFMYWTDWGTPAKIKKGGL--------NCYDIYSLVTENI--QCPNGITLDLLS	572
YWTD-5	573	--GRLYWVDSK-LHSISSIDV--------NGGNRKTILEDEKRLAHPFSLAVF--E	615
YWTD-6	616	--DKVFWTDII-NEAIFSANR-------LTGSDVNLLAEN---LLSPEDMVLFHNLTQPR	662
EGF-C	663	GVNWCERTTLSNGGCQYLCLPAPQINPHSPKFTCACPDGMLLARDMRSCLTEAEAAV	719
O-link	720	ATQETSTVRLKVSSTAVRTQHTTTRPVPDTSRLPGATPGLTTVEIVTMSHQALGDVAGR	778
IM	779	GNEKKPSSVRALSIVLPIVLLVFLCLGVFLLW	810
CD	811	KNWRLKNINSINFDNPVYQKTTEDEVHICHNQDGYSYPSRQMVSLEDDVA	860

(LBD-4 / LBD-5 boundary labeled: Hinge region)

Conclusions: The results of this work provide a powerful tool to classify LDLr pathogenic variants and also contribute to decipher pathogenic hot spots within the receptor. This study clearly shows that combination of several predictive software results in an more accurate prediction to make help clinicians in FH diagnosis.

SS037 / #528

Topic: *AS02 Lipids and Lipoproteins / AS02.04 Lipoprotein receptors*

A DIFFERENTIAL PROTEOMIC ANALYSIS ON HUH7 HUMAN HEPATOCARCINOMA CELL LINE NATURALLY OVEREXPRESSING OR HYPOEXPRESSING LDLR: NEW PROTEINS/PATHWAYS INVOLVED IN CHOLESTEROL AND TRIGLYCERIDES METABOLISM

SAAG SESSION 06: NEW PATHWAYS VIA LDL RECEPTOR

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Background and Aims: LDL receptor (LDLR), chiefly expressed in liver, is the main regulator of LDL plasma levels. The aim of this study is to identify differentially expressed proteins (DEPs) involved in LDLR modulation and/or in chol/TG metabolism.

Methods: HuH7 human hepatocarcinoma cell line was incubated with fluorescently labeled LDLs and sorted in two subpopulations naturally expressing high and low LDLR.

Results: The proteomic analysis on HuH7 High and Low pointed out an enrichment in 7 upregulated and 5 downregulated DEPs. Among the upregulated DEPs, CES1(carboxylestherase 1) is of greatest interest because it is highly expressed in liver and involved in TG metabolism and in the protection against hepatic steatosis. Among downregulated proteins, ACOT7 (acyl-CoA thioesterase 7), who hydrolyses acyl-CoA thioesters into free-fatty acids (FFA). Interestingly, the analysis on up- and down-regulated pathways unveiled the modulation of the FXR/RXR axis, thus leading to an important modulation in cholesterol metabolism (CYP7A1), biosynthesis (CYP51A1, FDFT1), transport (ABCA1, ABCG1-5-8), efflux (APOA4, CD36), lipoprotein synthesis (LPL, CETP, PLTP) and lipogenesis (SREBP-1c, FASN, SCD1, ACACA, MLXIPL).

Conclusions: The proteomic analysis shed light on several up- and down-regulated DEPs between HuH7 with High and Low LDLR expression. The Gene Ontology functional analysis revealed that CES1 and ACOT7 are annotated as involved in "lipid metabolism" and "fatty acid metabolism", and the Ingenuity pathway analysis confirmed the modulation of pathways involved in these processes. Therefore, it would be of great interest to further investigate their potential contribution into the hepatic tissue in relation to life-threatening pathologies such as familial hypercholesterolemia.

SS038 / #344

Topic: *AS02 Lipids and Lipoproteins / AS02.04 Lipoprotein receptors*

FUNCTIONAL ANALYSIS OF LDLR VARIANTS USING AUTOMATED SYSTEMS TO IMPROVE RARE-VARIANT ASSOCIATION STUDIES AND RISK ASSESSMENT IN HYPERCHOLESTEROLEMIA.

SAAG SESSION 06: NEW PATHWAYS VIA LDL RECEPTOR

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Background and Aims: Lack of functional information for most low-density lipoprotein receptor (LDLR) mutations limits the use of genetic tools for early diagnosis of familial hypercholesterolemia (FH) and risk assessment in cardiovascular disease (CVD). The goal of this study was an in-depth functional characterization of LDLR variants at large-scale, to improve rare-variant association studies and decision-making in the treatment of FH.

Methods: We combined open-source robotics with multiplexed high-content imaging and python-based image and data analysis to establish a semi-automated analysis pipeline, enabling large-scale functional characterization of LDLR variants regarding LDL uptake, LDLR expression and subcellular localization. LDLR variants were expressed in a LDLR deficient liver cell line using CRISPR technology. The multiparametric functional data was then integrated with genetic and health data from UK Biobank and FinnGen research project.

Results: So far, we have analyzed more than 240 LDLR variants, providing more than 1400 data points for LDL uptake, LDLR localization and expression. This allowed us to group LDLR variants based on their functional activity, shedding light on more than 60 variants of unknown significance, 100 likely pathogenic and 30 likely benign LDLR variants. We utilized the functional activity groups in rare-variant association studies with lipoprotein and CVD outcome data using UK Biobank whole-exome sequencing and FinnGen genotype data. This allowed us to highlight the benefits of including in-depth functional data in genetic studies.

Conclusions: Our detailed functional analysis of LDLR variants paves the way for improved characterization of FH patients and can guide new personalized medicine approaches for lipid-lowering therapy

Topic: *AS02 Lipids and Lipoproteins / AS02.04 Lipoprotein receptors*

FUNCTIONAL ANALYSES OF LDLR GENETIC VARIANTS FOUND IN FAMILIAL HYPERCHOLESTEROLEMIC PATIENTS, USING CRISPR/CAS9

SAAG SESSION 06: NEW PATHWAYS VIA LDL RECEPTOR

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Background and Aims: Background: Familial Hypercholesterolemia (FH) is one of the main causes of cardiovascular diseases, occurring mainly as a consequence of genetic variants in *LDLR* gene, from which many variants still lacks of functional studies. Using the CRISPR/Cas9 tool allows the construction of experimental models in order to contribute to a better functional characterization of these variants. **Objectives:** The objective is to functionally evaluate the *LDLR* variants rs879254797, rs750518671 and rs5928 that lacks functional studies.

Methods: Methods: Three *LDLR* variants were selected (rs5928, rs750518671 and rs879254797) for functional studies based mainly on in-silico prediction tools. sgRNAs were constructed, inserted in PX458 plasmids, cloned and purified. The constructs were then transfected in HepG2 cells, and isolated by cell sorting. The LDLR expression assay was evaluated by flow cytometry using antibody Alexa647 conjugated mouse anti-LDLR antibody, and by Western Blotting. Dil-LDL was used to evaluate LDLR activity by flow cytometry. It was also used Oil Red O, a lipid dye, to evaluate LDLR activity in bright field

Results: Results: Functional analyses of HepG2-rs879254797 transfected cells showed difference in LDLR expression, and docking molecular analyses show that changing an Asp by a Gly at position 373 decreases the interaction distance between LDLR and ApoB. Besides, it was verified histologically that HepG2- rs879254797 and HepG2-rs750518671 showed unexpected cellular processes, like cell-cell communication, vacuolization and pseudopods.

Conclusions: Conclusion: The HepG2-rs879254797 showed less LDLR expression compared to the WT, and also showed important histological result when incubated with LDL, that could suggest a pathological process.

SS040 / #301

Topic: *AS02 Lipids and Lipoproteins / AS02.08 Cellular lipid metabolism and lipid droplets*

APOB SECRETION AND INTRACELLULAR LIPID CONTENT ARE MODULATED BY ANGPTL3 AND PCSK9 IN HEPG2 CELLS.

SAAG SESSION 06: NEW PATHWAYS VIA LDL RECEPTOR

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Background and Aims: ANGPTL3 and PCSK9 are known regulators of lipoprotein metabolism. Patients harboring homozygous loss of function mutations in the *ANGPTL3* gene show reduced levels of circulating PCSK9 and ApoB, indicating a possible coordinate regulation of these two proteins. In addition, HepG2 cells KO for *ANGPTL3* shows intracellular accumulation of ApoB, and when knocked down for PCSK9, they show an increased lipid content. Therefore, this study aims to establish whether the two proteins modulate the secretion of ApoB and lipid droplets accumulation in HepG2 cells.

Methods: To verify potential protein-protein interaction co-immunoprecipitations (Co-Ip) experiments were carried out in HepG2 cells and culture media in standard growth conditions. Similar experiments were repeated after overexpression of *ANGPTL3*, *PCSK9,* or both through transient transfection. Western blotting was performed for protein secretion analysis. We used Oil-Red O - hematoxylin staining to quantify intracellular lipid droplet accumulation.

Results: The Co-Ip in baseline growth conditions highlighted a direct interaction of PCSK9 and ANGPTL3 proteins intracellularly and in culture medium. Cells overexpressing ANGPTL3, PCSK9, or both showed a similar secretion pattern of overexpressed targets. ApoB secretion appears to be tightly dependent on ANGPTL3 and PCSK9 overexpression. Surprisingly, ANGPTL3 overexpression determines a 3-fold increase in ApoB secretion and a 15% reduction in intracellular lipid accumulation. Conversely, the overexpression of PCSK9 determines a significant reduction in intracellular lipid accumulation of about 50%.

Conclusions: ANGPTL3 and PCSK9 are transcriptionally cross-regulated. The two proteins are in close intracellular interaction, they are finely regulated, and both promote apoB secretion and regulate intracellular lipid accumulation.

SS041 / #963

Topic: *AS02 Lipids and Lipoproteins / AS02.09 Lipid and lipoprotein metabolism*

THE LOW-DENSITY LIPOPROTEIN RECEPTOR FUELS CHOLESTEROL-MTORC1 AXIS DURING CD8 T CELL ACTIVATION

SAAG SESSION 06: NEW PATHWAYS VIA LDL RECEPTOR

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Background and Aims: Activation of T lymphocytes combines functional to metabolic rewiring of cell machinery, including cholesterol homeostasis. Here we evaluated the role of LDLR, as a key regulator of cholesterol cellular uptake, on T cell biology.

Methods: Immunophenotypic characterization of T cells from WT and LDLR KO mice was performed in vitro (anti-CD3/CD28) and in vivo (ovalbumin vaccination) coupled to proteomics and WB analysis on isolated T cells. T cells from FH (familial hypercholesterolemia) patients, carrying mutations in the LDLR gene, were tested.

Results: LDLR mRNA expression increased after in vitro activation of CD8, but not CD4 T cells, suggesting a different regulation of cholesterol homeostasis between T cell subsets. Functionally, deficiency of LDLR mainly dampened CD8 vs CD4 activation as demonstrated by in vitro proliferation (-35%, $p<0.01$) and INFγ production (-39.6%,$p<0.01$), and in vivo proliferation and cytokine production (↓IFNγ $p<0.001$,↓IL13 $p<0.01$,↓perforin $p<0.05$) after ovalbumin vaccination. Addition of LDL to serum free media increased by roughly 15%($p<0.01$) CD8 proliferation in WT but not in KO and in CD4 cells. By proteomic and WB analysis we associated this phenotype to a reduced activation of mTORC1 (pmTOR -40%,$p<0.01$) and impaired lysosomal organization (reduced lysotracker and LAMP-1 expression). CD8 T cells from FH patients proliferated less (-36%,$p>0.05$) compared to sex- and age-matched controls; in addition, CD8 from FH vaccinated for seasonal influenza were tested in vitro with virus-derived peptides, showing a decreased granzyme production (-60.3%,$p<0.01$) compared to CD8 from vaccinated controls.

Conclusions: LDLR plays a critical role in regulating the immunometabolic responses in CD8 Tcells by fuelling the cholesterol-lysosome-mTORC1 axis.

SS042 / #493

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.04 Inflammation, immunity and infection in atherosclerosis*

IDENTIFICATION ON PREVENTIVE MECHANISM OF GLUCAGON-LIKE PEPTIDE-1 (GLP-1) AGONIST FOR ATHEROSCLEROSIS MODEL

SAAG SESSION 07: INFLAMMATORY MECHANISMS IN CVD

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Background and Aims: Aim: The glucagon-like peptide-1 (GLP-1) agonist, a new class of diabetic medication, reduces progression of atherosclerosis lesions. However, mechanism for the protective effect has not been clearly identified. Therefore, we sought to investigate an anti-atherosclerotic and anti-inflammatory mechanism of GLP-1 in atherosclerosis model.

Methods: A total of 25 male ApoE-/- mice were fed a high fat diet (HFD) for 8 weeks. 25 mice were classified into 3 groups: NCD = normal chow diet fed, HFD = high fat diet fed, GLP-1= high fat diet fed treated with dulaglutide for 8 weeks. At follow up, blood and aorta were harvested for metabolic parameters, histological assessment and biochemical analysis. The THP-1 cell induced cell differentiation using PMA and then inducing inflammation was induced with LPS to confirm the anti-inflammatory effect of dulaglutide.

Results: At 8 week follow up, fasting blood glucose was significantly higher in the HFD group compared to the GLP-1 group (365.27±20.46mg/dL vs. 140.82±9.91mg/dL, p<0.001). The GLP-1 group showed significantly lower NLRP3 inflammasome expression (5.40±0.41% vs. 2.04±0.41%, p<0.001) as well as inflammatory expression (4.15±0.27% vs. 2.85±0.29%, p=0.002). From in vitro analysis, using THP-1 cells revealed significantly decreased NLRP3 inflammasome in GLP-1 group compared to LPS group (1.83±0.16% vs. 3.94±0.19, p<0.001). Also inflammation marker of NF-kB, IL-1β and TNF-α significantly decreased in GLP-1 treatment group.

Conclusions: Dulaglutide attenuated atherosclerotic plaque progression and NLRP3 inflammasome activation in atherosclerosis mice model. Our results may suggest potential clinical implication for dulaglutide for the treatment of atherosclerosis progression in atherosclerosis patients.

SS043 / #1001

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.04 Inflammation, immunity and infection in atherosclerosis*

HISTONE METHYLTRANSFERASE SET7 MEDIATES THE INFLAMMATORY RESPONSE IN ATHEROSCLEROTIC APOLIPOPROTEIN E - DEFICIENT MICE

SAAG SESSION 07: INFLAMMATORY MECHANISMS IN CVD

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Background and Aims: Dysregulated histone methylation-related epigenetic pathways are associated with atherosclerosis. Hence, the precise mechanistic links between histone methylation and atheroma formation remain incompletely understood. Histone methyltransferase SET7 specifically methylates histone H3 at lysine 4 (H3K4me1) and modulates the activity of transcription factors to induce gene expression. We aimed at elucidating the potential role of SET7 in the regulation of key inflammatory molecules in atherogenesis.

Methods: Non-atherosclerotic/atherosclerotic tissue specimens derived from patients subjected to extended carotid endarterectomy, apolipoprotein E-deficient (ApoE-/-) mice, and polarized pro-inflammatory (M1)/anti-inflammatory macrophages (Mac) were investigated by fluorescence microscopy, real-time PCR and Western blot. Male ApoE-/- mice fed a normal/atherogenic diet were randomized (n=15/group) to receive 5 mg/kg (R)-PFI-2-hydrochloride, a specific SET7 inhibitor, or its vehicle for 4 weeks.

Results: Significantly up-regulated SET7 mRNA and protein levels were detected in atherosclerotic human carotid arteries, atherosclerotic aorta of ApoE-/- (HD) mice, and in M1-Mac. A robust immunostaining of SET7 protein was detected in the area of infiltrated immune cells/Mac within human and mouse atherosclerotic lesions. Pharmacological inhibition of SET7 reduced the mRNA/protein up-regulation of selected pro-inflammatory markers (MCP-1, TNFα, NOS2), markers of immune cell infiltration (CD68, CD80, CD86, TLR2, TLR4) and cell adhesion molecules (ICAM-1, VCAM-1, E-Selectin) in the atherosclerotic aorta of mice. LSD1 blockade suppressed the up-regulation of MCP-1, TNFα and NOS2 expression in M1-Mac.

Conclusions: SET7 methyltransferase mediates the up-regulation of key inflammatory markers in experimental atherosclerosis. Pharmacological targeting of SET7 could become a supportive therapeutic option in atherosclerosis. Work supported by UEFISCDI (PN-III-P4-ID-PCE-2020-1898, PN-III-P1-1.1-TE-2021-0180).

SS044 / #1248

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.04 Inflammation, immunity and infection in atherosclerosis*

DISEASE-ASSOCIATED CELL STATES IN ATHEROSCLEROSIS DEFINED BY SPATIAL TRANSCRIPTOMICS

SAAG SESSION 07: INFLAMMATORY MECHANISMS IN CVD

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Background and Aims: Atherosclerotic coronary artery disease is one of the leading causes of mortality in the developed world. Atherosclerotic lesion development encompasses endothelial dysfunction, macrophage infiltration and smooth muscle cell dedifferentiation into other cell types. Yet how these disease states situate in the lesions or interact with each other, remains unknown.

Methods: Here, we used the Molecular Cartography platform by Resolve Biosciences to confirm lesional cell states identified by single cell RNA-Seq and to interrogate their location in thoracic aorta of $LDLR^{-/-}$ $ApoB^{100/100}$ mice during atherosclerosis progression. We used bioinformatic analysis methods to define distinct cell type clusters and their spatial distribution, as well as several other properties of atherosclerotic tissues such as spatially distinct gene expression patterns and cell-cell interactions.

Results: Our results demonstrate that combining single cell RNA-seq, spot-based cell segmentation and image-based spatial transcriptomics can be used to identify specific cell types and several individual cell states, as well as cell-cell interactions in a detailed manner from atherosclerotic lesions.

Conclusions: We were able to combine image-based spatial transcriptomics, Bayesian segmentation and bioinformatic analysis methods to create a detailed description of atherosclerotic tissue. We hope this will help in building a deeper understanding of vascular biology and the disease progression of atherosclerosis.

SS045 / #14

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.04 Inflammation, immunity and infection in atherosclerosis*

MODULATION OF HOMING RECEPTORS ON MDSCS AND TREGS AUGMENTS EXISTING INFLAMMATION

SAAG SESSION 07: INFLAMMATORY MECHANISMS IN CVD

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Background and Aims: The possible role of homing pertaining to Tregs and MDSCS in the development of atherosclerosis has not been elucidated yet. Therefore, we investigated expression of homing receptors-ligand pair (CX3CR1-CX3CL1/CCL26 and CCR5-CCL5/CCL4/CCL3) in MDSCs and Tregs and endothelial cell.

Methods: A case-control study involving atherosclerosis patients with early (risk factor exposed) to advance disease (angio positive/CABG) and healthy controls was conducted (n=15 in each group). Various assays like flow cytometry, qRTPCR, Immunofluorescence microscopy and ELISA were performed to determine the expression levels of various receptors and ligands to identify phenotype.

Results: Although frequency of Tregs was reduced, homing receptor, CCR5 was upregulated in Tregs with disease severity. They exhibited elevated levels of CD80/86, TGF-β1, IL-10, IFN-γ, IL-4, IL-17F and IL-22. They also co-expressed negative (PD1, TIM3) and positive (GITR) immune check-points. Frequency of M-MDSCs were higher while PMN-MDSCS were lower in patients. Patient M-MDSCs expressed higher levels of CCR5, CD86, MMP9, CD36, and IFN. Calprotectin (S100A8/S100A9), and TGF-β1 were decreased in all the groups, while iNOS was elevated in only RF group. Co-expression of (PD1, CD86, & HMGB1) and (CD70 and GITRL) were observed. HUVECs cultured in presence of inflammatory cytokines expressed higher chemokine ligands CX3CL1, and CCL5. Tregs and M-MDSCs from healthy controls displayed similar gene expression pattern under inflammatory conditions *in vitro*. *p<0.05, **p<0.01, ***p<0.001

Conclusions: Altered phenotype of M-MDSCs and Tregs, particularly in presence of dysfunctional endothelium might affect their functionality as suppressor cells in atherosclerosis, which may be ameliorated by targeting CCR5.

SS046 / #121

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.04 Inflammation, immunity and infection in atherosclerosis*

BMP-7 ATTENUATES TLR4-NLRP3 INFLAMMASOME MEDIATED PYROPTOSIS IN VASCULAR SMOOTH MUSCLE CELLS IN ATHEROSCLEROTIC PLAQUES

SAAG SESSION 07: INFLAMMATORY MECHANISMS IN CVD

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Background and Aims: Atherosclerosis (ATH) is an inflammation-mediated disease in which cell death underlies the formation of lesions along the intima layer of vascular walls resulting in vessel narrowing, decreased blood-flow, and increased risk of lesion rupture leading to myocardial infarction and stroke. The current study was undertaken to investigate whether inflammation in ATH can induce pyroptosis in vascular smooth muscle cells (vSMC's). To study this, we established disturbed flow-induced hemodynamic injury to the vascular wall using our partial left carotid artery ligation (PLCA) model. We hypothesize that, Bone morphogenetic protein-7 (BMP-7) attenuates PLCA- induced pyroptosis in vascular SMCs at both acute (D5) and mid stages (D28) of atherosclerosis.

Methods: ApoE KO mice (10±2 weeks old) were divided into three groups: control (Sham), PLCA and PLCA+BMP-7. Blood velocity function was examined with echocardiography, and carotid arteries were collected. Pyroptosis markers were analyzed using RT-PCR, immunohistochemistry, and western blotting at both stages of atherosclerosis.

Results: A decreased trend in the plaque formation in PLCA+BMP-7 group compared with PLCA was observed. A significant increase ($p<0.05$) in pyroptosis markers TLR4, NLRP3, Caspase1, IL1β and IL-18 were observed in PLCA group compared to control in both acute and mid stage of atherosclerosis. Whereas BMP-7 treatment significantly attenuated the pyroptosis at both stages. A significant ($p<0.05$) decrease in blood velocity in the PLCA group was improved with BMP-7 treatment.

Conclusions: Our data suggests that both in acute- and mid-stages of atherosclerosis there is a significant increase in pyroptosis markers which was significantly attenuated upon BMP-7 treatment.

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.04 Inflammation, immunity and infection in atherosclerosis*

TARGETING SINGLE INFLAMMATION MARKER IL-1B DOES NOT ALTER DEVELOPMENT OF ADVANCED ATHEROSCLEROTIC PLAQUES IN HIGH-FAT DIET-FED APOE KNOCKOUT MICE

SAAG SESSION 07: INFLAMMATORY MECHANISMS IN CVD

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Background and Aims: Despite intensive research, novel anti-inflammatory therapies to treat atherosclerosis and prevent cardiovascular events remain highly sought after. Anti-inflammatory therapies targeting the inflammasomes represent a promising strategy. The most prominent inflammasome is the nucleotide-binding domain and leucine-rich repeat pyrin domain (NLRP) 3, which activates the pro-inflammatory interleukins (IL) IL-1β and IL-18. Hence, we here evaluated the potential of blocking the NLRP3 and IL-1β as late therapy intervention in advanced atherosclerosis.

Methods: High-fat diet-fed ApoE knockout mice, which underwent tandem stenosis, were used for investigating systemic and intraplaque inflammation, lipid profile and plaque stability after blocking IL-1β formation using the NLRP3 inhibitor MCC950 (0.3 mg/ml), or the IL-1β neutralizing antibody (10 mg/kg BW). Data was statistically analyzed using one-way ANOVA with Tukey correction or unpaired student's t-test.

Results: MCC950 did not alter systemic secretion of NLRP3-related pro-inflammatory interleukins (n=6-10). Furthermore, formation, composition and stability of advanced atherosclerotic plaques remained unchanged (n=6-16). In contrast, the IL-1β neutralizing antibody attenuated systemic inflammation (n=9-11, $p<0.05$). However, capturing systemic pro-inflammatory IL-1β lacked localized effects on the formation and stability of vulnerable atherosclerotic plaques.

Conclusions: Despite the expectation that an anti-inflammatory therapy has the potential to stabilize advanced vulnerable plaques, the development and stability of advanced atherosclerotic plaques was not

affected by NLPR3 inflammasome or IL-1β inhibition in our model of unstable atherosclerosis. Thus, the precise role of inflammation in plaque development needs to be further investigated, with the aim to improve our understanding of the end-stage of atherosclerosis and, most importantly, to develop novel treatment options for plaque stabilisation.

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.04 Inflammation, immunity and infection in atherosclerosis*

LOSS OF FUNCTION AND MISSENSE VARIANTS IN CCR2 AND PROTECTION AGAINST ATHEROSCLEROTIC DISEASE

SAAG SESSION 07: INFLAMMATORY MECHANISMS IN CVD

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Background and Aims: MCP-1 governs monocyte recruitment to atherosclerotic lesions. Coherent evidence from genetic analyses and large-scale epidemiological studies supports an association between circulating MCP-1 levels and risk of coronary artery disease and atherosclerotic stroke. Still, it remains unknown if pharmacologically targeting CCR2, the cognate receptor of MCP1, would offer protection against cardiovascular disease.

Methods: We analyzed whole-exome sequencing data from 171,917 participants (40-69 years at baseline) of the population-based UK Biobank study. We searched for predicted loss-of-function (LOF) or damaging missense (REVEL>0.50) variants within the *CCR2* gene that showed associations with decreases in monocyte count (p<0.05). We tested associations with risk of coronary artery disease and ischemic stroke over a mean follow-up to 14 years.

Results: A total of 31 predicted LOF/damaging missense variants were identified in the *CCR2* gene, 5 of which were associated with decreases in monocyte count. A total of 259 individuals were heterozygote carriers of these variants (0.12%). Heterozygote carriers of the predicted LOF/damaging missense *CCR2* variants were at a 48% lower lifetime risk of a combined cardiovascular endpoint (coronary artery disease, ischemic stroke, cardiovascular death, 16,918 events, OR: 0.52, 95%CI: 0.31-0.89, p=0.016) and a lower risk of myocardial infarction, ischemic stroke or cardiovascular death over follow-up (HR: 0.54, 95%CI: 0.32-0.89, p=0.015). These variants were not associated with traditional vascular risk factors including circulating LDL-cholesterol levels, blood pressure, or glycemic status.

Conclusions: LOF and damaging missense variants in the *CCR2* gene are associated with lower burden of atherosclerotic disease. In conjunction with previous evidence, our findings highlight the high translational potential of CCR2-targeting approaches for atheroprotection.

SS049 / #47

Topic: *AS02 Lipids and Lipoproteins / AS02.01 Lp(a)*

LONG-TERM LIPID APHERESIS REDUCES CARDIOVASCULAR EVENTS IN HIGH-RISK PATIENTS WITH ISOLATED LIPOPROTEIN(A) ELEVATION

SAAG SESSION 08: LP(A) IN CVD MANIFESTATIONS

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Background and Aims: Elevated lipoprotein(a) (Lp(a)) is an established risk factor for cardiovascular disease (CVD). To date, the only approved treatment to lower Lp(a) is lipoprotein apheresis (LA). Here we report our long-term experience with LA and its effectiveness in reducing CVD events in patients with isolated Lp(a) elevation.

Methods: This retrospective open-label, single-center study included 39 individuals with significant Lp(a) elevation > 60 mg/dL but low LDL-C < 55 mg/dl (corrected for Lp(a) cholesterol) who had indication for LA due to severe CVD with at least one recurrent cardiovascular event despite maximal dietetic and medical therapy. The primary end-point of this study was the incidence of any cardiovascular event (determined by medical records) before and after initiation of LA therapy.

Results: Mean LA treatment duration was 10 years (min-max: 1-25 years). Median Lp(a) was reduced from 102.5 to 26.3 mg/dL after LA (-74.3%, $p < 0.0001$, **Figure 1**). Mean corrected LDL-C was reduced from 49.9 to 24.3 mg/dL after LA (-51.3%, $p < 0.0001$). Prior LA, 139 CV events occurred (event rate 0.72 events/patient/year). During LA, 98 CV events occurred (event rate 0.23 events/patient/year; -0.49, $p = 0.0002$, **Figure 2**). The probability for cardiovascular events during LA was 72% lower (Hazard Ratio: 0.28, 95% CI: 0.16 to 0.48) compared to pre-LA.
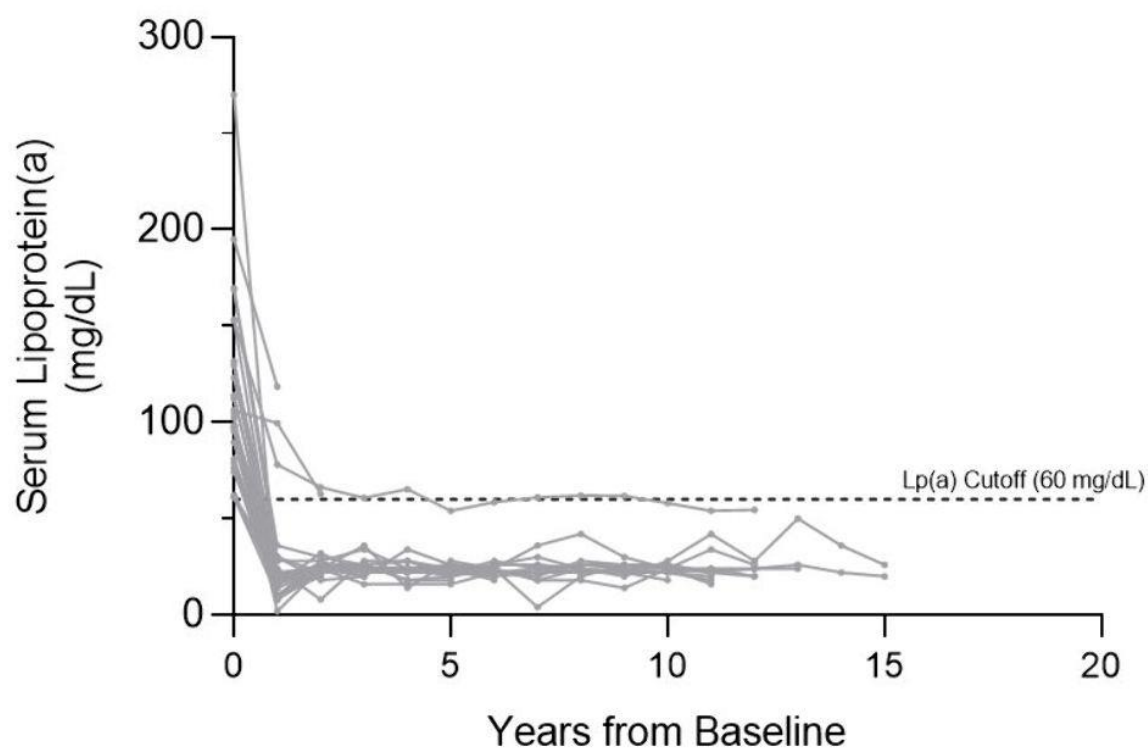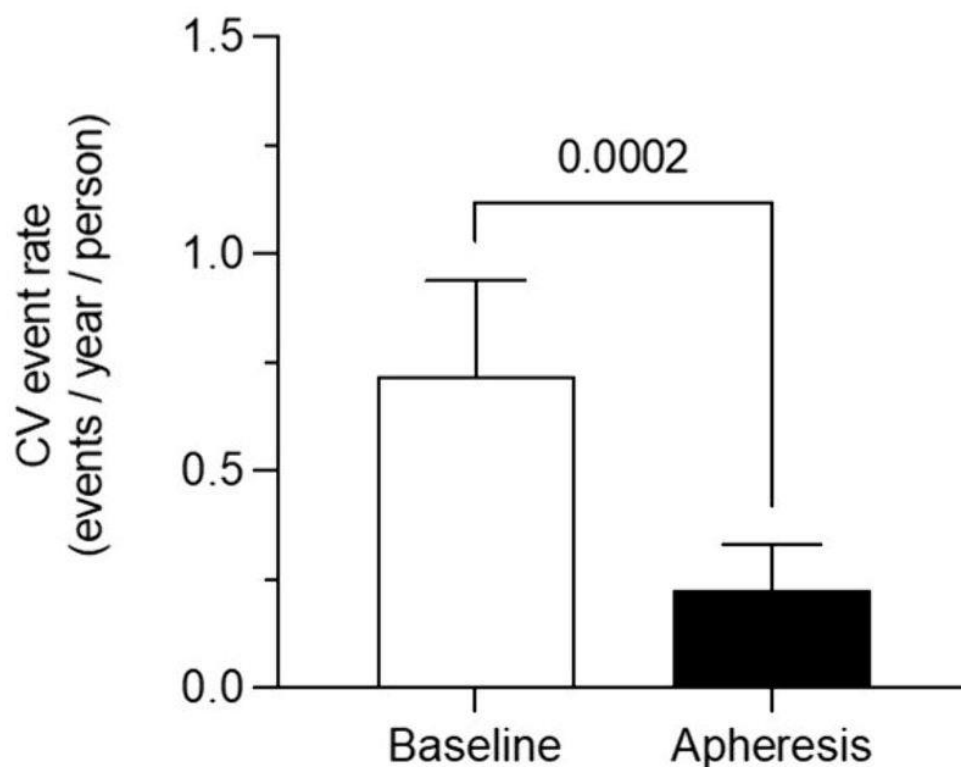
Conclusions: In a heterogenous CV high-risk cohort with isolated Lp(a) elevation, LA led to a significant reduction in Lp(a) levels and consequently reduced risk for CV events. We recommend LA for patients with isolated Lp(a) elevation who continue to experience CV events despite optimal drug and dietetic therapy.

SS050 / #365

Topic: *AS02 Lipids and Lipoproteins / AS02.01 Lp(a)*

LIPOPROTEIN(A) AND INTIMA-MEDIA THICKNESS IN CHILDREN WITH FAMILIAL HYPERCHOLESTEROLEMIA: A 20-YEAR FOLLOW-UP STUDY

SAAG SESSION 08: LP(A) IN CVD MANIFESTATIONS

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Background and Aims: Elevated lipoprotein(a) [Lp(a)] and familial hypercholesterolemia (FH) are both independent risk conditions for cardiovascular disease (CVD). Although signs of atherosclerosis can be observed in children with FH, it is unknown whether elevated Lp(a) is an additional risk factor for atherosclerosis in young FH patients. Therefore, we assessed the contribution of Lp(a) levels to arterial wall thickening (measured by carotid intima-media thickness [cIMT]) in children with FH during long-term follow-up.

Methods: We conducted a 20-year follow-up study of 214 children (aged 8-18 years) with heterozygous FH who were randomized in a pravastatin-trial between 1997-1999. We used linear mixed-effects models to evaluate the association between Lp(a) and cIMT during follow-up (baseline, and 2, 10, 20 years hereafter). We adjusted for sex, age, LDL_{cor}, statin use and BMI.

Results: In 200 children, at least one cIMT in addition to an Lp(a) level was available for the same visit. At baseline, median (IQR) Lp(a) was 18.5 (9.4-35.7) nmol/L and mean (SD) cIMT was 0.448 (0.051) mm. During follow-up, higher Lp(a) levels contributed significantly to progression of cIMT (ß-adjusted [95% CI]: 0.007 [0.001-0.013] mm per 50 nmol/L increase Lp(a), p=0.017).

Conclusions: Lp(a) levels contribute significantly to early signs of atherosclerosis in children with FH during long-term follow-up, suggesting that Lp(a) is an independent and additional risk factor in those already at increased CVD risk. Lp(a) measurement in young patients with FH is crucial to identify those at even higher CVD risk. These patients may serve as potential target population for Lp(a)-lowering therapy, when available in the future.

Topic: *AS02 Lipids and Lipoproteins / AS02.01 Lp(a)*

LIPOPROTEIN (A) AND ATHEROSCLEROTIC CARDIOVASCULAR DISEASE IN ADULTS WITH HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLAEMIA: A CROSS-SECTIONAL STUDY FROM THE EAS FAMILIAL HYPERCHOLESTEROLAEMIA STUDIES COLLABORATION (FHSC)

SAAG SESSION 08: LP(A) IN CVD MANIFESTATIONS

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Background and Aims: To investigate the distribution of lipoprotein (a) [Lp(a)] and its association with atherosclerotic cardiovascular disease (ASCVD) in adults with heterozygous familial hypercholesterolaemia (HeFH).

Methods: Cross-sectional analysis of adults with a clinical or genetic diagnosis of HeFH and available data on Lp(a) from the FH Studies Collaboration (FHSC) registry. Assessment was performed at the time of patients' inclusion in the registry. Individuals with untreated LDL-cholesterol ≥ 13 mmol/L (500 mg/dL) were excluded (likely homozygous FH). Association of Lp(a) with presence of ASCVD was assessed using logistic regression adjusting for traditional risk factors, uncorrected LDL-cholesterol and lipid-lowering treatment.

Results: 2887 HeFH adults were included (54.6% females; 62.7% genetically diagnosed; median age 48 years [IQR: 37-58]). Median Lp(a) levels were 22.7 mg/dL (IQR: 9.0-59.3); values at 80th, 90th, and 95th percentiles were 71.2, 109 and 147 mg/dL. 846 patients (29.3%) had Lp(a) >50 mg/dL. There was an independent association of log[Lp(a)] with presence of coronary artery disease (CAD) (OR: 1.77, 95% CI: 1.41-2.22), but not with stroke (OR: 1.15, 95% CI: 0.72-1.83) or peripheral artery disease (OR: 1.28, 95%

CI: 0.82-1.98). Compared with the lowest Lp(a) tertile group, subjects in the second and third tertile groups had higher odds of CAD
(Figure).

Odds Ratio for Coronary Artery Disease
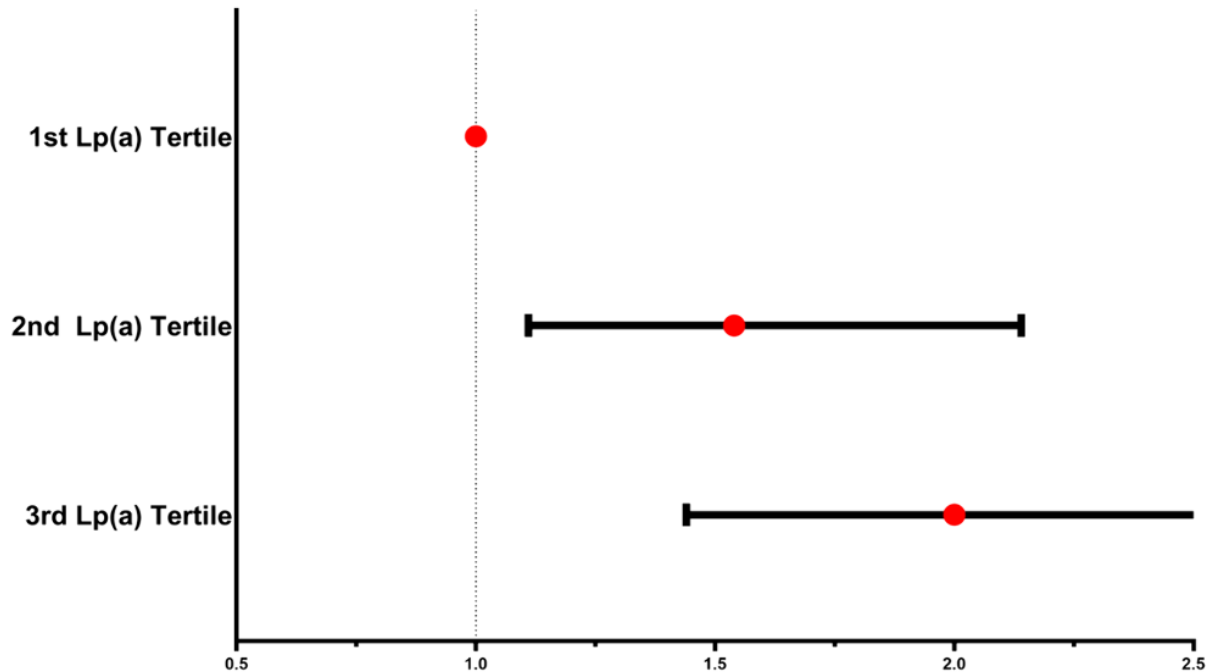
Conclusions: Almost 1 in 3 HeFH adults had Lp(a) >50 mg/dL. High Lp(a) levels were independently associated with the presence of CAD. In addition to optimal LDL-cholesterol control, Lp(a)-lowering intervention may be needed to further reduce CAD risk among HeFH individuals with high Lp(a).

SS052 / #629

Topic: *AS02 Lipids and Lipoproteins / AS02.01 Lp(a)*

GENETIC VARIABILITY OF LIPOPROTEIN(A) CONTROLS VASCULAR INFLAMMATION/REDOX SIGNALLING AND PREDICTS ADVERSE CARDIOVASCULAR OUTCOMES IN CORONARY ARTERY DISEASE

SAAG SESSION 08: LP(A) IN CVD MANIFESTATIONS

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Background and Aims: Background: Lipoprotein(a) [Lp(a)] has an established link with cardiovascular disease, however the underlying mechanisms are incompletely understood. **Aims**: We investigate the prognostic value of *LPA* genetic variants that determine Lp(a) levels, in patients with coronary artery disease.

Methods: Methods: We genotyped 880 cardiac surgery patients for 7 SNPs in *LPA* that account for >40% of Lp(a) levels. We compared subjects homozygous for ≥2 alternative *LPA* alleles (alternative group) to those without (reference group). Coronary inflammation was measured using coronary CT angiography (CCTA) by applying Perivascular Fat Attenuation Indexing (FAI-Score) in the left anterior descending (LAD), left circumflex (LCx) and right coronary arteries (RCA). The CaRi-HEART risk score was measured using the CaRi-HEART medical device. Arterial redox state was quantified from internal mammary artery (IMA) samples using lucigenin chemiluminescence and the pan-NOX inhibitor Vas2870. Patients were followed up for a median of 7.8 years.

Results: Results: Alt. patients had significantly increased: arterial NOX-derived O_2^{-} *(A)*; FAI-Scores in LAD, LCx, and RCA *(B)*; and CaRi-HEART 8-year cardiac mortality risk *(C)*. Images of CCTA FAI mapping of LADs from ref. vs alt. patients are shown (D). Alt. patients had a significantly elevated risk for cardiovascular mortality in non-diabetics *(E)* but not in diabetics (adj HR=3.87e-07, p=0.986). IMA RNAseq pathway enrichment analyses revealed a significant reduction in PI3K/Akt signalling in alt. patients *(F)*.

Conclusions: Conclusions: We demonstrate for the first time that Lp(a) leads to increased coronary inflammation in humans, which could mediate the increased risk of cardiovascular events associated with high Lp(a)

levels.

(A)

(B)

(C)

(D)

(E)

(F)

*p<0.05; **p<0.01; ***p<0.001

SS053 / #938
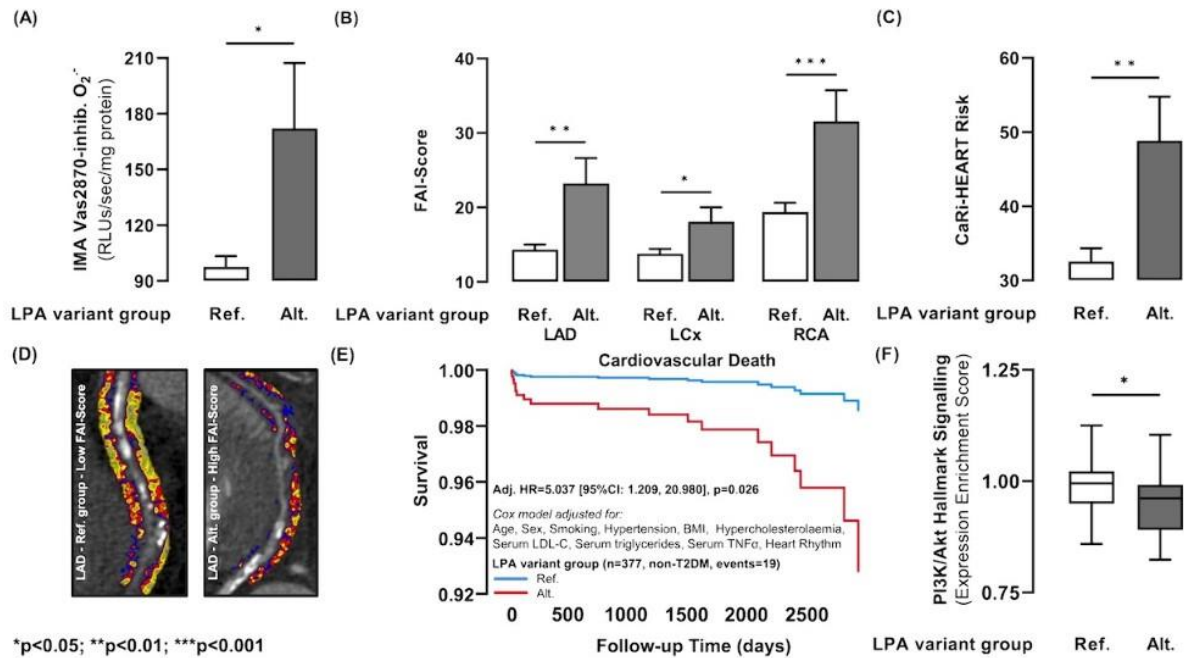
Topic: *AS02 Lipids and Lipoproteins / AS02.01 Lp(a)*

LIPOPROTEIN(A) AND THE AGE OF MANIFESTATION OF CORONARY HEART DISEASE

SAAG SESSION 08: LP(A) IN CVD MANIFESTATIONS

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Background and Aims: Elevated lipoprotein(a) [Lp(a)] is a recognized as a significant risk enhancer for premature ASCVD, but the difference in the age of manifestation of coronary heart disease (CHD) in patients with normal and elevated Lp(a) levels has not yet been established.

Methods: The study enrolled 300 patients aged 60±10 years. The CHD group included 200 patients with CHD debut before 55 years in men and before 60 years in women. The control group included 100 patients without CHD and stenotic atherosclerosis in any vascular beds. CHD risk factors and concentration of lipids, Lp(a) were determined in all patients

Results: According to the Cox proportional hazards model, the concentration of Lp(a) ≥30 mg/dL led to the development of CHD 7 years earlier than in patients with normal Lp(a) levels, regardless of gender, age, baseline levels of LDL-C and HDL-C (figure 1). The relative risk of early coronary heart disease in patients with hyperLp(a) was 1.66 (95% CI 1.22 – 2.17). According to logistic regression analysis, when gender, hypertension, obesity, type 2 diabetes mellitus and a family history of CVD are included in the model, an increase of LDL-C level by 1 mmol/L (1.69 (1.28-2.22), Lp(a) by 10 mg/dL (1.13 (1.05-1.22) as well as male gender (OR 2.78, (95% CI 1.45-5.30) were defined as independent predictors of early CHD.

Conclusions: Male gender, LDL-C and Lp(a) are predictors of early CHD, but hyperLp(a) was associated with earlier manifestation of CHD regardless of gender, age and baseline levels of LDL-C and HDL-C.

SS054 / #1342

Topic: *AS02 Lipids and Lipoproteins / AS02.01 Lp(a)*

LP(A) LEVELS RELATED IN SEVERITY AND DISABILITY OF ISCHEMIC STROKE

SAAG SESSION 08: LP(A) IN CVD MANIFESTATIONS

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Background and Aims: Lipoprotein(a) [Lp(a)] concentration is a causal risk factor for atherosclerotic cardiovascular disease.The association of Lp(a) and stroke-related neurologic deficits and stroke functional outcomes is unclear. The aim was to analyze whether high Lp(a) is associated with stroke severity and unfavorable functional outcomes.

Methods: We included 300 consecutive patients admitted for acute ischemic stroke or transient ischemic attack (TIA) who had Lp(a) determined on admission. The National Institutes of Health Stroke scale (NIHSS) was used to assess the severity of the neurological deficit and the modified Rankin scale (mRS) the degree of disability. The association between Lp(a) levels and mRS and NIHSS values was measured using Spearman correlation. Patients were divided into two groups according to low (<75nmol/L) or high (>75nmol/L) Lp(a) values.

Results: Baseline data: mean age 71.5 years (SD 11.6), 59.7% men, 23.7% smokers, 76.3% hypertension, 40% diabetes, 60.3% dyslipidemia, 21.3% chronic kidney disease, 20% previous stroke, 46.7% previous statin treatment, 41% Lp(a) >75nmol/L. A positive correlation was observed between Lp(a) values with mRS values at discharge [RhoSpearman 0.116, $p=0.045$ (CI 95%: -0.003-0.227)] and NIHSS values at the acute phase [Rho Spearman 0.125, $p=0.03$ (CI 95%: 0.009-0.238)] and at discharge [RhoSpearman 0.120, $p=0.041$ (CI 95%: 0.001-0.235)].

Conclusions: In patients with acute ischemic stroke or TIA, the elevation of Lp(a) > 75nmol/L correlates with greater neurological deficit in the acute phase and at discharge and with a worse functional prognosis at discharge.

SS055 / #651

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.08 Novel risk factors and biomarkers*

SMOKING AND DIABETES ATTENUATE BENEFICIAL EFFECTS OF PSCK9 INHIBITORS ON ARTERIAL WALL PROPERTIES IN PATIENTS WITH HIGH LIPOPROTEIN (A) LEVELS.

SAAG SESSION 08: LP(A) IN CVD MANIFESTATIONS

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Background and Aims: Elevated lipoprotein (a) (Lp(a)) and low-density lipoprotein cholesterol levels are significant residual risk factors for cardiovascular events. Treatment with protein convertase subtilisin kexin type 9 (PCSK9) inhibitors reduces the levels of both. Less is known about effects of PCSK9 inhibitors on functional and morphological properties of the arterial wall. To determine whether treatment of patients with coronary artery disease and high Lp(a) levels with alirocumab and evolocumab influences lipoprotein levels and functional (flow-mediated dilation [FMD]) andmorphological (carotid intima-media thickness [c-IMT], pulse-wave velocity [PWV]) properties of the arterial wall.

Methods: One hundred patients with coronary artery disease after myocardial infarction before 55 year and with high Lp(a) were randomised to lipid-lowering therapies without PCSK9 inhibitors (control;N=31), or with alirocumab 150 mg SC (N=35) or evolocumab 140 mg SC (N=34), every 2 weeks. All patients underwent blood sampling for biochemical analyses and ultrasound measurements for FMD,c-IMT and PWV.

Results: There were no significant changes in FMD for the control (10.7% ±6.6% to 11.1% ±4.4%, p=0.716) and alirocumab (10.7% ±5.9% to 11.2% ±5.3%, p=0.547) groups, while evolocumab promoted significant increase (11.2% ±6.8% to 14.1% ±6.6%, p<0.0001). For risk factors of current smoking and diabetes, only patients with neither showed significant improvements in FMD (p=0.003)c-IMT (p=0.027) and PWV (p=0.039).

Conclusions: These data show that for patients with coronary artery disease and high Lp(a) levels, beneficial effects of PCSK9 inhibitors on functional and morphological properties of the arterial wall can be attenuated by specific risk factors, such as smoking and diabetes.

Topic: *AS02 Lipids and Lipoproteins / AS02.01 Lp(a)*

DOES THE LIPOPROTEIN(A) GENOTYPE INFLUENCE THE DIAGNOSIS OF FAMILIAL HYPERCHOLESTEROLEMIA?

SAAG SESSION 09: LP(A): TAKE TO WORK FOR CLINICIANS

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Background and Aims: Evidence suggests that LPA genotypes associated with elevated lipoprotein(a) [Lp(a)] levels may result in a phenotype suggestive of clinical familial hypercholesterolemia (FH). This study aimed at determining the prevalence of two Lp(a) raising variants in FH individuals enrolled in the Italian LIPIGEN study.

Methods: We selected adults with a clinical diagnosis of FH. We defined FH subjects as mutation-positive (FH/M+), with a causative variant on LDLR, or mutation-negative (FH/M−). For each subject, the genetic predisposition to high Lp(a) levels was evaluated, calculating an Lp(a) genetic score by summing the number of risk-increasing alleles inherited at rs3798220 and rs10455872 variants.

Results: Overall, in the 4.6% of 1695 clinically diagnosed FH patients, the phenotype could not be explained by a monogenic or polygenic aetiology, but only by genotype associated with high Lp(a) levels. Among 765 FH/M− subjects and 930 FH/M+ patients, 21.0% and 10.2% were characterized by at least one of the variants associated with higher Lp(a), respectively. We found that FH/M− subjects had higher levels of Lp(a) than patients in the FH/M+ group (median values 41 mg/dL [9-103] vs 19 mg/dL [8-41], p-value <.0001). Overall, the adjustment of LDL-C levels based on Lp (a) concentrations reduced from to 68% to 42% the proportion of subjects with LDL-C level ≥190 mg/dL, which is one of the criteria considered in the clinical diagnosis of FH.

Conclusions: Our study supports the importance of measuring Lp(a) to appropriately perform the FH diagnosis, and to avoid unnecessary genetic tests.

SS057 / #325

Topic: *AS02 Lipids and Lipoproteins / AS02.01 Lp(a)*

MEASURING LIPOPROTEIN(A) IN MOLAR UNITS: IS IT WORTH THE FUSS?

SAAG SESSION 09: LP(A): TAKE TO WORK FOR CLINICIANS

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Background and Aims: Consensus statements advocate that lipoprotein(a) [Lp(a)] should be measured with assays using calibrators traceable to the WHO/IFCC reference material and in molar units. The aim of this study was to compare two commercially available immunoturbidimetric Lp(a) assays differing in their calibration and reporting units, and assess their clinical concordance.

Methods: The Abbott Alinity c Lp(a) assay, routinely used in our laboratory, standardised to an internal reference material giving results in mg/dL, and the Randox assay standardised against the WHO/IFCC reference material measuring levels in nmol/L were used to analyse eighty patient serum samples spanning a wide concentration range. The strength of their agreement in stratifying CVD risk according to the Lp(a)-dependent HEART UK classification was assessed by calculating Cohen's κ index. Assay-specific cutpoints in mg/dL were derived from Passing-Bablok regression analysis.

Results: Lp(a) concentrations ranged between 3.2-266.3 mg/dL with the Alinity and 3.63-605.18 nmol/L with the Randox assay. Table 1 presents the classification of patients into CVD risk grades. The assays gave discrepant results in patients with high and very high risk, with the one reporting results in mg/dL categorizing more cases into the very high risk group; notably these groups included small numbers of patients. The evaluation of the overall strength of agreement of the results showed $\kappa=0.815$ (p<0.001).

Table 1. Classification of patients into different CVD risk grades as proposed by the HEART UK consensus statement based on the Lp(a) results obtained with different assays.

Lp(a) cutpoints		Alinity c (mg/dL)	Randox (nmol/L)
nmol/L	mg/dL	N (%)	N (%)
≤32	≤14.8	18 (22.5)	19 (23.8)
>32-90	>14.8-39.1	23 (28.8)	24 (30.0)
>90-200	>39.1-85.2	26 (32.5)	26 (32.5)
>200-400	>85.2-169.0	8 (10.0)	10 (12.5)
>400	>169.0	5 (6.2)	1 (1.2)

Conclusions: The two assays demonstrated very good clinical concordance. The difference in classifying patients with high and very high Lp(a) levels did not substantially change their risk assessment, and, until targeted treatments for Lp(a) become available, is unlikely to have meaningful clinical implications.

SS058 / #477

Topic: *AS02 Lipids and Lipoproteins / AS02.01 Lp(a)*

LIPOPROTEIN(A) SERUM CONCENTRATIONS IN CHILDREN ARE INDEPENDENT OF AGE, SEX, OR BODY MASS INDEX

SAAG SESSION 09: LP(A): TAKE TO WORK FOR CLINICIANS

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Background and Aims: Lipoprotein(a) (Lp(a)) is an independent, inherited risk marker of premature atherosclerotic cardiovascular disease (ASCVD). The current ESC/EAS guidelines recommend measuring Lp(a) in every individual once in a lifetime. Data on Lp(a) serum concentrations in children are limited.

Methods: We analyzed 1107 Lp(a) measurements from 628 children aged between 5 years and 18 years enrolled in the population-based LIFE CHILD (German civilization diseases cohort study). Lp(a) was quantitated using the Cobas/Roche assay. At least 1 and up to 8 yearly follow-up visits (mean time between visits 14.3 months) were reported for 266 children.

Results: At the first visit, median Lp(a) serum concentration was 8.7 mg/dL (IQR 3.8-29.5). 24.7% of all children showed Lp(a) levels ≧ 30mg/dL. 71 children (11.3%) exhibited Lp(a) values between 30 and 50 mg/dL, while 84 children (13.4%) had values ≧ 50 mg/dL. Lp(a) concentrations did not significantly correlate with age, sex, puberty status or body mass index (BMI). There was a significant correlation with Lp(a) and higher LDL-cholesterol levels (r=0.17, p<0.0001).Repeat Lp(a) measurements per individual approximately 1 year apart were highly intercorrelated over time (mean absolute intra-individual standard deviation 3.5 ± 4.6 mg/dL).

Conclusions: In conclusion, Lp(a) serum concentrations in children between 5 and 18 years are largely independent of age, sex, puberty status or BMI. These data indicate that measurement of Lp(a) can help to identify children at increased lifetime risk for ASCVD and suggest that Lp(a) could be used for reverse family screening. Therefore, we believe that Lp(a) may be a valuable addition to screening examinations in children.

SS059 / #1242

Topic: *AS02 Lipids and Lipoproteins / AS02.01 Lp(a)*

EFFECT OF HIGH DOSE OMEGA-3 FATTY ACIDS ON ARTERIAL INFLAMMATION IN PATIENTS WITH ELEVATED LIPOPROTEIN(A): A PILOT STUDY

SAAG SESSION 09: LP(A): TAKE TO WORK FOR CLINICIANS

Qidi Ying[1], Natalie Ward[2], Dick Chan[1], Jing Pang[1], Trevor Mori[1], Carl Schultz[1,3], Girish Dwivedi[1,4,5], Roslyn Francis[1,6], Gerald Watts[1,7]
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Background and Aims: Elevated plasma lipoprotein(a) [Lp(a)] is a causal risk factor for atherosclerotic cardiovascular diseases (ASCVD), partly driven by its pro-inflammatory effects. Recent positron emission tomography/computed tomographic (PET/CT) imaging results show that subjects with high Lp(a) have increased arterial inflammation. Omega-3 fatty acids (ω-3FAs) have been reported to exhibit pleiotropic effects for primary and secondary CVD prevention. This pilot study investigated the effect of high dose ω-3FAs on arterial inflammation in patients with elevated Lp(a) and stable coronary artery disease (CAD) receiving lipid-lowering therapies.

Methods: 15 patients with elevated Lp(a) concentrations (>50 mg/dL) and stable CAD completed this 12-week open-labelled pilot study with 4 g/day ω-3FAs supplementation. Arterial inflammation was assessed by maximal standardized uptake value (SUVmax) and most diseased segment (MDSSUV) in the aorta using ^{18}F-fluorodeoxyglucose (FDG)-PET/CT imaging.

Results: High dose ω-3FAs significantly decreased plasma Lp(a) (-5%, $P<0.01$) and triglycerides (-17%, $P<0.01$). After exclusion of 3 patients who developed inflammatory conditions at follow-up, ω-3FAs supplementation was associated with a significant decrease in SUVmax (-4%, $P<0.05$), a borderline decrease in MDSSUV (-4%, $P=0.10$), and an increase in high-density lipoprotein-cholesterol (HDL-cholesterol, +8%, $P<0.001$). The changes in SUVmax and MDSSUV were positively associated with the change in Lp(a) (r=0.661 and 0.581, both $P<0.05$; Figure 1A) and inversely with HDL-cholesterol (r= -

0.805 and -0.706, both P<0.01; Figure 1B).

Figure 1. Correlations between percentage change in SUVmax and (A) Lp(a) and (B) HDL-cholesterol after ω-3FAs supplementation in patients with elevated Lp(a).
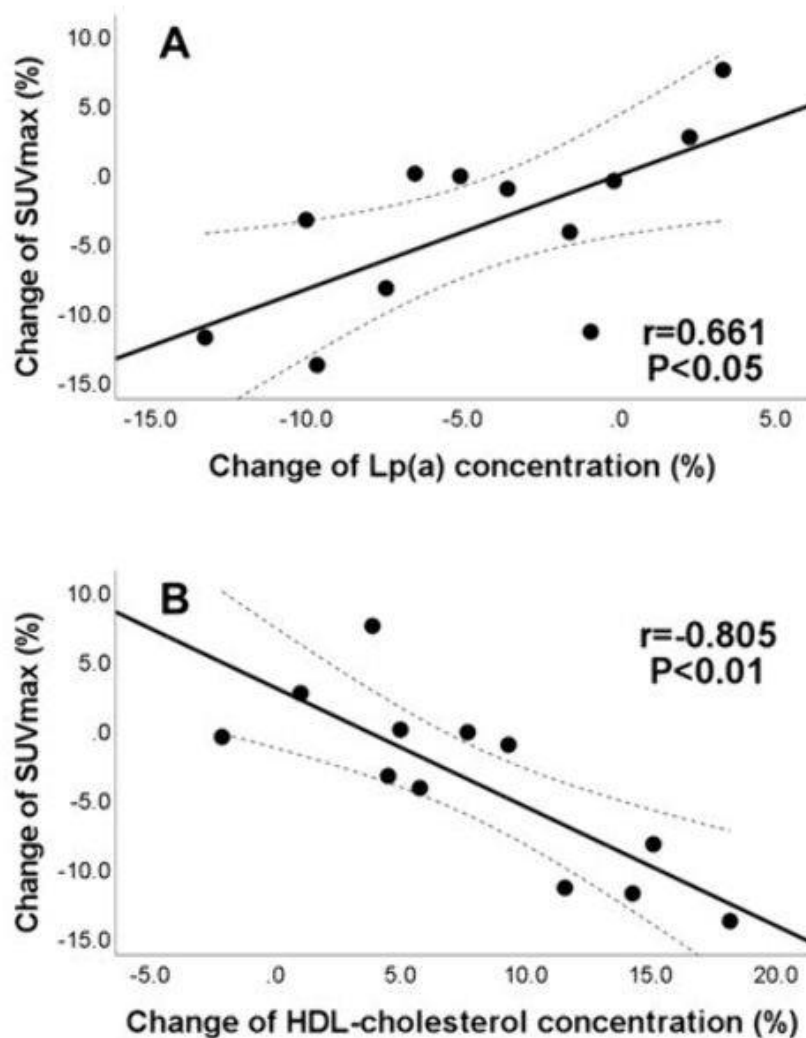
Correlations presented as mean ± 95% confidence interval.

Conclusions: High dose ω-3FAs may improve arterial inflammatory conditions in patients with elevated Lp(a) and stable CAD. These findings provide evidence for the benefits of ω-3FAs in patients with elevated Lp(a) receiving treatment with background statins.

SS060 / #300

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.01 Epidemiology of cardiovascular diseases and risk factors*

META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS EVALUATING THE EFFECT OF PCSK9 INHIBITORS ON LIPOPROTEIN(A) LEVELS

SAAG SESSION 09: LP(A): TAKE TO WORK FOR CLINICIANS

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Background and Aims: We aimed to investigate to what extent PCSK9 inhibitors (PCSK9i) affect Lp(a) level, an independent risk factor for cardiovascular diseases.

Methods: This meta-analysis was conducted according to the PRISMA guidelines. Databases were searched from inception to November 2022. Inclusion criteria were: (1) randomized controlled trials (RCTs) in adults (≥18 years), phase II, III or IV; (2) English language; (3) reporting the effects on Lp(a) levels; (4) with intervention duration more than 3 weeks. Pooled estimates were assessed by a random-effects model. Between-study heterogeneity was tested and measured by Cochrane's Q test and I^2 statistics. Subgroup analyses were conducted based on median baseline Lp(a) levels.

Results: Overall, 39,271 participants from 51 RCTs were included in our meta-analysis. An additional absolute reduction of -5.97 mg/dL (95%CI -7.31 to -4.63) of Lp(a) levels was observed in patients treated with PCSK9i compared to placebo. The percentage change was -26.35% (95%CI -28.90 to -23.80). In subgroup analysis, trials with baseline Lp(a) levels <10 mg/dL (7 RCTs) showed a statistically significant pooled reduction of -1.40 mg/dL; trials with baseline Lp(a) levels between 10 and 30 mg/dL (19 RCTs) reported a significant decreasing effect (-4.76 mg/dL), that was even higher when median baseline Lp(a) was 30 to <50 mg/dL (9 RCTs) or ≥50 mg/dL (5 RCTs): -9.73 and -10.50 mg/dL, respectively. These different absolute changes translated into not statistically different percentage drops in Lp(a) among different groups (P=0.10).

Conclusions: PCSK9i seems to lower Lp(a) levels. Further research is necessary to understand whether it translates into a clinically relevant cardiovascular benefit.

SS061 / #1035

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.01 Epidemiology of cardiovascular diseases and risk factors*

LIPOPROTEIN(A) AND OTHER CAUSES FOR ELEVATED LDL CHOLESTEROL IN CHILDREN NEGATIVE FOR FAMILIAL HYPERCHOLESTEROLEMIA – PRELIMINARY DATA

SAAG SESSION 09: LP(A): TAKE TO WORK FOR CLINICIANS

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Background and Aims: Lifelong exposure to elevated low-density lipoprotein cholesterol (LDL-C) causes an increased risk of atherosclerotic cardiovascular disease. More than 5% of children meeting phenotypic criteria for Familial hypercholesterolemia (FH) are not geneticaly confirmed. High Lipoprotein(a) (Lp(a)), obesity, anorexia, or hypothyroidism were all associated with high LDL-C.

Methods: We retrospectively analyzed data of 545 children (60.9% female, age 7.23±2.68 years) who were referred to the University children's hospital in Ljubljana by the Slovenian National screening program for FH. They also had to have available Lp(a), thyroid stimulating hormone (TSH) and body mass index (BMI) at the first visit. We divided them into three groups based on results of genetic testing for FH (positive, negative, and variant of unknown significance (VUS)); the FH negative group was further divided based on LDL-C (LDL-C<3.5 mmol/L (135 mg/dL) and LDL-C≥3.5 mmol/L).

Results: In the FH-positive group, the VUS group, and FH-negative group with LDL<3.5 mmol/L, and the FH-negative group with LDL ≥3.5 mmol/L, the respective percentages of Lp(a) above 30 mg/dL were 20.6%, 28.9%, 28.9%, and 39.5%. The difference statistically significant (p=0.009; Chi-squared test). Analyzing the percentages of BMI <5 percentile or >95 percentile and TSH levels above 4 mE/L, there were no significant differences in the incidences between the groups.

Conclusions: Elevated Lp(a) values are an important cause of elevated LDL-C values in FH-negative children. Although TSH, and low or high BMI impact LDL-C, they are not more often present in FH-negative children with higher LDL-C compared with children with normal LDL-C or children with genetically confirmed FH.

SS062 / #434

Topic: *AS02 Lipids and Lipoproteins / AS02.01 Lp(a)*

IMPROVED MUTATION SCREENING IN THE KIV-2 COPY NUMBER VARIATION OF THE LPA GENE FROM SHORT-READ WHOLE-EXOME-SEQUENCING DATA

SAAG SESSION 09: LP(A): TAKE TO WORK FOR CLINICIANS

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Background and Aims: Elevated lipoprotein(a) [Lp(a)] concentrations increase the cardiovascular disease risk up to 3-fold and are primarily controlled by the *LPA* gene. A 5.6-kb copy number variation (KIV-2 CNV) encodes up to 70% of *LPA* and contains many causal mutations. However, it is unresolved in reference databases due to its repetitive sequences and other homologous kringle domains, which result in unspecific read alignments and prevent *LPA* mutation screening. Here, we adapted our previously developed KIV-2 variant calling approach to whole-exome-sequencing (WES) data.

Methods: We used WES data of 24 samples with known KIV-2 variant patterns (Coassin, Schönherr et al., J Lipid Res 60, 2019) to develop and benchmark a novel method that extracts KIV-2 reads from aligned WES data using experimentally identified optimal regions based on the presence of KIV-2 units subtype B (KIV-2B). We applied our method to 199,119 UK Biobank (UKB) individuals.

Results: We show that isolating the correct KIV-2 reads is key and even minor shifts in the read extraction region dramatically affect the variant calling (F1 score, i.e. harmonic mean of precision and sensitivity: 5-100%), with the optimal region being KIV-2B specific. The developed KIV-2B specific approach led to mean F1-scores >90% in set-up and benchmarking samples and improved 1.4 to 2.4-fold compared to published strategies. In UKB individuals, our approach detected >700 KIV-2 mutations, including 37 nonsense and 8 splice-site mutations, and successfully reproduced and validated previous findings.

Conclusions: Our approach maximizes the utility of publicly available datasets in *LPA* screening and is customizable to other unresolved genes.

SS063 / #363

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.01 Endothelial cell function and biology*

YBX GENES REGULATE ENDOTHELIAL-TO-MESENCHYMAL TRANSITION (ENDMT)

SAAG SESSION 10: ENDOTHELIAL CELL BIOLOGY

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Background and Aims: Endothelial-to-mesenchymal transition (EndMT) was detected in vulnerable and ruptured atherosclerotic plaques. Stress stimuli induce EndMT, while the cold shock proteins YBX1 and YBX3 are regulated during cell and tissue stress. This study investigates the influence of the *YBX* family on EndMT.

Methods: YBX level were measured after induction of EndMT in human EC using qPCR and single cell sequencing and spatial transciptomics of vascular sections from Ldlr$^{-/-}$ mice after HFD. Impact of YBX knockdown during EndMT was analyzed via qPCR. YBX interacting mRNAs during EndMT were detected using RNA immunoprecipitation with subsequent qPCR.

Results: *YBX1* and *YBX3* show high baseline expression in human EC, while they are also present in the left ventricle and left atrium. *Ybx1*, *Ybx3* are increased in murine plaques compared to wt vessles ($p < 0.05$), while *YBX1* is also elevated during EndMT (n=6; $p<0.05$). Single cell sequencing revealed, that *YBX1* and *YBX3* expression is increased in EndMT-positive cell fraction (EndMT-: n=4274, EndMT+: n=2469, $p<0.05$). Interestingly, YBX1, YBX3 physically interact with the mRNA of *TGF-β1* (641 and 550 fold vs. IgG) and *TGF-β2* (3030 and 1803 fold vs. IgG). A pooled-siRNA-based knockdown of *YBX1* or *YBX3* during EndMT induction led to selective inhibition of EndMT markers. Here, the *YBX1*-knockdown resulted in a 95% reduction of *VCAN* ($p<0.05$), whereas the *YBX3*-knockdown showed a 70% reduction of *SM22* ($p<0.05$) compared to

EndMT.

Wt   Ldlr$^{-/-}$ HFD

Cluster

1
2
3
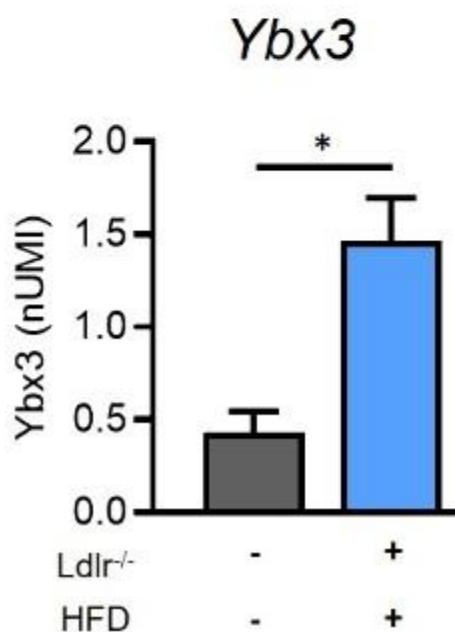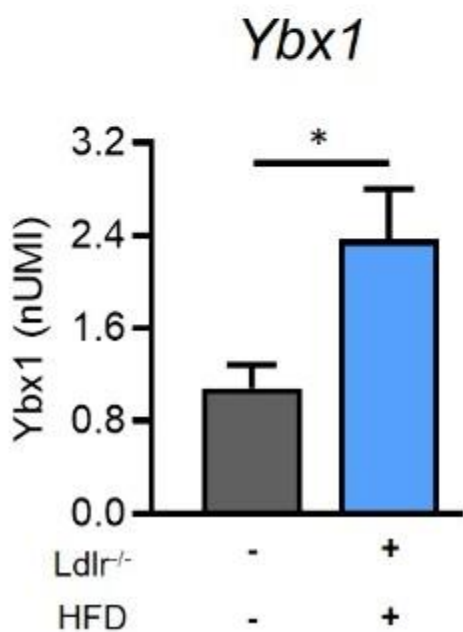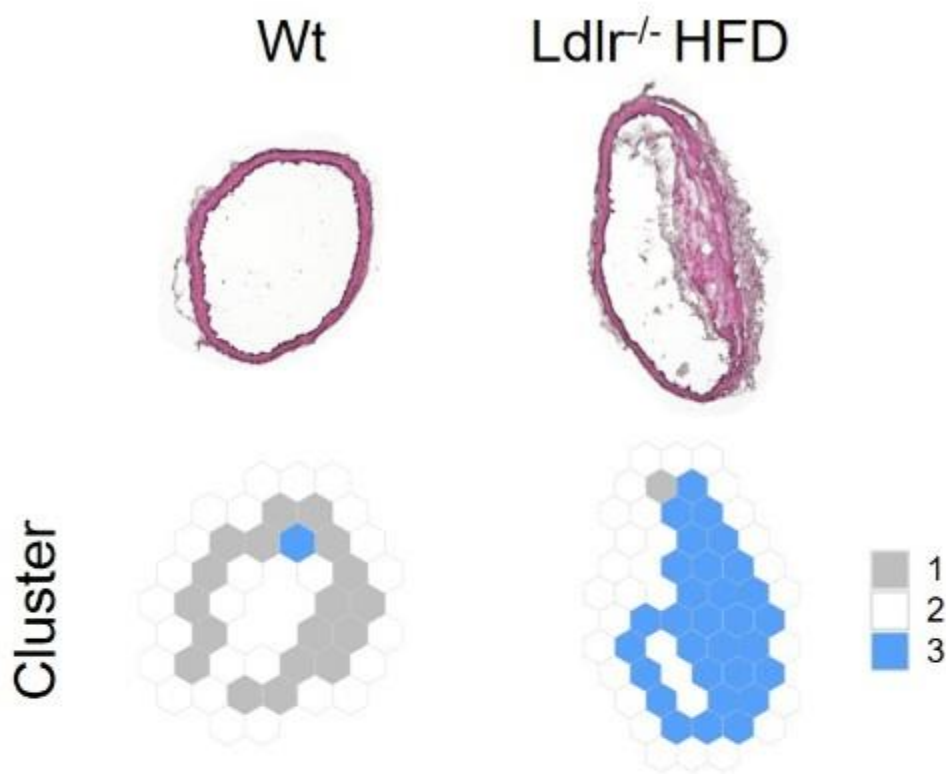
Ybx1

Ybx3

Conclusions: *YBX1* and *YBX3* are upregulated during EndMT and in atherosclerotic plaques, they interact with mRNAs of the *TGF-β* signaling pathway and modulate key components of the EndMT process.

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.01 Endothelial cell function and biology*

M6A RNA METHYLATION IS INTEGRAL FOR ENDOTHELIAL PLASTICITY

SAAG SESSION 10: ENDOTHELIAL CELL BIOLOGY

Simon Tual-Chalot[1], Giorgia Ciliberti[2], Maria Polycarpou-Schwarz[2], Michael Amponsah-Offeh[2], Francesca Bonini[2], Aikaterini Gatsiou[1], Konstantinos Stellos[2]
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Background and Aims: N6-methyladenosine (m6A) is the most abundant and conserved cotranscriptional modification in eukaryotic RNAs. m6A modification is added by the m6A writers such as METTL3/14 and WTAP. Whether m6A RNA methylation controls vascular homeostatic processes remain yet elusive.

Methods: The role of WTAP in vascular homeostasis was determined with a constitutive vascular endothelial cell (iEC)-restricted WTAP. Primary human and murine vascular EC culture assays and confocal microscopy were used to assess the EC-specific m6A effects. Molecular studies involved MeRIP-seq, stability, RNAi and gain- and loss of function mutant protein assays in primary ECs.

Results: WTAP is the major regulator of m6A levels in endothelial cells. Strikingly, EC-specific WTAP deletion in mice resulted in prenatal embryonic lethality evidencing a requirement of EC-WTAP in life. WTAP-deficient ECs exhibited impaired angiogenic potential and reduced levels of junctional molecules. Characterisation of the endothelial cell methylome transcriptome revealed that VE-cadherin, an essential gene of the endothelial-to-mesenchymal transition process, is among the most extensively methylated transcripts. VE-cadherin transcript exhibited decreased methylation levels in WTAP-deficient ECs. Validation gene expression experiments confirmed VE-cadherin among the highly downregulated targets after WTAP silencing. Mechanistically, WTAP regulates VE-cadherin mRNA stability and expression through the interaction with the RNA-binding protein Human Antigen R (HuR). HuR silencing phenocopied the reduced VE-cadherin expression levels initially observed in WTAP-deficient ECs. Conversely, overexpression of HuR was sufficient to restore the normal VE-cadherin expression levels in WTAP-deficient ECs.

Conclusions: m6A RNA methylation is essential for the maintenance of the endothelial fate in a WTAP/HuR-dependent manner.

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.01 Endothelial cell function and biology*

TRAIL-TRAIL-R LIGATION REGULATES EC-PERICYTE CROSSTALK TO GENERATE STABLE MICROVESSEL NETWORKS IN ISCHEMIA

SAAG SESSION 10: ENDOTHELIAL CELL BIOLOGY

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Background and Aims: Endothelial cell (EC)-pericyte crosstalk is essential for generating stable capillary development, a process disrupted in cardiovascular disease. Circulating TNF-related apoptosis-inducing ligand (TRAIL) levels are suppressed in peripheral artery disease (PAD) and $Trail^{-/-}$ mice have impaired angiogenesis. The contribution of EC-specific TRAIL to angiogenesis and vessel stabilisation in ischaemia is unknown.

Methods: Angiogenesis was quantified in the Matrigel plug, aortic sprouting and hindlimb ischaemia (HLI) models using $Trail^{EC-/-}$ and $Trail^{EC+/+}$ mice. scRNA-sequencing was used to identify cell clusters and interactions.

Results: EC and pericyte content in plugs from $Trail^{EC-/-}$ was ~50-60% less than $Trail^{EC+/+}$ mice, with a ~50% reduction in mRNA expression of angiogenesis and pericyte markers. Aortic segments from $Trail^{EC-/-}$ had reduced microvascular sprouts and $Trail^{-/-}$ ECs had ~50% reduction in tubule formation compared to wildtype ECs. Capillary density as a measure of EC-pericyte interaction (CD31+SMA+) was significantly reduced in $Trail^{EC-/-}$ ischaemic limb skeletal muscle, consistent with reduced blood perfusion to the lower limbs. Furthermore, vascular leak was increased in lung, liver, spleen, and colon of $Trail^{EC-/-}$ vs. $Trail^{EC+/+}$ mice. Importantly, MD5-1, a mAb that binds and activates the TRAIL receptor, restored blood perfusion and increased CD31+SMA+ capillaries in $Trail^{EC-/-}$ mice. ScRNA sequencing of $Trail^{EC-/-}$ and $Trail^{EC+/+}$ ischaemic limbs identified multiple EC clusters and differences in EC-pericyte interactions dependent on TRAIL.

Conclusions: These studies provide novel TRAIL-dependent EC-pericyte interactions mediating stable blood vessel formation in ischaemia. TRAIL could be used as a potential new therapy to stimulate stable capillary networks in PAD and other ischaemic vascular diseases.

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.01 Endothelial cell function and biology*

THE ROLE OF THE NUCLEAR RECEPTOR REV-ERBA DURING INTRAPLAQUE NEOVASCULARIZATION

SAAG SESSION 10: ENDOTHELIAL CELL BIOLOGY

Cecilia Bellengier[1], Lise Ferri[1], Meryem Tardivel[2], Antonino Bongiovanni[2], Stephane Delhaye[1], Christian Duhem[1], Quentin Thorel[1], Aurore Hebras[1], Bettina Ram[1], Mouna Amaouche[1], Mélissa Leriche[1], Alicia Mayeuf-Louchart[1], Yasmine Sebti[1], Bart Staels[1], Hélène Duez[1], Benoit Pourcet[1]
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Background and Aims: Plaque instability is now recognized as the most deleterious event during atherogenesis leading to plaque rupture. Among destabilizing processes, intraplaque neovascularization has been correlated to plaque progression and rupture. The nuclear receptor Rev-erbα displays anti-atherogenic properties by improving the lipoprotein metabolism and promoting anti-inflammatory activities. The role of Rev-erbα on intraplaque neovascularization has never been established.

Methods: To assess the role of Rev-erbα on intraplaque neovascularization, we developed an original approach by analyzing the intraplaque neovessel content of 3DISCO-whole cleared brachiocephalic artery of aged 18-month-old *Rev-erbα+/+ LDLr-/-* and *Rev-erbα-/- LDLr-/-* mice, fed a chow diet (n=5 per group). Thoracic aortas from these mice were used to analyze the whole transcriptome and to identify the endothelial cell content.

Results: Unexpectedly, we observed in whole brachiocephalic artery that intraplaque neovessels sprout from the luminal endothelium in aged *LDLr-/-* mice, a feature found in human and large animals. We also observed unperfused intraplaque neovessels budding from isolated organized endothelial cells, suggesting the presence of vasculogenic nuclei. Strikingly, *Rev-erbα* deficiency promotes the development of a more complex and immature intraplaque vascular network compared to control. Accordingly, *Rev-erbα* deficiency is associated with an increase of the endothelial cell and endothelial progenitor cell content, as well as an induction of the pro-angiogenic program and the expression of genes involved in endothelial progenitor cell recruitment.

Conclusions: Rev-erbα may prevent intraplaque neovascularization by inhibiting the angiogenic and vasculogenic activity of endothelial cells and endothelial progenitor cells and then represents a new anti-atherogenic target.

SS067 / #1250

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.01 Endothelial cell function and biology*

ALPHA-LINOLENIC ACID: A NOVEL SENOLYTIC AGENT IN ENDOTHELIAL CELLS

SAAG SESSION 10: ENDOTHELIAL CELL BIOLOGY

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Background and Aims: Aging is a strong risk factor for atherosclerosis. Endothelial senescence leads to impaired angiogenesis, inflammation and oxidative stress, which are underlying pathological mechanisms of atherosclerosis. The omega-3 fatty acid (n-3 FA) α-linolenic acid (ALA) is known to reduce cardiovascular mortality and its benefits in the prevention of vascular aging are gradually emerging. We aimed to determine the senolytic effects and mechanisms of ALA on senescent endothelial cells.

Methods: We studied the effect of ALA treatment in primary human aortic endothelial cells (HAECs) in proliferating (PECs), replicative senescence (SECs) and H_2O_2 induced-premature senescence (H-SECs) in order to determine cell viability, apoptosis, senescent markers and endothelial function. Student's t-test and one-way ANOVA were used to determine differences between groups.

Results: We found that ALA differentially decreased senescent endothelial cells viability in a concentration- and time-dependent manner and effectively reduced β-galactosidase-positive cell numbers and ɣ-H2AX expressions (telomere shortening marker). We also found that ALA promoted only in senescent ECs pro-apoptotic effects by increasing BAX and decreasing Bcl-2. To further characterize the mechanisms, we observed an increase of SIRT1, a well-known cellular rejuvenating regulator, in both proliferating and senescent ECs. In terms of endothelial function, ALA upregulated eNOS in both

ECs.

a) ALA reduced cell viability of senescent ECs
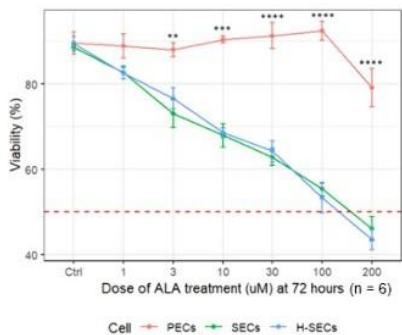
b) ALA decreased SA-β-Gal positive cells in senescent ECs
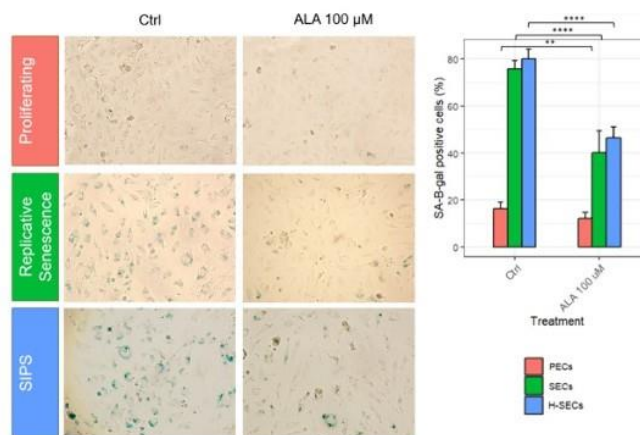
c) ALA upregulated SIRT1 expression in ECs
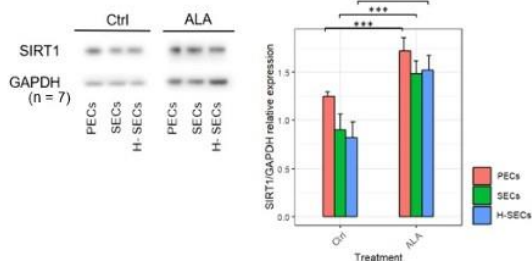
Data are presented as mean ± SD.
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$

Conclusions: ALA exerts senolytic effects by differentially inducing apoptosis in senescent endothelial cells. This could contribute to a nutritional role of ALA in the prevention of age-related vascular dysfunction and atherosclerosis.

Topic: *AS02 Lipids and Lipoproteins / AS02.12 Adipose tissue biology and pathology*

OPPOSING EFFECT OF CHLOROGENIC ACID ON INDUCTION OF BEIGE ADIPOCYTE PHENOTYPE IN 3T3-L1 ADIPOCYTES AND ANGIOGENIC PHENOTYPE IN ENDOTHELIAL CELLS

SAAG SESSION 10: ENDOTHELIAL CELL BIOLOGY

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Background and Aims: Browning of white adipose tissue (WAT) has emerged as an anti-obesity strategy. Adipogenesis involves adipocyte development associated with new blood vessel formation through angiogenesis. Chlorogenic acid (CGA), a dietary polyphenol, was reported to improve glucose tolerance and modulate lipid metabolism. This study investigated the effect of CGA on induction of brown-like phenotype in 3T3L1 adipocytes and its effect on endothelial cells.

Methods: CGA-induced browning of 3T3 L1 adipocytes was studied by analysis of morphology and lipid accumulation in treated cells, and determining levels of markers such as UCP1, perilipin, PGCIα by ELISA and immunoblotting. Angiogenesis was studied using HUVECs in culture and Chorio-allantoic membrane assay.

Results: Treatment of 3T3L1 pre-adipocytes with CGA altered lipid distribution from large unilocular to small multilocular type along with increase in brown adipocyte markers UCP-1 and PGC1α and decrease in cellular triglyceride content. CGA also induced trans-differentiation of white adipocytes to beige adipocytes as indicated by morphology and increase in UCP-1 levels. Increased phosphorylation of AMP-dependent kinase (AMPK) and reversal of this effect of CGA by dorsomorphin, an inhibitor of AMPK, suggested that the AMPK pathway is involved in inducing beige adipocyte phenotype by CGA. CAM assay and analysis of angiogenic markers in HUVECs in culture indicated CGA inhibited endothelial cell proliferation and angiogenic phenotype.

Conclusions: CGA promoted differentiation of 3T3-L1 pre-adipocytes into beige adipocytes, apparently mediated by the AMPK pathway. This suggests CGA could protect against obesity related complications. However in vitro studies suggested CGA inhibited angiogenesis.

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.01 Endothelial cell function and biology*

PERICYTE-ENDOTHELIAL CELL CROSSTALK IN CARDIAC MICROVASCULAR DYSFUNCTION

SAAG SESSION 10: ENDOTHELIAL CELL BIOLOGY

Mandy Grootaert[1], Steven Simmonds[1], Ilona Cuijpers[1], Paolo Carai[1], Nazha Hamdani[2], Stephane Heymans[3], Elizabeth Jones[1]
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Background and Aims: Aim: Pericytes, the mural cells enveloping the micro-vessels, play essential role in vascular remodelling and control vascular functions (e.g. blood flow and tone). Capillary damage results in microvascular dysfunction, highly associated with metabolic comorbidities, yet little is know about the role of pericytes herein. Here, we aim to understand the mechanisms of pericyte loss in metabolic syndrome (MetS) induced-cardiac microvascular dysfunction, and how EC respond to pericyte dysfunction.

Methods: Methods: We characterised cardiac microvascular dysfunction in several animal models of MetS, including the Zucker fatty spontaneously hypertensive (ZSF1) obese rat and the diabetic db/db mouse given 1% salt for 8wks. Pericyte-EC co-cultures were used *in vitro*.

Results: Results: In the ZSF rat hearts, pericyte loss was the earliest microvascular change, occurring prior to loss of capillaries and cardiomyocyte dysfunction. Db/db+salt mice showed pericyte and capillary density loss prior to diastolic dysfunction. Pericyte loss was also present in biopsies from HFpEF patients. Exposure of pericytes to oxidative stress (OX) or high glucose resulted in downregulation of cell cycle and upregulation of interleukin pathways. OX-treated pericytes induced EC inflammation and decreased VE-Cadherin expression when co-cultured. Next, we will perform pericyte lineage tracing in db/db+salt mice followed by scRNAseq to elucidate the role and fate of dysfunctional cardiac pericytes. These data will be revealed at the conference.

Conclusions: Conclusion: We propose a protective role for pericytes in maintaining capillary integrity and EC function, whereas pericyte dysfunction enhances EC reactivity to inflammation, thereby accelerating the effects of the comorbidities and initiating microvascular dysfunction in the heart.

SS070 / #215

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.04 Inflammation, immunity and infection in atherosclerosis*

RECRUITED AND RESIDENT CARDIAC MACROPHAGE PHENOTYPES CONVERGE IN THE HEALING INFARCT BUT NOT IN THE FAILING HEART EXPOSED TO CONTINUOUS PRESSURE OVERLOAD.

SAAG SESSION 11: MONOCYTES AND MACROPHAGES IN CVD

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Background and Aims: Resident macrophages represent 5% of the cells in the healthy heart, although the number increases with monocyte recruitment after cardiac injury. We aim to identify how macrophage origin, tissue location and cardiac injury type determine macrophage phenotypes in ischaemic and non-ischaemic cardiac injury.

Methods: We use a inducible $CX_3CR_1^{Yfp\ CreER/+}$:$R26^{tdT/+}$ mouse line to track resident and recruited macrophage fluxes in the heart following ischemia and reperfusion (I/R) injury and pressure overload after transversal aortic constriction (TAC).

Results: Recruited macrophages outnumbered the resident macrophage pool within the infarct area, but reached a 1:1 equilibrium at 4 weeks post I/R. In the remote myocardium the 1:1 equilibrium established in the first week. In TAC-injured hearts, the 1:1 ratio set in the first week and continued after 8 weeks even cardiac function deteriorated. Gene expression analysis of resident and recruited macrophages from the infarct and remote areas showed differential expression profiles (inflammation, migration, proliferation) within the first week. Unexpectedly, the transcriptional changes converged at a new steady state profile in both cardiac macrophages subsets at 4 weeks, but distinct from the uninjured heart. After TAC surgery, however, transcriptional profiles remained differentially regulated at all time points, and distinct from those observed post I/R injury.

Conclusions: Ischemic and non-ischemic, acute and chronic cardiac injuries induce a transient peak of monocyte-derived macrophages recruitment that lead to a permanent integration into the pool of tissue resident macrophages. The type of injury and macrophage localization within the changing tissue microenvironment determine partial or complete override of ontogenic cell programs.

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.04 Inflammation, immunity and infection in atherosclerosis*

MONOCYTE PRIMING DURING ACUTE MYOCARDIAL INFARCTION ALTERATIONS OF THE BONE MARROW MONOCYTE RESERVOIR SECONDARY TO MYOCARDIAL INFARCTION

SAAG SESSION 11: MONOCYTES AND MACROPHAGES IN CVD

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Background and Aims: Myocardial infarction (MI) inflicts a sterile wound on the heart which recruits thousands of monocytes from the blood. Apart from the quantitative response, little is known whether the bone marrow (BM) supplies qualitatively different monocytes during the course of MI.

Methods: We combined in vivo experiments (MI mouse model and competitive adoptive transfer experiments) with invitro assays in order to characterize the leukocytes and evaluate their recruitment capability.

Results: We isolated BM Ly6C^{high} monocytes from steady state and infarcted mice and we examined their transcriptomes. We found 672 differentially expressed genes (DEG). Most of DEG were enriched in "cell adhesion and recruitment" pathways, then we experimentally demonstrated that BM provides monocytes that are primed for better recruitment at the early inflammatory phase following coronary ligation (12h after MI). In contrast, monocytes displayed worse recruitment capacities at the end of the inflammatory phase (72h after MI). The RNA sequencing data revealed that – in monocytes with poor recruitment capacities – the cell adhesion molecule CD209 was significantly downregulated. To investigate the molecular role of CD209, we overexpressed it in THP-1 cells, which showed enhanced adhesion to endothelial cells (HUVECs) compared to control. Finally, we infarcted wild type and CD209 knockout (CD209$^{-/-}$) mice and found decreased numbers of Ly6C^{high} monocytes in infarcted hearts of CD209$^{-/-}$ mice in comparison to control.

Conclusions: The BM responds to MI and provides an altered monocyte repertoire in terms of numbers and function. Consequently, the heart–BM axis may represent a novel therapeutic target to beneficially modify post-MI cardiac remodeling.

SS072 / #779

THE IMPACT OF CX43-DEPENDENT HETEROCELLULAR COUPLING BETWEEN CARDIOMYOCYTES AND MACROPHAGES IN THE TIME COURSE POST MYOCARDIAL INFARCTION

SAAG SESSION 11: MONOCYTES AND MACROPHAGES IN CVD

Benedict Heinz[1], Tamara Vico[1], Christina Zehender[1], Bianca Dufner[1], Callum Zgierski-Johnston[2], Thomas Kok[2], Peter Kohl[2], Dirk Westermann[1], Ingo Hilgendorf[1]
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Background and Aims: Gap junction protein *Connexin43* (Cx43) connects cardiomyocytes with one another, and macrophages with cardiomyocytes and thereby modulates their electrical activity. We aim to define the impact of this interaction after myocardial infarction (MI), when a massive migration of macrophages into the forming scar tissue takes place.

Methods: We employed Mϕ-specific $CX_3CR_1Cre:Cx43^{fl/fl}$ (KO) mouse model, and we performed optical mapping and arrhythmia induction to assess electrical conduction patterns at baseline. As a model of MI, permanent LAD-ligation injury was performed followed by cardiac function analysis.

Results: We were able to reproduce the impaired AV node conduction described by Hulsmans et al. (2017), which is reflected in a different Wenckebach cycle length in pacing experiments of KO and WT mice at baseline. Moreover, we found a higher risk of developing spontaneous or induced ventricular arrhythmia in KO compared to WT hearts. After MI, we observed a non-significantly higher mortality in KO animals. However, the ejection fractions, cardiac outputs, and stroke volumes were significantly lower in KO mice at d28 post MI compared to WT mice, while both groups did not differ in cardiac function at baseline and d3 post MI.

Conclusions: Heterocellular coupling between cardiac macrophages and cardiomyocytes via Cx43 plays a role in the development of arrhythmia and tissue remodeling after MI. Decoding myocyte/non-myocyte crosstalk will inform us on how to optimize and steer interactions for cardiovascular benefit.

SS073 / #924

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.04 Inflammation, immunity and infection in atherosclerosis*

MACROPHAGES DERIVED FROM LPS-STIMULATED MONOCYTES FROM PATIENTS WITH ASYMPTOMATIC ATHEROSCLEROSIS WERE CHARACTERIZED BY PROLONGED AND INCREASED PRO-INFLAMMATORY ACTIVITY

SAAG SESSION 11: MONOCYTES AND MACROPHAGES IN CVD

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Background and Aims: The pro-inflammatory reaction of human monocytes and macrophages in response to a pathogen is characterized by a subsequent attenuation and the tolerance formation. We decided to study the pro-inflammatory response of monocytes from patients with preclinical atherosclerosis and the subsequent decrease in the pro-inflammatory activity of cells during differentiation into macrophages.

Methods: The study included 46 healthy patients with normal intima-media thickness (IMT) of the carotid arteries and 26 patients with atherosclerotic plaque and thickened IMT. CD14+ monocytes were isolated from blood, stimulated with 1µg/ml LPS for 1 day and cultured for 7 more days without LPS. Secretion of IL-1β,IFN-α2,IFN-γ,TNF-α,CCL2,IL-6,IL-8,IL-10,IL-12p70,IL-17A,IL-18,IL-23 and IL-33 were measured in supernatants at each stage by ELISA.

Results: We found no differences in the generation of cytokines by monocytes in response to LPS. However, further in the course of cell differentiation into macrophages, the cells of patients with atherosclerosis were characterized by increased secretion of the cytokines CCL2 and IL-6 on days 1-6 in culture. Moreover, after changing the medium, 7-day-old macrophages from atherosclerotic patients retained increased secretory activity compared to macrophages from healthy donors. This was manifested in increased basal secretion of the cytokines CCL2, IL-6, and IL-8. The degree of secretion of these cytokines significantly ($p < 0.01$) correlated directly with IMT.

Conclusions: Macrophages derived from LPS-stimulated monocytes from patients with asymptomatic atherosclerosis are characterized by prolonged and increased pro-inflammatory activity. The causes of chronification of inflammation in the vascular wall may lie at the level of circulating monocytes. Supported by RSF (Grant No.22-15-00273).

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.04 Inflammation, immunity and infection in atherosclerosis*

MINOCYCLINE DECREASES SYSTEMIC INFLAMMATION, MODIFIES MONOCYTE SUBPOPULATIONS, AND INCREASES SURVIVAL IN A MOUSE MODEL OF DIET-INDUCED CORONARY ARTERY DISEASE.

SAAG SESSION 11: MONOCYTES AND MACROPHAGES IN CVD

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Background and Aims: Experimental and clinical evidence supports the critical role of inflammation in atherosclerosis (1), independent of the cholesterol level (2). The purpose of this study is to evaluate the effect of short-term treatment with minocycline, a tetracycline antibiotic, that has been shown to possess anti-inflammatory properties, on survival, inflammation, and monocyte subpopulations in a diet-induced myocardial infarction mouse model (3).

Methods: SRB1 KO/apoE hypomorphic mice, aged 2-3 months, fed with high cholesterol diet (HFD) were randomly assigned to 2 groups: Control (HFD-Control) and minocycline (HFD-MIN). Minocycline was administered in the drinking water at a dose of 0.05 mg/mL. Atherosclerosis was induced by feeding a high fat, high cholesterol diet (15% fat, 1.25% cholesterol, 0.5% cholate). Survival analysis was evaluated by the Kaplan-Meier method, and the log-rank test to compare survival curves. Systemic inflammation and monocyte sub-populations were compared by Mann–Whitney U test.

Results: Minocycline administration significantly improved mean survival in SRB1 KO/apoE hypomorphic mice by 35% (30.4 vs 22.5 days, P=0.006) and significantly reduced IL-6 levels (259.7 vs 398.4 pg/mL, P=0.04). Moreover, minocycline attenuated the pro-inflammatory Ly6Chigh monocytes sub-population (57% vs 35.9%, P= 0.006); and increased Ly6Clow subset (43% vs 61%, P= 0.006) without affecting total blood monocytes (2.9% vs 4.6%, P= 0.3).
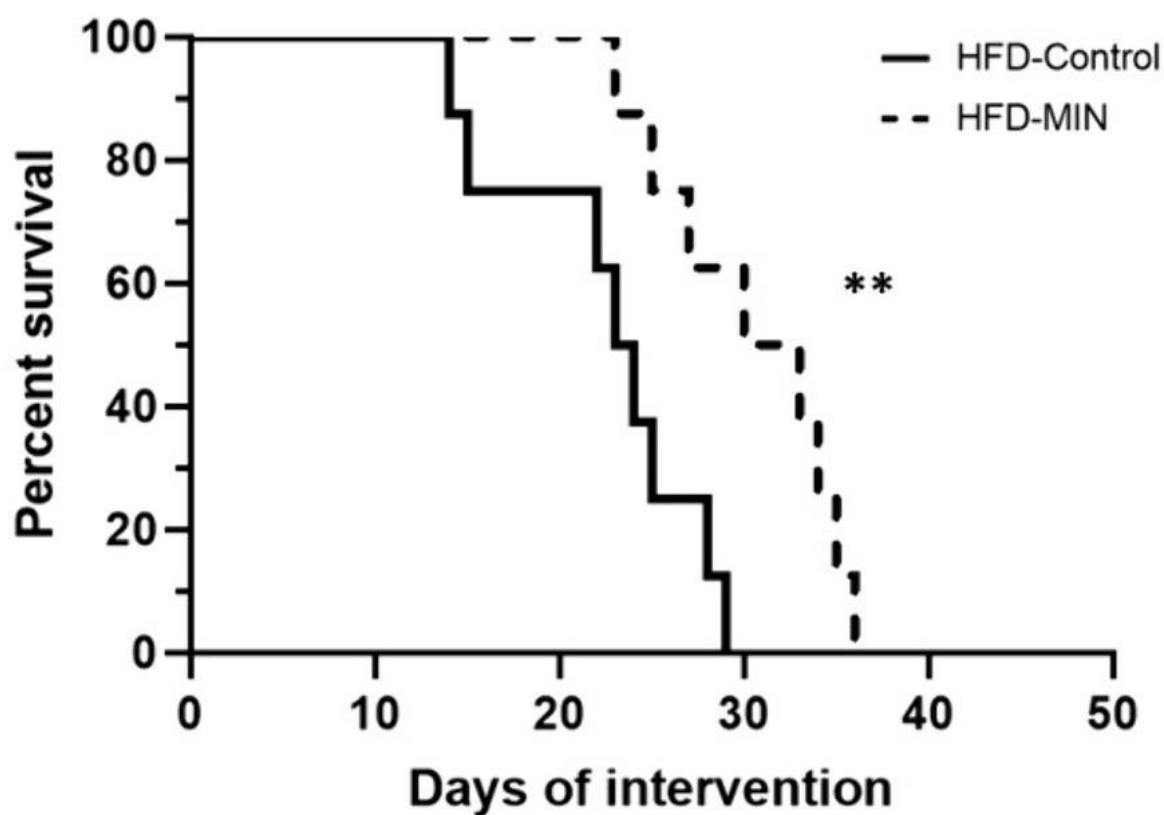
Figure 1. Effect of Minocycline on survival of SRB1 KO/apoE-hypomorphic mice fed atherogenic diet. Kaplan–Meier survival curves of: HFD-Control (n= 8) and HFD-MIN (n= 8). **P<0.005
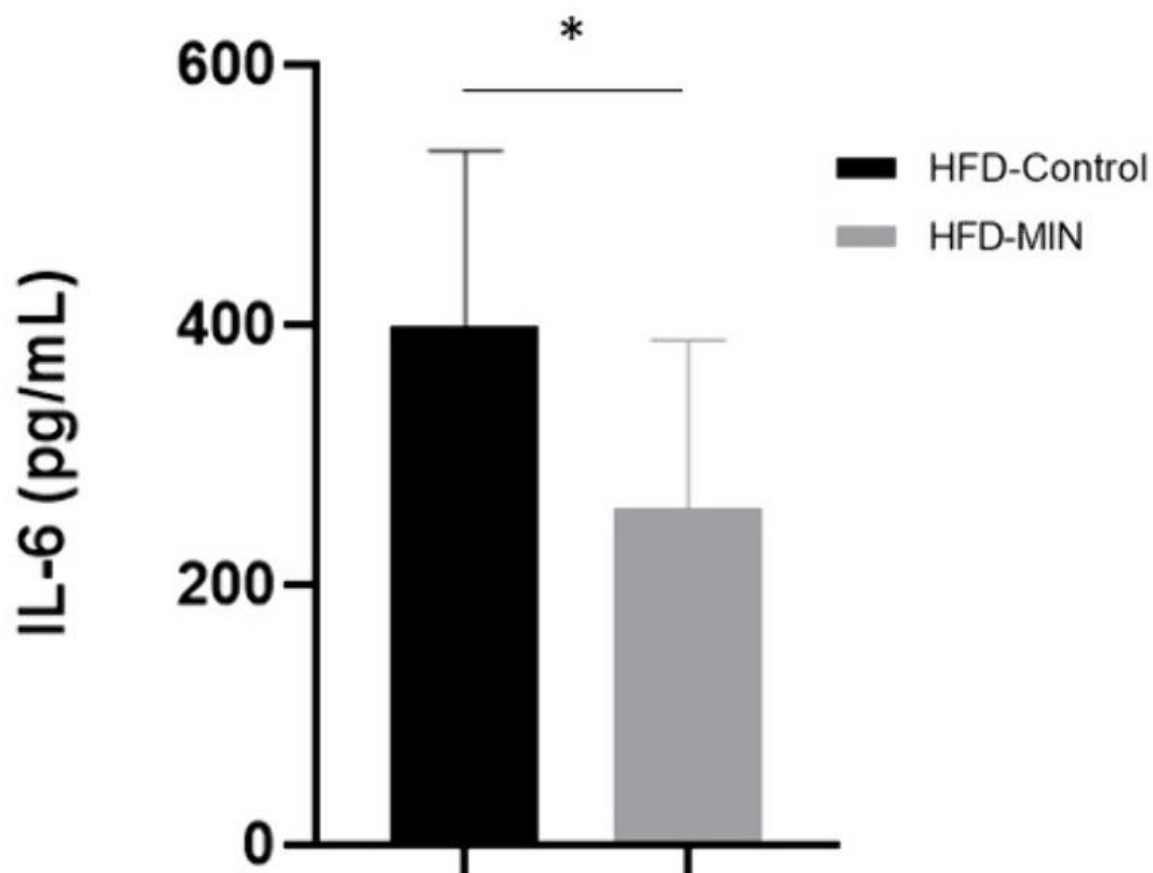
Figure 2. Effect of Minocycline on plasma IL-6 levels in diet-fed SRB1 KO/apoE-hypomorphic mice. HFD-Control (n=9) and HFD-MIN (n=9) after 21 days of feeding HFD and minocycline administration. *P<0.05
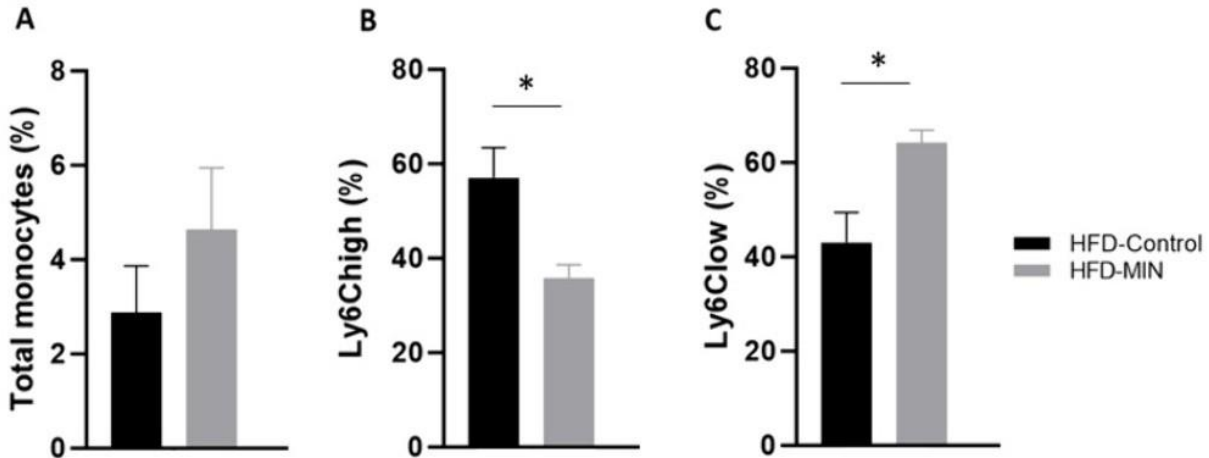
Figure 3. Effect of Minocycline on Blood Monocyte content and Subsets in SRB1 KO/apoE-hypomorphic mice fed atherogenic diet. (A) Flow cytometric quantification of % monocytes in the sample (B) Flow cytometric quantification of Ly6Chigh and (C) Ly6Clow monocyte subsets expressed as percentage of cells over total monocytes. HFD-Control (n= 9) and HFD-MIN (n= 10). Data were represented as mean ± SEM. *P<0.05

Conclusions: High fat diet decreased the survival and caused early death in this animal model and short-term treatment with minocycline successfully improved survival in SRB1 KO/apoE-hypomorphic mice fed an atherogenic diet, impacting systemic inflammation by reducing plasma IL-6 levels and shifting toward a more "reparative" phenotype on circulating monocyte subsets.

SS075 / #179

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.03 Macrophages in lipid metabolism and atherosclerosis*

EICOSAPENTAENOIC ACID (EPA) IMPAIRS ABCA1- AND SR-BI- MEDIATED CHOLESTEROL EFFLUX FROM CHOLESTEROL-LOADED HUMAN THP-1 MACROPHAGES BY REDUCING THE CHOLESTERYL ESTER MOBILIZATION FROM LIPID DROPLETS

SAAG SESSION 11: MONOCYTES AND MACROPHAGES IN CVD

Hani Dakroub[1], Maxime Nowak[1], Delphine Rousseau-Ralliard[2], Jean-Louis Paul[1], Natalie Fournier[1]
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Background and Aims: A diet rich in n-3/n-6 polyunsaturated fatty acids (PUFAs), especially eicosapentaenoic acid (EPA) (C20:5 n-3), is cardioprotective. We previously reported that EPA increases the key antiatherogenic ABCA1-mediated cholesterol efflux pathway from cholesterol-normal human THP-1 macrophages. We investigated here the consequences of the membrane incorporation of several PUFAs on the cholesterol efflux pathways from cholesterol-loaded THP-1 macrophages.

Methods: Human THP-1 macrophages were supplemented (or not: control cells) with 70 µM EPA, 50 µM arachidonic acid (AA) (C20:4 n-6) or 15 µM docosahexaenoic acid (DHA) (C22:6 n-3) for a long time to mimic a chronic exposure. Macrophages were thereafter cholesterol-loaded by incubation with acetylated LDL and isotopic cholesterol efflux to lipid-free apolipoprotein (apo) AI (ABCA1 pathway) or to HDL (SR-BI pathway) was measured after 24 h of incubation.

Results: EPA decreased ABCA1-mediated cholesterol efflux (-14%) without altering ABCA1 expression, whereas AA and DHA had no effect. Compared to control cells, phospholipid fraction of EPA cells exhibited higher levels of EPA (21.3% vs 1.5%), which was associated with lower levels of AA (5.7% vs 10.3%). Moreover, EPA also reduced cholesterol efflux to HDL (-19%). EPA incorporation did not hinder efflux in free cholesterol-loaded THP-1. Conversely, EPA reduced the neutral hydrolysis of cytoplasmic cholesteryl ester (CE).

Conclusions: In conclusion, the beneficial *in vitro* effect of EPA observed in cholesterol-normal THP-1 macrophages is abolished when the THP-1 macrophages are cholesterol-loaded, mimicking foam cells observed within human atherosclerotic plaques. We are currently investigating the mechanism(s) by which EPA reduces the cholesteryl ester mobilization from lipid droplets.

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.04 Inflammation, immunity and infection in atherosclerosis*

CSF2RA INHIBITION SUPPRESSES PRO-INFLAMMATORY RESPONSES AND ACCELERATES REPARATIVE REMODELLING POST-MYOCARDIAL INFARCTION

SAAG SESSION 11: MONOCYTES AND MACROPHAGES IN CVD

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Background and Aims: The accumulation of divergent macrophage populations differentially directs tissue injury and reparative processes after acute myocardial infarction (MI), with colony stimulating factors (CSFs) proposed to orchestrate macrophage polarisation towards pro-inflammatory (Csf2) and pro-fibrotic (Csf1) phenotypes. In this study, we evaluated the effect of Csf2 receptor (Csf2ra)-deficiency or inhibition on post-MI remodelling.

Methods: We performed proteomics on the secretome of Csf1- and Csf2-polarised human macrophages, and exposed human cardiac fibroblasts to the secretomes, in conjunction with loss-/gain-of-function assays. Additionally, mice with macrophage-specific deletion of Csf2ra (Csf2ra-mac-KO), and C57Bl/6J mice with pharmacological inhibition of Csf2ra, were subjected to LAD-induced MI, alongside relevant controls.

Results: The secretome of Csf2-polarised macrophages increased fibroblast migratory and contractile capacity, and proteomics revealed increased Cathepsin Z expression (log-fold-change=2.09) alongside reduced CXCL10 levels (log-fold-change=2.14). Gain-/loss-of-function experiments revealed CXCL10/CXCR3 promoted a reparative fibroblast phenotype, while Cathepsin Z degraded CXCL10. *In vivo*, Csf2ra mac-KO increased fibroblast ($p<0.01$) and anti-inflammatory macrophage density ($p<0.001$) 28-days post-MI, and improved cardiac function ($p<0.05$). Similarly, Csf2ra inhibition accelerated fibroblast ($p<0.05$), anti-inflammatory macrophage ($p<0.01$), and capillary accumulation ($p<0.05$), which was associated with reduced infarct size ($p<0.01$) and enhanced cardiac function ($p<0.01$). Furthermore, Csf2ra inhibition reduced Cathepsin Z levels ($p<0.001$) and concomitantly increased CXCL10 expression ($p<0.05$).

Conclusions: Together these novel findings reveal Csf2-polarised macrophages drive pro-inflammatory responses post-MI through a Cathepsin-Z/CXCL10-mediated mechanism and crosstalk with cardiac fibroblasts, preventing scar resolution. Providing direct translational potential, our interventional outcomes demonstrate that Csf2ra inhibition accelerates reparative remodelling, through enabling favourable Csf1-driven pro-fibrotic responses and therefore promoting post-MI recovery and limiting heart failure risk.

SS077 / #899

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.05 Inherited dyslipidemias*

THE ASSOCIATION BETWEEN STATIN ADHERENCE AND PULSE WAVE VELOCITY IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA

SAAG SESSION 12: THE CHALLENGES IN FH MANAGEMENT

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Background and Aims: Familial Hypercholesterolemia (FH) leads to severely elevated levels of LDL-c and increased risk for premature atherosclerosis. Statins slow the progression of atherosclerosis. Pulse wave velocity (PWV), as a parameter for arterial stiffness, may assess the effect of statins on atherosclerosis. The aim of this study is to evaluate the association between PWV and adherence to statins in patients with FH.

Methods: For this cross-sectional study 214 eligible patients with FH participated in a double-blind, placebo-controlled trial on the efficacy and safety of pravastatin in children. After twenty years of follow-up, PWV measurements of the carotid arteries using 4D Flow MRI were made **(Figure)**. Statin adherence was assessed using the Medication Adherence Self-Report Inventory: use of ≥80% of prescribed statins over the last month was defined as 'adherent'.
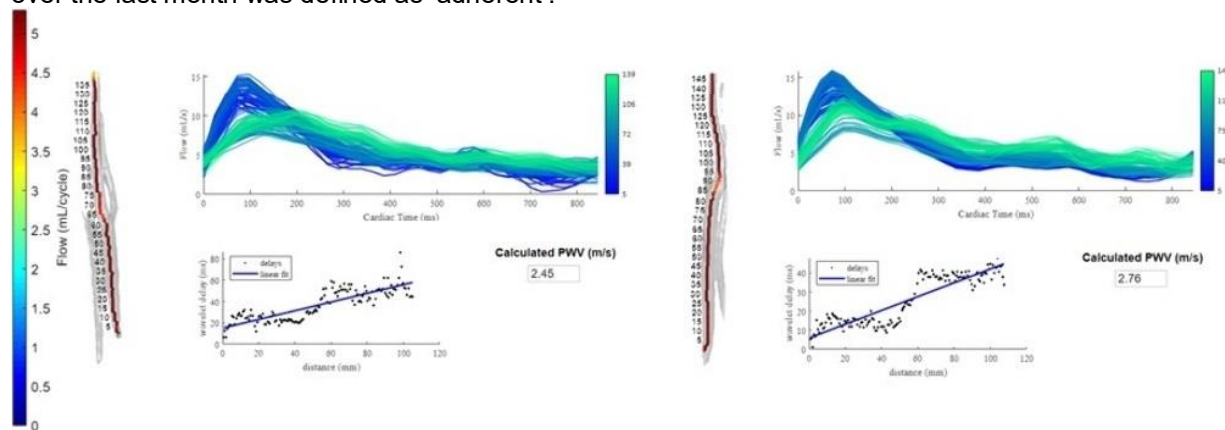
Figure
4D flow PWV measurements of the carotid arteries

Results: We included 108 patients (mean [SD] age: 32.1 [3.3] years, 52 (48.1%) males). Adherent patients had lower mean (SD) PWV than patients who were not (4.02 [1.42] m/s vs. 4.31 [1.15] m/s; p=0.290). After adjustment for confounders (age, sex, LDL-c, height), this association remained not

significant (p=0.217). Similar results were observed when different definitions of adherence were used **(Table)**.

Table   Mean difference in PWV between adherent and non-adherent patients according to the different definitions for adherence

Adherence	Mean PWV[a,b]	95% CI[c] lower	upper	Difference[a]	p-value[d]
≥80% last month	4.02	3.69	4.35	-0.29	0.290
<80% last month	4.31	3.91	4.72		
≥80% last year	4.05	3.70	4.41	-0.15	0.561
<80% last year	4.20	3.84	4.57		
≥90% last month	4.03	3.68	4.38	-0.22	0.410
<90% last month	4.25	3.88	4.61		
≥90% last year	4.00	3.58	4.40	-0.21	0.393
<90% last year	4.21	3.89	4.54		

[a] = in meters per second (m/s), [b] PWV = pulse wave velocity, [c] CI = confidence interval
[d] = unadjusted

Conclusions: We found lower PWV in patients with FH that were adherent compared with non-adherent patients, but not significant. Possible explanations for being non-significant may be technical aspects of the 4D flow MRI measurements (variable coverage of carotid arteries, low temporal resolution). Further follow-up and improvement of techniques is needed to determine possible changes of PWV in these FH patients in the coming years.

SS078 / #79

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.05 Inherited dyslipidemias*

IMPACT OF CORONARY ARTERY CALCIUM ON THE PROGNOSIS OF HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA

SAAG SESSION 12: THE CHALLENGES IN FH MANAGEMENT

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Background and Aims: We aimed to examine the association between CAC and MACE and to estimate CAC progression in patients with FH and understand the prognostic value of the CAC score in primary prevention settings.

Methods: Data of patients with FH admitted to Kanazawa University Hospital between 2000 and 2020, who underwent CAC measurement and were followed up (N = 622, male = 306), were retrospectively reviewed. Risk factors for MACEs, including death associated with cardiovascular disease, unstable angina, myocardial infarction, and staged revascularization, were determined using the Cox proportional hazard model.

Results: The median follow-up duration was 13.2 years. We observed 132 MACEs. Patients with MACEs had a significantly higher median CAC score than those without MACEs (104 *vs.* 0; $p < 2.2 \times 10^{-16}$). The event rate per 1,000 person-years for CAC scores of 0 (N = 283 [45.5%]), 1–100 (N = 260 [41.8%]), and >100 (N = 79 [12.7%]) was 1.2, 17.0, and 78.8, respectively. Log (CAC score+1) was a significant predictor of the occurrence of MACEs (hazard ratio: 3.24; 95% confidence interval: 1.68–4.80; $p = 5.6 \times 10^{-5}$) in the multivariate Cox regression analysis. The risk discrimination of MACEs was enhanced by adding CAC to other conventional risk factors (C-statistics: 0.833–0.934; $p = 1.4 \times 10^{-15}$). The regression equations between age and the CAC score in male and female were $Y = 0.074X - 1.52$ and $Y = 0.087X - 3.21$, respectively.

Conclusions: CAC score helps in further risk stratification in patients with FH. Furthermore, the average age of CAC onset was 21 years in male and 37 years in females.

SS079 / #1312

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.05 Inherited dyslipidemias*

GENETICALLY DETERMINED FAMILIAL HYPERCHOLESTEROLEMIA INTO REDUCED PROGENITOR CELLS WITH IMPAIRED ENDOTHELIAL FORMING POTENTIAL

SAAG SESSION 12: THE CHALLENGES IN FH MANAGEMENT

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Background and Aims: Genetically determined Familial Hypercholesterolemia (FH) causes premature coronary atherosclerosis. As we previously demonstrated that FH induces premature hematopoietic aging, we now aim to evaluate whether this results into premature impaired ability of the bone marrow derived Endothelial Progenitor Cells (EPCs).

Methods: EPCs and Circulating Endothelial Cell (CEC) (the latter marking endothelial dysfunction) were characterized for blood count (Flow-Cytometer) and EPCs were isolated from 113 genetically confirmed Heterozygous FH (all on cholesterol lowering treatments) and from 40 normocholesterolemic subjects ("controls", none on treatment) (i) to count the number of Endothelial Colony Forming Units ("EC-CFUs") generated ex vivo for up to seven days and (ii) to study the ex-vivo proliferation (Ki67$^+$ cells).

Results: Heterozygous Familial Hypercholesterolemia (HeFH) presented reduced blood EPCs but higher CEC vs controls (for EPCs: 192,80±118,80 vs. 320,60±142,50 cells/mL, p-value <0,001; for CEC: $7.9*10^3±5.3*10^3$ vs $4.3*10^3±2.9*10^3$ cells/mL, p-value <0,001). After three days, EC-CFU generated from the EPCs of FH were less than those generated from EPCs of controls (4,68±6,48 vs 9,00±3,74 EC-CFU/well, p-value <0,001). This reduction holds constant also after seven days. EPCs from HeFH displayed evident impairment in cell proliferation vs those from controls, still at the third day (109,58±40,63 vs. 185,40±77,48 Abs. count, p-value 0,005) and the seventh day (100,25±34,55 vs. 172,73±86,16 Abs. count, p-value=0,015).
These cell impairments did not correlate with markers of atherosclerosis (CT-Coronary Artery Calcium score).

Conclusions: EPCs are reduced and appear dysfunctional in FH who are still treated. In depth analyses are warranted to unravel developmental aspects of this cell compartment in FH.

SS080 / #588

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.05 Inherited dyslipidemias*

LONG-TERM EFFECTIVENESS AND SAFETY OF LOMITAPIDE: REAL-WORLD EXPERIENCE FROM ITALY IN PATIENTS WITH HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA

SAAG SESSION 12: THE CHALLENGES IN FH MANAGEMENT

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Background and Aims: To evaluate the long-term effectiveness and safety of lomitapide in patients with homozygous familial hypercholesterolemia (HoFH).

Methods: Low-density lipoprotein (LDL-C) changes from baseline to last visit, concomitant lipid-lowering therapies, adverse events (AEs), and cardiovascular events were retrospectively assessed in 13 HoFH patients from two centers in Italy.

Results: Median (IQR) age at diagnosis of HoFH was 21 (13–37) years. Patients received lomitapide for up to 6.5 years for a median of 44 months (IQR: 12–55; max: 79); median lomitapide dose was 20 mg/day. LDL-C decreased by 69% during lomitapide treatment from a mean (SD) of 311.1 (149.8) mg/dL at baseline to 96.5 (51.7) mg/dL at last follow-up (Figure). At last visit, 5 patients (38%) achieved LDL-C <100 mg/dL; all had LDL-C <70 mg/dL. Two patients received apheresis at baseline, with both discontinuing during lomitapide treatment. Lomitapide was discontinued in 2 patients (15%) due to gastrointestinal AEs and temporarily interrupted due to diarrhea in 1 patient (8%). Median on-treatment alanine or aspartate transaminase values remained <3X the upper limit of normal at 45 IU/L (IQR: 32.5–68.5) and 39 IU/L (IQR: 28.5–53.5), respectively (n=11). Hepatic elasticity remained normal (range 4.0–6.3 kPa) among patients with hepatic elastography (n=4) during follow-up. Hepatic steatosis was reported as mild (n=2) or moderate (n=2). Cardiovascular events were reported in 9 patients before lomitapide treatment and in 1 patient during treatment.

Conclusions: Lomitapide was effective at reducing LDL-C in this long-term real-world retrospective analysis. Adverse events were uncommon, and hepatic elasticity remained

normal.

Figure: LDL-C levels at baseline and last visit
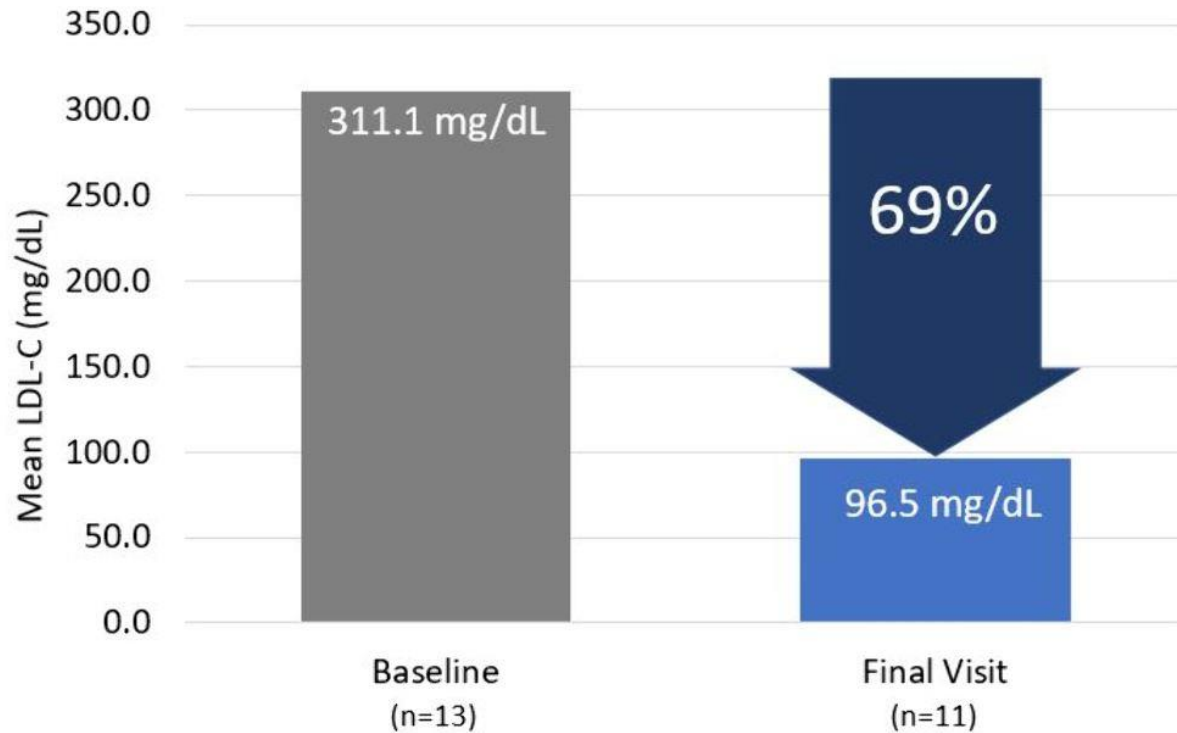
Topic: *AS03 Dyslipidemia and Risk Factors / AS03.05 Inherited dyslipidemias*

HOFH IS A LIFE LIMITING CONDITION: CLINICAL CHARACTERISTICS AND LIFESPAN OF DECEASED HOFH PATIENTS IN THE HICC REGISTRY

SAAG SESSION 12: THE CHALLENGES IN FH MANAGEMENT

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Background and Aims: Homozygous familial hypercholesterolemia (HoFH) is a rare, genetic disorder characterized by severe LDL hypercholesterolaemia, stenotic aortic valve and root disease, and premature accelerated atherosclerotic cardiovascular disease (ASCVD). Premature cardiovascular death due to HoFH has been reported in children. Here we present a contemporary assessment of the clinical characteristics and life-courses of deceased HoFH patients.

Methods: We analysed the data of all deceased patients in the HoFH International Clinical Collaborators (HICC) registry. The HICC registry includes 751 patients with HoFH who were alive in 2010 or later.

Results: In total, 37 patients (49% women) were deceased at registry entry. The median [IQR] age of diagnosis was 12 [5-23] and 92% presented with xanthomas. Median [IQR] untreated LDL-cholesterol level was 15.6 [13.2-19.5] mmol/L and last known LDL-cholesterol level 9.4 [5.1-13.4] mmol/L. Most (28 (76%)) patients died of confirmed cardiovascular causes at a median [IQR] age of 32 [17-52] years (Figure 1).

The majority (26 (70%)) had experienced (recurrent) ASCVD prior to death, with a median [IQR] age of onset of 28 [16-39] years. The most common diagnoses or interventions were aortic stenosis 15 (41%), myocardial infarction 11 (30%), angina pectoris 11 (30%), aortic valve replacement 8 (22%), CABG 8 (22%) and PCI 7 (19%).
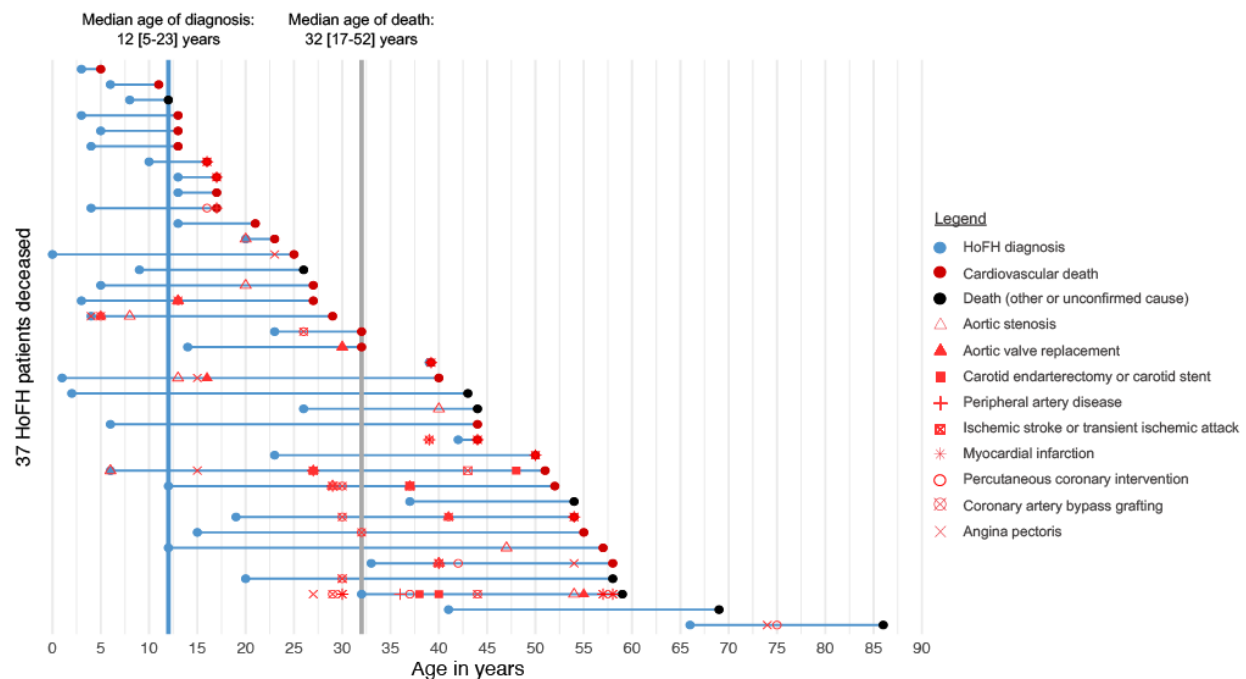
Conclusions: Approximately half of the deaths occurred before the third decade of life, mostly from cardiovascular causes. These data stress the potentially life-limiting nature of HoFH and need for early diagnosis and treatment.

SS082 / #913

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.05 Inherited dyslipidemias*

CCTA RESULTS IN CHILDREN WITH HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA UPON INTENSIVE LIPID LOWERING TREATMENT WITH EVINACUMAB

SAAG SESSION 12: THE CHALLENGES IN FH MANAGEMENT

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Background and Aims: Homozygous FH (HoFH) is a severe lipid disorder, which results in extremely elevated LDL-C, early ASCVD and can even lead to death in childhood. Drastic lipid lowering in HoFH is of vital importance to prevent premature ASCVD, but remains challenging. Evinacumab, a novel monoclonal antibody directed against ANGPTL3, has shown reductions in LDL-C levels up to 50% on top of background lipid-lowering therapy in HoFH patients ≥12 years of age. The aim of this study is to determine the effects of evinacumab in children with HoFH on treatment (goals) and coronary CT angiography (CCTA) results.

Methods: Children (6-18 years old) with HoFH who were treated with evinacumab were eligible. General characteristics, as well as LDL-C levels, presence of ASCVD and CCTA results, were compared before and after the start of evinacumab.

Results: Seven children were studied. LDL-C levels were further reduced with a mean 33% since the start of evinacumab, while reducing the frequency of apheresis in all patients from weekly to once per four weeks. One child even was prevented from starting apheresis. None of them developed ASCVD. Visual CCTA interpretation revealed subtle non-calcified plaques regression in three patients, while none of the patients had evident plaque

progression.

Tables and figures

Table 1. Patient characteristics

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Age (years)							
At diagnosis	2	1	0	11	3	5	8
At start evinacumab	11	6	11	11	12	17	18
Gender	male	female	female	female	female	male	female
Mutations							
Name	4 kb duplication exon 11-12	c.131G>A p.(Trp44*)	c.314-1G>A p ?	c.1359-1G>A p.?	c.313+1G>A p.?	c 2417_2418insG p.(Phe807Leufs*10)	4 kb duplication exon 11-12
	4 kb duplication exon 11-12	c.681C>A p.(Asp227Glu)	c.917C>T p.(Ser306Leu)	c.1775G>A p.(Gly592Glu)	c.313+1G>A p.?	c.910G>A p.(Asp304Asn)	4 kb duplication exon 11-12
Type	negative/negative	negative/defective	negative/defective	negative/defective	negative/negative	negative/defective	negative/negative
LLT Treatment	rosuvastatin 20 mg ezetimibe 10 mg apheresis	rosuvastatin 5 mg ezetimibe 10 mg apheresis	rosuvastatin 10 mg ezetimibe 10 mg apheresis	rosuvastatin 20 mg ezetimibe 10 mg	rosuvastatin 20 mg ezetimibe 10 mg apheresis	rosuvastatin 20 mg ezetimibe 10 mg apheresis	rosuvastatin 20 mg ezetimibe 10 mg apheresis
Frequency of LA							
at start evinacumab	1/week	1/week	1/2 weeks	N/A	1/week	1/week	1/week
recent	1/4 weeks	1/4 weeks	1/4 weeks	N/A	1/4 weeks	1/4 weeks	1/4 weeks
Lipid levels (mmol/L)							
LDL-C at diagnosis	14.18	16.50	14.15	13.60	20.82	16.89	15.46
LDL-C at start evinacumab (pre-LA)	5.62	5.57	5.88	8.57	5.36	4.16	4.00
Recent LDL-C (pre-LA)	4.21	3.66	3.74	*	4.02	*	2.28
ASCVD							
Clinical ASCVD at start evinacumab	0	0	0	0	0	0	0
Clinical ASCVD recent	0	0	0	0	0	0	0

* Results are awaited

Table 2. CCTA results before and after start evinacumab

Patient	CCTA results before start evinacumab			CCTA results after start evinacumab			Change
	Calcified plaque	Non-calcified plaque	Number of segments⁺	Calcified plaque	Non-calcified plaque	Number of segments⁺	
1	No	No	0	No	No	0	No change
2	No	No	0	No	No	0	No change
3	Yes	Yes	1	Yes	No	1	Regression
4	No	No	0	*	*	*	*
5	Yes	Yes	2	Yes	No	2	Regression
6	Yes	No	1	*	*	*	*
7	No	Yes	2	No	Yes	2	Regression

Results are awaited. ⁺number of segments include the number of coronary artery segments and the aortic root as additional segment.

Conclusions: In HoFH children, treatment with evinacumab resulted in superior LDL-C target attainment, and allowed reduction in apheresis. Subtle non-calcified plaque regression was observed on repeat CCTA imaging in three patients, illustrating that the potential of evinacumab can play a crucial role in the treatment of these HoFH patients.

SS083 / #367

Topic: *AS04 Clinical Vascular Disease / AS04.12 Prevention and treatment of cardiovasculardisease; miscellaneous*

MACHINE LEARNING IDENTIFICATION OF CARRIERS OF PATHOGENIC AND LIKELY PATHOGENIC VARIANTS LINKED TO FAMILIAL HYPERCHOLESTEROLAEMIA IN THE UK BIOBANK.

SAAG SESSION 12: THE CHALLENGES IN FH MANAGEMENT

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Background and Aims: To evaluate the performance (sensitivity, specificity, positive predictive value [PPV]) of machine learning (ML) models for large-scale screening of familial hypercholesterolaemia (FH) in Electronic Health Records (EHR).

Methods: Cross-sectional analysis using UK Biobank data with identification of carriers of FH-related likely pathogenic/pathogenic genes variants, and comparison of ML models with Dutch Lipid Clinic Network (DLCN) and Simon Broome (SB) clinical criteria for FH. Genotypes were defined according to a simplified version of the FH variant interpretation guidelines published by the ClinGen variant curation expert panel for the *LDLR* gene and ClinVar for *LDLR*, *APOB* and *PCSK9* genes. The best-performing ML models derived in the derivation dataset were compared against modified versions of DLCN and SB criteria within the validation dataset.

Results: 1,031 of the 454,710 participants had an FH-causing variant. In the validation dataset (N=91,205;N_{FH}=210), a stacked generalisation ensemble model presented the highest specificity and PPV and high sensitivity (Figure). The ensemble outperformed both the modified versions of DLCN and SB criteria in terms of sensitivity and PPV but was slightly less specific than the modified SB criteria

(Figure).

Performance of machine learning models and clinical criteria

Receiver Operating Chracteristic: best machine learning model and clinical criteria
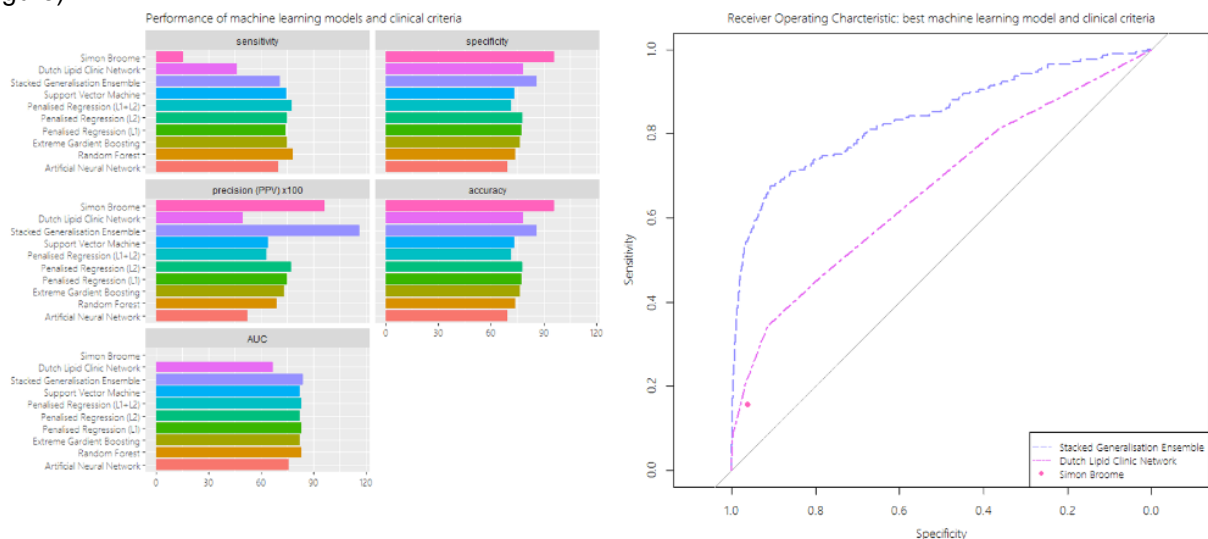
Conclusions: Current clinical diagnostic criteria for FH include variables which are not routinely captured in EHR and thus require modification. Modified versions of standard clinical criteria yield lower sensitivities than ML models in the biobank and are thus less appropriate for large-scale screening programs. Among ML models, a stacked generalisation ensemble provided higher predictive performances and may present the optimal solution for automated case-finding of FH in the general population.

SS084 / #772

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.13 Other*

CHRONIC KIDNEY DISEASE INDUCED ATHEROSCLEROSIS: THE REGULATORY ROLE OF MIR-26B

SAAG SESSION 13: MICRORNAS IN DIABETES, ATHEROSCLEROSIS AND STATIN TREATMENT

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Background and Aims: Chronic kidney disease (CKD) has been clearly associated with atherosclerosis and both involve complex pathological processes. MicroRNAs (miRs) are known to mediate such processes, however, the role of many miRs remains to be elucidated. Previously found to be upregulated in atherosclerotic tissues, miR-26b is the focus of this study where we investigate its regulatory role in CKD-enhanced atherosclerosis.

Methods: Atherosclerosis and CKD were induced in *mir26b* full-body knockout mice on an ApoE-deficient background to evaluate disease development. The effects of miR-26b overexpression on inflammation and fibrosis were assessed in human kidney cells (HK-2). Lastly, associations between miR-26b expression and CKD parameters were evaluated in a CKD patient cohort (FOSU study).

Results: MiR-26b deficiency resulted in a 1.8-4.2 fold increase in plaque size after 12w and 4w Western-type diet, respectively. Furthermore, lesional collagen content in miR-26b-deficient mice was 1.4-3.4 fold higher, compared to wildtype controls. Kidney collagen content and fibrotic gene expression showed no significant changes in miR-26b-deficient mice, however, IL-6/TNF-α were downregulated. Overexpression of miR-26b in HK-2 cells corroborated the pro-inflammatory effect of miR-26b. Correspondingly, analysis of the CKD cohort revealed strong correlations between plasma miR-26b expression and various CKD parameters (e.g. glomerular filtration rate and serum urea), supporting a role for miR-26b in CKD development.

Conclusions: In conclusion, our findings show that miR-26b exacerbates CKD progression while exerting an atheroprotective role, most likely by aiding in plaque stability. Overall, these results indicate a tissue-

specific regulatory role of miR-26b in CKD-induced atherosclerosis that could be exploited for potential mechanistic or therapeutic insights.

SS085 / #347

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.03 Diabetes, insulin sensitivity and resistance*

STATIN-INDUCED MIR-33A AND MIR-27B UP-REGULATION CONTRIBUTES TO THE DEVELOPMENT OF NEW-ONSET TYPE 2 DIABETES.

SAAG SESSION 13: MICRORNAS IN DIABETES, ATHEROSCLEROSIS AND STATIN TREATMENT

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Background and Aims: Although Statin effectiveness and safety has been widely demonstrated, increasing evidence suggest that long-term statin treatment could be responsible for an increased risk of new-onset type 2 diabetes mellitus (T2DM) by 10-12 %. Although the mechanism by which statin treatment induces T2DM remains not fully understood, some studies support the hypothesis that statins may disrupt glucose homeostasis through both mpaired insulin secretion and diminished insulin sensitivit. In this study we sought to unravel the molecular mechanism by which statin treatment induces T2DM through studying miRNAs implicated in β-cell insulin and glucose homeostasis.

Methods: EndoC-βh cells were treated with statins and expression levels of miR-33a and miR-27b along with their target genes were studied. To confirm that the effects of statin treatment on insulin and glucose homeostasis were due to the overexpression of miR-27b and miR-33a, levels of the target genes were studied in mimic- and antagoMIR-transfected EndoC-βh cells. Ca^{2+} mobilization and insulin secretion was studied in all the conditions.

Results: Statin treatment negatively affects insulin sensitivity, release of Ca^{2+} from the endoplasmic reticulum and, insulin secretion by causing the overexpression of miR-27b, which targets several genes involved in the aforementioned processes. This miR-27b overexpression occurs upon statin -induced miR-33a upregulation.

Conclusions: The increased incidence of *de novo* T2DM in patients treated with statins could be due to a deregulation of the microRNAs miR-27b and miR-33a caused by the treatment, which leads to a downregulation of some key target-genes involved in insulin and glucose homeostasis.

SS086 / #607

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.14 Epigenetics and microRNA*

LONG-TERM LDL-APHERESIS TREATMENT AND DYNAMICS OF CIRCULATING MIRNAS IN PATIENTS WITH SEVERE FAMILIAL HYPERCHOLESTEROLEMIA

SAAG SESSION 13: MICRORNAS IN DIABETES, ATHEROSCLEROSIS AND STATIN TREATMENT

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Background and Aims: Familial hypercholesterolemia (FH) is an autosomal-dominant disorder caused by mutations within the *LDLR*, *APOB*, and *PCSK9* genes, characterized by high plasma levels of total- and low-density lipoprotein cholesterol (LDL-C). LDL apheresis (LA) serves as an extracorporeal circulation device to eliminate LDL-C from plasma. MicroRNAs (miRNAs) are important posttranscriptional gene regulators involved in the pathogenesis of atherosclerosis. Our study aimed to monitor the dynamics of twenty pre-selected circulating miRNAs in patients under long-term apheresis treatment.

Methods: Plasma samples from 12 FH patients (men=50%, age=55.3±12.2 years; mean LA overall treatment time=13.1±7.8 years) were collected before each apheresis therapy every 6th month due to the 4- years of treatment. Completely 8 follow-up (FU) samples were measured in each patient. Twenty pre-selected circulating miRNAs were measured using quantitative PCR.

Results: Dynamic changes in the relative quantity of 6 miRNAs (miR-92a, -21, -126, -122, -26a, and miR-185; all $P<0.04$) during follow up time were identified. Apheresis overall treatment time has influenced circulating miR-146a ($P<0.04$). In LDLR mutation homozygotes (N=5) compared to heterozygotes, we found higher plasma levels of miR-181, -126, -155, and miR-92a (all $P<0.03$). Treatment with PCSK9 inhibitors (N=6) affected plasma levels of 7 miRNAs (miR-126, -122, -26a, -155, -125a, -92a, and miR-27a; all $P<0.04$).

Conclusions: Long-term monitoring has shown, that LA in the patients with severe familiar hypercholesterolemia influences plasma circulating miRNAs involved in endothelial dysfunction, cholesterol homeostasis, inflammation, and plaque development. The longer is the treatment using LA the better is the miRNA milieu depicting the potential cardiovascular risk.

SS087 / #631

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.14 Epigenetics and microRNA*

UPREGULATION OF MICRORNAS BY STATINS IN HEPATOCYTES CAN ACT AS INTER-CELLULAR OR -TISSUE MESSENGERS AS EXOMIRS.

SAAG SESSION 13: MICRORNAS IN DIABETES, ATHEROSCLEROSIS AND STATIN TREATMENT

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Background and Aims: Although the exact mechanism of microRNA participation in extracellular signalling is not well understood, it has been shown that mature miRNAs are likely involved in intercellular communication, a process by which microRNAs are transferred between cells by lipid-based carriers, including exosomes, microvesicles and lipoproteins, as exomiRs. Previous work in our lab has demonstrated that statin treatment induces upregulation of miR-33 and miR-122 in hepatocytes The objective of this work has been to determine wether these exomiRs have any effect in adipocytes, when liberated to the extracellular medium in lipoproteins/exosomes.

Methods: Adipocytes where incubated with exovesicules excreted from Huh7 hepatoma cells that have been previously incubated for 48 hours with atorvastatin. Then, microRNA tracing from Huh7 media to adipocytes was performed by qRT-PCR. Specifically, miR-33 and miR-122 were quantified in adipocytes by qRT-PCR after have been exposed to the exovesicles secreted by Huh7 cells treated with Atorvastatin. In addition, the effect of microRNA delivery to adipocytes was analyzed by measuring the mRNA levels of miR-33 and miR-122 targets: G6PC2 and CPTA1.

Results: Here, we demonstrate that statin treatment in hepatocytes induces and upregulation of miR-33 and miR-122 that can be transported as exomiRs in lipoproteins/exosomes and target other tissues as adipocytes where these microRNAs downregulate the expression of their targets G6PC2 and CPTA1.

Conclusions: Atorvastatin treatment affects microRNA pattern in liver, which at the same time can affect other tissues than liver, such as adipose tissue, by delivery of the upregulated microRNAs through extracellular vesicules (exomiRs), where microRNAs downregulate target protein expression.

SS088 / #800

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.14 Epigenetics and microRNA*

EPICARDIAL AND VISCERAL ADIPOSE TISSUE EXPRESSION LEVELS OF MIR-1247-5P AND PGC-1A GENE ARE ASSOCIATED WITH OBESITY

SAAG SESSION 13: MICRORNAS IN DIABETES, ATHEROSCLEROSIS AND STATIN TREATMENT

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Background and Aims: Epicardial adipose tissue (EAT) is a visceral adipose tissue (VAT) that surrounds the heart and coronary arteries. It was previously found that during coronary artery disease (CAD) progression, EAT miRNA and mRNA expression profile is changed. In this study, it is aimed to identify the associations between known CAD risk factors and expression levels of miRNA and mRNAs.

Methods: Epicardial adipose tissue (EAT) samples (63 CAD; 30 non-CAD), and visceral adipose tissue (VAT) samples from 65 individuals (46 CAD; 19 non-CAD) were collected. Anthropometric and biochemical measurements are done. *PPARGC1A* which encodes PGC-1α and miR-1247-5p expression levels were examined in EAT and VAT samples using real-time PCR.

Results: Body mass index (BMI) and EAT miR-1247-5p expression levels were negatively correlated in the study population ($p=0.011$), male ($p=0.004$), and in the CAD group ($p<0.001$). In addition, *PPARGC1A* expression levels in EAT samples were also negatively correlated with BMI in the study population ($p=0.024$) and in the CAD group ($p=0.027$). In male individuals, EAT *PPARGC1A* expression levels were negatively correlated with BMI ($p=0.003$) and fasting glucose levels ($p=0.026$). In addition, VAT *PPARGC1A* expression levels were negatively correlated with BMI in the study population ($p=0.002$), male ($p=0.010$), and in the CAD group ($p=0.001$).

Conclusions: The results obtained in the study show that the EAT and VAT expression levels of miR-1247-5p and *PPARGC1A* which is the key regulator of cellular energy metabolism are negatively associated with obesity. Moreover, this is the first study that demonstrated the association between EAT miR-1247-5p expression levels and BMI.

SS089 / #1247

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.09 Aortic valve stenosis*

DIABETES PROMOTES EARLY PROGRESSION OF CALCIFIC AORTIC VALVE DISEASE IN TWO ANIMAL MODELS OF DIABETIC CAVD

SAAG SESSION 14: NAFLD AND DIABETES

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Background and Aims: Calcific aortic valve disease (CAVD) is the most common valvulopathy in the Western world. Diabetes contributes to the progression of CAVD, but the pathophysiological mechanisms are still not completely understood. Therefore, we aimed to assess the molecular processes leading to diabetic CAVD. In particular, we investigated the effects of a high glucose treatment in isolated VICs and of hyperglycemic conditions on two animal models of CAVD.

Methods: LDLr$^{-/-}$ and LDLr$^{-/-}$:ApoB$^{100/100}$ mice were fed a diabetogenic (HFD) or control diet (NC) for 6, 12 and 26 weeks; then, the aortic valves were collected for bulk RNA sequencing analyses. For the *in vitro* system, non-human primate VICs were treated with low- or high-glucose media (5.5 mM or 25 mM) for 5 days and inorganic phosphate (2.6 mM) to induce their osteogenic differentiation. Total RNA was extracted for gene expression analysis and calcium deposition was determined through a colorimetric assay.

Results: The transcriptomic analyses detected an overexpression of inflammatory, immune, and diabetes-related pathways in HFD-fed LDLr$^{-/-}$:ApoB$^{100/100}$ mice. Moreover, HFD downregulated cardioprotective genes (including TBX5, GATA5, and NKX2-5) in both animal models of CAVD. In our *in vitro* system, high glucose treatment did not affect calcium deposition, while it modified osteogenic markers and significantly downregulated cardioprotective genes.

Conclusions: Diabetogenic diet induces inflammatory and immune processes, accelerating early progression of CAVD. Moreover, hyperglycemic conditions downregulate cardioprotective genes in both our *in vivo* and *in vitro* systems. These preliminary findings give new insights on the pathophysiology of diabetic CAVD and may lead to the discovery of novel therapeutic targets.

Topic: *AS02 Lipids and Lipoproteins / AS02.11 Liver metabolism and steatosis*

ROLE OF OPA1-MEDIATED MITOCHONDRIAL DYNAMICS IN KUPFFER CELLS ON SYSTEMIC METABOLISM

SAAG SESSION 14: NAFLD AND DIABETES

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Background and Aims: Kupffer Cells are resident macrophages that are essential for liver pathophysiology as they play a crucial role in the innate immune response. OPA1 (Optic Atrophy 1) is a dynamin-related protein located in the inner mitochondrial membrane with a pro-fusion activity that modulates mitochondrial dynamic controlling oxidative phosphorylation. Since mitochondria are pivotal for physiological energy demand by KCs, this project aims to study the role of mitochondrial dynamic within these cells and its impact on systemic lipid metabolism exploiting mice selectively lacking OPA1 in KCs.

Methods: Mice C57BL/6J OPA1 Flox/Flox Clec4F-Cre+ and control Cre- littermates (N=7:7) were fed with Chow or High Fat Diet (HFD) for 20 weeks. The metabolic phenotype was assessed by indirect calorimetry through metabolic cages. Blood and liver were collected for immunophenotyping by flow cytometry analysis and for total lipids dosages. Liver histology was characterized with tissue stainings. Statistical significance was assessed through Unpaired T-test.

Results: OPA1 Flox/Flox Clec4F-Cre+ showed less energy expenditure (-9.44%; $p<0.05$), less O_2 consumption (-9.44%; $p<0.05$), less CO_2 production (-9,48%; $p<0.01$), despite an increase in movement (+26.6%) compared to Cre- control mice under ChowD; no differences were reported under HFD. Systemic immune profile was similar, while the percentage of KCs in the liver was reduced in Cre+ mice (-25% $p<0.05$). No significant differences in cholesterol and triglycerides levels were observed as well as in liver histology, glucose and insulin sensitivity tests under ChowD or HFD.

Conclusions: Our data suggest that OPA1-mediated mitochondrial function in KCs differently impacts systemic metabolic response to Chow or HFD feeding.

SS091 / #521

Topic: *AS02 Lipids and Lipoproteins / AS02.11 Liver metabolism and steatosis*

AN INTEGRATED UNDERSTANDING OF THE METABOLIC BENEFITS OF A NOVEL DOUBLE-TARGETED GENETICALLY ENGINEERED PROBIOTIC EXPRESSING ALDAFERMIN INTERVENTION WITH DIETARY CHANGE ON NAFLD

SAAG SESSION 14: NAFLD AND DIABETES

Ambrin Farizah Babu, Valeria Iannone, Johnson Lok, Carlos Gomez-Gallego, Giuseppe D'Auria, Ruben Vazquez-Uribe, Jussi Pihlajamaki, Morton Sommer, Kati Hanhineva, Hani El-Nezami, Marjukka Kohlemainen
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Background and Aims: Lifestyle changes toward a healthy diet and increased physical activity are the cornerstone interventions in the treatment of non-alcoholic fatty liver disease (NAFLD), the most common liver disease worldwide. However, due to its increased prevalence, new therapeutic approaches targeting the gut-liver-axis such as the use of microbial therapeutics and gut-hormonal interventions have been suggested.

Methods: The present study introduces a seven-week double-targeted intervention using the probiotic *Escherichia coli* Nissle 1917 genetically engineered to continuously express aldafermin (a non-tumorigenic analog of a human intestinal peptide hormone, fibroblast growth factor 19) along with dietary change (EcNA). The safety, efficacy, and mechanisms of action of the EcNA intervention were demonstrated using a high-fat-diet-induced NAFLD mouse model.

Results: The beneficial effects of the EcNA intervention were evidenced by the decrease in body weight, liver steatosis, and plasma concentrations of aspartate aminotransferase and cholesterol. Comprehensive integrated transcriptomics and non-targeted metabolomic analyses further revealed alterations in NAFLD-related genes and metabolites along with a switch in pathways related to the amino acid and lipid metabolism. Key alterations in gut microbial metabolism and associated receptor-signaling pathways were also observed.

Conclusions:
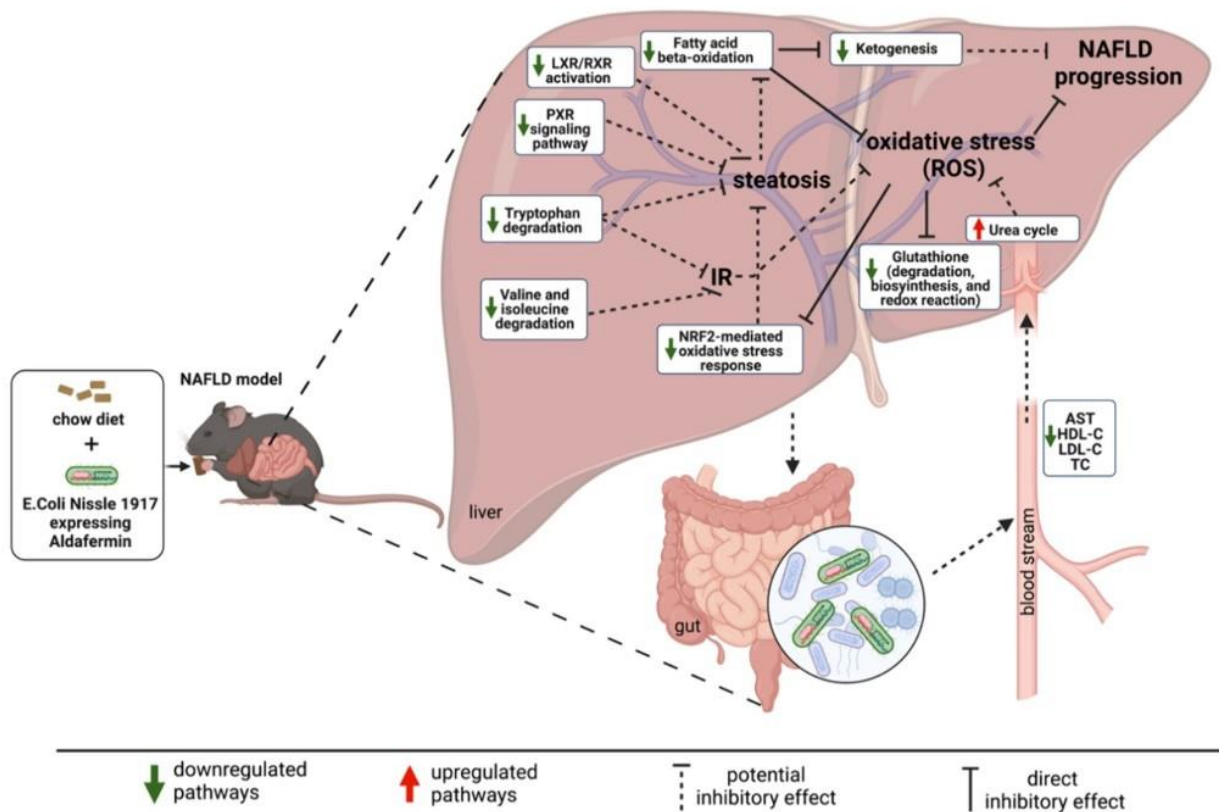
These results suggest the potential efficacy of EcNA in ameliorating NAFLD by decreasing insulin resistance, steatosis, and oxidative stress; and highlight the potential of exploring multi-targeted interventions combining microbial therapeutics with the diet for NAFLD.

SS092 / #786

Topic: *AS02 Lipids and Lipoproteins / AS02.11 Liver metabolism and steatosis*

GLUCOKINASE REGULATORY PROTEIN (GCKR) IN MEDIATING THE GENETIC RISK OF NAFLD

SAAG SESSION 14: NAFLD AND DIABETES

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Background and Aims: Glucokinase regulatory protein (GCKR) is one of the most pleiotropic loci of the human genome, and is associated with T2D, NAFLD, and CAD, along with associations to blood level of lipids, lipoproteins, carbohydrates, and amino acids. Altered *GCKR* expression through CRISPR perturbation of rs780094 enhancer demonstrated a broad trans-effect on gene expression that includes genes involved in the control of carbohydrate metabolism. Prominently involved in this trans-effect are also genes that regulate cholesterol synthesis and lipoprotein uptake/secretion by the liver. In this study we try to address the current knowledge gap in understanding the functional bases for GCKR associations by studying the cellular, tissue specific and systemic effects of GCKR deficiency in mouse.

Methods: We developed a transient *GCKR* knock-down mouse model using antisense oligonucleotides (ASOs) wherein two candidate ASOs were administered for 6 weeks under specific diet. With the help of this model we try to address our aims, primarily by functional characterization of the mouse model using liver phenotyping, RNA-seq, metabolomics and histopathology.

Results: Our results show increase in basic metabolite levels like HDL, LDL and total cholesterol. These results are recapitulated by our RNA-seq and histopathology analysis. Results from liver tissue staining also revealed increased macrosteatosis and hepatic inflammation in mice fed with HFD. These findings are in line with our hypothesis that GCKR deficiency induces systemic metabolic dysregulation and exacerbates NAFLD phenotype.

Conclusions: We aim to define the physiological consequences of GCKR deficiency with our work, thereby laying the groundwork for development of novel therapeutic strategies for related metabolic conditions.

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.03 Diabetes, insulin sensitivity and resistance*

CORRELATION OF FATTY INDEX LIVER WITH INCIDENT DIABETES RISK IN STATIN-TREATED PATIENTS: A 6-YEAR RETROSPECTIVE STUDY

SAAG SESSION 14: NAFLD AND DIABETES

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Background and Aims: To investigate the correlation of fatty index liver index (FLI) with the development of type 2 diabetes mellitus (T2DM) in patients initiating statin-therapy.

Methods: A retrospective observational study including 1,241 individuals with dyslipidemia and followed-up for ≥3 years. Patients with T2DM and those receiving lipid-lowering treatment at baseline visit were excluded. Models with clinical and laboratory parameters were used to assess the effect of FLI on incident T2DM risk. FLI index is used as a prognostic score for the diagnosis of non-alcoholic fatty liver disease (NAFLD) and takes into account body mass index (BMI), waist circumference (waist), triglycerides (TG) and gamma-glutamyltransferase (gGT) levels [FLI=$e^y/(1+e^y)$×100, y=0.953×ln(TG,mg/dL)+0.139×BMI,kg/m^2+0.718×ln(gGT,U/L)+0.053×waist,cm-15,745].

Results: Among 882 subjects 11% developed T2DM during follow-up (6 years; IQR:4-10 years). After taking into account subjects' sex, age and metabolic syndrome parameters, multivariate analysis revealed that age (HR:1.05; 95% CI:1.01-1.09, $p<0.05$), fasting plasma glucose (HR:1.09; 95% CI:1.06-1.13, $p<0.05$) and FLI (HR:1.02; 95% CI:1.01-1.04, $p<0.05$) were significantly and independently associated with the risk of incident T2DM. Subjects with probable NAFLD (FLI ≥60) had a 3-fold increased risk of new-onset T2DM compared to subjects with FLI <60 (adjusted HR:3.14; 95% CI:1.50-6.59, $p<0.05$). ROC curve analysis showed that FLI had a significant predictive value for assessing incident T2DM risk (C-Statistic:0.67; 95% CI: 0.58-0.77, $p<0.05$). Higher FLI was associated with reduced T2DM-free survival (log-rank=15.46, $p<0.05$).

Conclusions: FLI is significantly and independently associated with new-onset T2DM risk in dyslipidemic patients initiating statin therapy.

SS094 / #463

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.04 Adipose tissue homeostasis*

THE EFFECT OF GLUCAGON-LIKE PEPTIDE 1 AGONISTS ON WHITE AND BROWN ADIPOSE TISSUE ACCORDING TO MAGNETIC RESONANCE SPECTROSCOPY IN OBESE PATIENTS.

SAAG SESSION 14: NAFLD AND DIABETES

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Background and Aims: assessment the ratio and dynamics of changes in the ratio of white and brown adipose tissue using magnetic resonance spectroscopy (MRS) in patients with cardiovascular diseases, obesity, diabetes mellitus before and after glucagon-like peptide type 1 (GLP 1) agonists treatment

Methods: 15 patients with a body mass index (BMI) over or equal 30 kg/m2, cardiovascular diseases and type 2 diabetes mellitus were included. Initially and after 6 months of therapy with GLP 1 agonists, MRS of the adipose tissue of the supraclavicular region, liver and subcutaneous adipose tissue of the neck was performed

Results: In obese patients on the therapy with GLP 1 agonists, there was a positive trend - decrease of the volume of adipose tissue in the supraclavicular region - (0.95[0.94;0.96] versus 0.93[0.91; 0.95] p<0.001), as well as a significant decrease in the level of triglycerides in the liver (0.13±0.1 versus 0.06±0.04

Characteristics of patients on GLP-1 agonist therapy. (n=15)

Parameters	Value
Age, years	$52,8 \pm 9,8$
Men	8 (53,3 %)
Smoking	1 (6,7%)
Arterial hypertension	13 (86,7%)
CHD	4 (26,7 %)
Myocardial infarction	1 (6,7 %)
Coronary bypass surgery	0 (0%)
Coronary artery stenting	3 (20 %)
Atherosclerosis of the carotid arteries	5 (33,3 %)
Metabolic syndrome	3 (20 %)
Diabetes mellitus type 2	5 (33,3 %)
Fatty liver	10 (66,6 %)
Therapy	
Statins	13 (86,7 %)
Statins and esetimib	2 (13,3 %)
Metformin	3 (20 %)
Sulfonylurea	0 (0 %)
Empagliflozin	1 (6,7 %)
Liraglutid	4 (26,7 %)
Semaglutid	11 (73,3 %)

$p < 0.01$)

Parameters	1 visit n=15	2 visit n=15	P value
Weight, kg	99,2±16,8	87,2±13,5	**0,04**
BMI, kg/m^2	33,7±5,0	29,6±4,0	**0,02**
Waist circumference, cm	108,5±16,0	95,2±12,8	**0,02**
Laboratory indicators			
TC, mmol/l	5,5±1,9	4,1±1,3	**0,03**
LDL-C, mmol/l	3,5±1,7	2,2±1,2	**0,02**
HDL-C, mmol/l	1,1 [1,0;1,5]	1,4 [1,1;1,6]	0,13
TG, mmol/l	2,1±0,9	1,5±0,1	**0,008**
Glucose, mmol/l	5,2 [4,8;5,3]	5,1 [5,0;5,5]	0,96
Magnetic resonance spectroscopy indicators			
	1 visit	**2 visit**	**P value**
Supraclavicular region, percentage of triglycerides	0,95[0,94;0,96]	0,93[0,91;0,95]	**<0,001**
Subcutaneous fat, percentage of triglycerides	0,98[0,97;0,98]	0,98[0,96;0,99]	0,63
Liver, percentage of triglycerides	0,13±0,1	0,06±0,04	**<0,01**

Conclusions: A decrease in the level of triglycerides and an increase in the water level in the supraclavicular region, the liver, according to the MRS, can be considered as an increase in the volume of brown adipose tissue against on the therapy with GLP 1 agonist.

SS095 / #828

Topic: *AS04 Clinical Vascular Disease / AS04.03 NASH and other ectopic lipid diseases*

FIBROSIS 4 SCORE AND VASCULAR CHANGES IN DIABETES MELLITUS TYPE 1

SAAG SESSION 14: NAFLD AND DIABETES

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Background and Aims: Metabolic associated fatty liver disease and cardiovascular diseases are closely linked. In this respect, fibrosis 4 score (FIB4S), ((age x AST)/(platelets x (sqr(ALT)), is used to rule out advanced liver fibrosis but it could also indicate risk for cardiovascular disease. The aim of our study was to analyze association between FIB4S and macro- and microvascular parameters in patients with diabetes mellitus type 1 (DMT1).

Methods: Patients with DMT1 were included into the study (260 men and 260 women, mean age 43.5 ± 1.3 years; median of duration of diabetes: 21.5 years, IQR 14.5-28.6 years). FIB4S was correlated with ankle to brachial index (ABI), toe to brachial index (TBI), interbranch index of pulse wave analysis obtained by photoplethysmography, reflecting mainly microvascular involvement:Oliva-Roztocil Index (ORI) and to pulse pressure (PP). The association between FIB4S and vascular parameters was analyzed by multivariate analysis standardized for duration of diabetes, HbA1c, sex, smoking, waist circumference and non-HDL cholesterol.

Results: FIB4S above risk values of 2.67 was present in 9 men and 2 women. FIB4S significantly correlated with all vascular parameters under study: ABI, TBI, ORI, and PP (r=0.14, r= - 0.29, r= 0.34, and r=0.34); including multivariate regression (p=0.003, p=0.001, p=0.009, and p= 0.001).

Conclusions: In DMT1, FIB4S index even when in normal range was associated with less favorable values of vascular but mainly of microvascular parameters and with decreased arterial compliance; the latter reflected by positive correlation with ABI and PP. FIB4S index might be considered for vascular risk assessment in patients with DMT1.

SS096 / #1200

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.03 Macrophages in lipid metabolism and atherosclerosis*

GLUTAMINE METABOLISM SHAPES MACROPHAGE PLASTICITY

SAAG SESSION 15: MODULATING INFLAMMATION IN ATHEROSCLEROSIS

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Background and Aims: Glutamine metabolism is considered as a "fuel for the immune system" and we demonstrated that conversion of glutamine to glutamate through glutaminase Gls1 activity supported macrophage clearance and repair functions. Unexpectedly, modulating exogenous glutamine concentrations in culture medium exerted Gls1 independent metabolic reprogramming indicating that macrophages can rely on alternative glutamine pathways. However, the underlying mechanisms remain poorly understood.

Methods: Macrophages express another isoform called Gls2 and could modulate their flux through an intrinsic synthesis depending on glutamine synthase (Gs). To test their roles in the metabolic and functional plasticity of macrophages, we generated Gls1 and Gls2 double deficient macrophages and modulated GS activity with the methionine sulfoximine (MSO) inhibitor.

Results: Metabolic activity indicates that Gls2 deficiency had no major effect on glycolysis (i.e,ECAR) or oxygen consumption rate (i.e,OCR). But, GS inhibition with MSO reduced OCR and this effect was additive to Gls1 deficiency upon IL-4 stimulation. This suggested that glutamine synthesis and degradation are exquisitely linked through a feedback loop to support mitochondrial reprogramming. Testing canonical macrophage M2 markers or efferocytosis functionality (flow cytometry) confirmed that absence of Gls1 reduced both functions and this was independent of Gls2 expression. Inhibition of GS also reduced macrophage repair and clearance functions. We confirmed by RNA sequencing that the reduced glutamate generation in Gls1 deficient cells impacted mitochondrial oxidative phosphorylation genes. GS inhibition indicated modulation of mitochondrial oxidative phosphorylation expression and the hexosamine pathway genes.

Conclusions: Our results provide a deeper understanding on how glutamine homeostasis controls the metabolic flexibility and macrophage plasticity of macrophages.

SS097 / #647

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.03 Macrophages in lipid metabolism and atherosclerosis*

REVERSING ATHEROSCLEROSIS BY THE SPECIFIC REMOVAL OF OXIDIZED CHOLESTEROL WITH CYCLODEXTRIN DIMER

SAAG SESSION 15: MODULATING INFLAMMATION IN ATHEROSCLEROSIS

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Background and Aims: Cardiovascular disease (CVD) is the world's biggest killer. Current treatments for CVD are failing to deliver on promises to beat this deadly and complex family of diseases. New approaches that go beyond targeting LDL are needed. Cyclarity Therapeutics has developed a novel class of specifically engineered, dimerized cyclodextrin (CD) molecules for the encapsulation of toxic oxidized cholesterol. Oxidized cholesterol accumulates over time and causes dysfunction in many cell types, linking it to several age-related diseases including atherosclerosis. Here, we present a synergistic rational drug design strategy for developing CDs to remove atherogenic oxidized cholesterol (primarily 7-ketocholesterol (7KC)) from cells and tissues.

Methods: A combination of in silico, in vitro, and ex vivo methods are used to implement a synergistic rational drug design strategy for developing CDs to remove atherogenic oxidized cholesterol (primarily 7-ketocholesterol (7KC)) from cells and tissues.

Results: Our results thus far indicate that our lead compound, UDP-003, can both prevent and reverse the formation of atherogenic foam cells in-vitro. UDP-003 binds and clears 7KC from in-vivo and ex-vivo systems selectivity. IND enabling studies, including pivotal GLP studies in rodent and non-rodent species, are complete and show that UDP-003 has an excellent safety profile with no significant clinical liabilities. UDP-003 has been awarded the Innovative Licensing and Access Pathway (ILAP) Innovation Passport by the MHRA.

Conclusions: Our data suggest that targeted removal of 7KC from foam cells with UDP-003 has the potential to prevent and reverse the formation of atherosclerotic plaques. This innovation represents the first disease-modifying therapeutic approach to treating atherosclerotic disease.

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.11 Plaque remodelling*

ATHEROSCLEROTIC PLAQUES IN THE CAROTID ARTERY OF HYPERCHOLESTEROLEMIC YUCATAN MICROSWINE: EFFECT OF TLR4 ANTAGONISM

SAAG SESSION 15: MODULATING INFLAMMATION IN ATHEROSCLEROSIS

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Background and Aims: Rupture of vulnerable atherosclerotic plaque is a major cause of acute cardiovascular events. Prevention of atherosclerotic plaque formation and stabilization of vulnerable plaque will be of therapeutic significance to decrease the number of acute events. Chronic inflammation within the plaque leads to plaque vulnerable leading to rupture and thrombus formation. Toll-like receptor (TLR)-4 plays a critical role in chronic inflammation and atherosclerotic plaque formation. Thus, we inhibited TLR-4 signaling with a selective inhibitor, TAK-242, in a microswine model of carotid artery atherosclerosis.

Methods: Hypercholesterolemic Yucatan microswine were subjected to intimal injury in carotid artery with balloon angioplasty to induce fatty streaks and plaque formation. Swine were treated with vehicle or TAK-242 at the time of intimal injury and sacrificed after 5-6 months. Carotid arteries were evaluated for vessel wall thickness, blood flow, blood volume, lumen area using color doppler ultrasound, angiography, and optical coherence tomography (OCT) at baseline, 6-weeks after surgery, and before sacrifice at 5-6 months after the initial surgery.

Results: TAK-242 decreased neointimal hyperplasia and plaque formation. Histomorphologically, neointimal hyperplasia with significantly increased inflammation and elastin degradation was found in the vehicle-treated group compared to TAK-242. Decreased mRNA expression of TLR-4, MyD88, TREM-1, and COLI and increased COLIII and MMP-9 expression were found in TAK-242-treated swine compared to vehicle-treated swine. Further, the gene and protein expression for plaque vulnerability biomarkers (MMP-7, IL-6, IL-12/23, CD36) were significantly decreased with TAK-242 compared to vehicle.

Conclusions: These findings support the therapeutic efficacy of inhibiting TLR-4 signaling to prevent the occurrence of TIA and stroke.

SS099 / #169

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.01 Epidemiology of cardiovascular diseases and risk factors*

PLASMA C-REACTIVE PROTEIN IS ASSOCIATED WITH AN ADVERSE AND PRO-INFLAMMATORY PLAQUE PHENOTYPE

SAAG SESSION 15: MODULATING INFLAMMATION IN ATHEROSCLEROSIS

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Background and Aims: Low-grade inflammation plays an important role in atherosclerotic cardiovascular disease (ASCVD) with high sensitivity C-reactive protein (hsCRP) as a central biomarker. Plaque characteristics have emerged as major determinants for ASCVD risk, but the association with low-grade inflammation remains obscured.

Methods: In this study, we evaluated plaque differences and future ASCVD events in patients with high (≥2 mg/L) versus low (<2 mg/L) hsCRP plasma levels before undergoing carotid or femoral endarterectomy (CEA/FEA) in the Athero-Express study. Cox regression for ASCVD outcomes and logistic regression for plaque phenotype analyses were multivariable adjusted.

Results: A total of 1344 patients were included. Patients with a hsCRP ≥2 mg/L (627; 46.7%) had a higher risk of MACE and CAD compared to patients with low CRP, HR 1.46 (95% CI: 1.11-1.90, p=0.006) and 2.00 (95% CI: 1.02-2.93; p=0.04) respectively. On plaque characteristics, these patients had increased vessel density, OR 1.53 (95% CI 1.18-2.10, p =0.004) and increased calcification, OR 1.45 (95% CI 1.09-1.94; p=0.01). Soluble endothelial adhesion molecule (sICAM-1) and pro-inflammatory cytokine IL-6 plaque levels were significantly higher in the high CRP group (1073.88 [699.07, 1554.49] vs. 863.36 [562.31, 1387.95]; p=0.029 and 43.55 [13.22, 122.32] vs. 34.00 [11.28, 89.89]; p=0.048

respectively).

Low-grade inflammation and plaque phenotype and future ASCVD events

1344 CEA/FEA patients included

hsCRP ≥2 mg/L in 627 patients (46.7%)

3 year follow-up

Adverse plaque characteristics

Intraplaque inflammation

Hazard ratios

MACE: 1.46 (95% CI: 1.11-1.90, p=0.006)

CAD: 2.00 (95% CI: 1.02-2.93; p=0.04)

Odds ratios

Vessel density: 1.53 (95% CI 1.18-2.10, p=0.004)

Calcification: 1.45 (95% CI 1.09-1.94; p=0.01)

Plaque levels

sICAM-1: 1075 [699, 1554] vs. 863 [562, 1388]; p=0.029

IL-6: 44 [13, 122] vs. 34 [11, 90]; p=0.048

Conclusions: Plasma hsCRP levels of ≥2 mg/L are associated with vulnerable plaque characteristics and a higher ASCVD risk. These data suggest that a systemic pro-inflammatory state is reflected in both an adverse and pro-inflammatory plaque phenotype and an increased ASCVD risk.

SS100 / #526

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.07 Environmental risk factors*

EXPOSURE TO AIR POLLUTION FINE PARTICULATE MATTER (PM2.5) PROMOTES ADIPOSE TISSUE INFLAMMATION AND OBESITY BY IMPAIRING THERMOGENESIS

SAAG SESSION 15: MODULATING INFLAMMATION IN ATHEROSCLEROSIS

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Background and Aims: Clinically, exposure to air pollution fine particulate matter ($PM_{2.5}$) is associated with the development of cardiometabolic disorders. We aim to study the mechanisms that link $PM_{2.5}$ inhalation with inflammation, impaired metabolism, and obesity.

Methods: C57BL/6 mice received 1 mg/kg of a $PM_{2.5}$ surrogate (ROFA, Residual Oil Fly Ash) or saline (control) by intranasal instillation, or inhaled $PM_{2.5}$ in a real-life mice model of exposure to polluted urban air for 16 weeks.

Results: ROFA-exposed mice showed a biphasic lung inflammatory cell recruitment, with neutrophils peaking at 6 h and macrophages at 72 h, together with increased proinflammatory TNF-α, IL-6, and CCL2. mRNA sequencing of sorted alveolar macrophages from ROFA-exposed mice revealed a proinflammatory gene expression signature and upregulated pathways for lipid metabolism. Differentially expressed genes, including CCL3, were validated by a customized cytokine bead assay in BAL and plasma, and were increased for up to 72 h in ROFA-exposed mice. Decreased metabolic gene expression (*Ucp1, Elovl3, Adrb3*) in brown adipose tissue suggests reduced lipolysis and thermogenesis, despite ongoing white adipose tissue inflammation. In metabolic cages, despite enhanced physical activity, ROFA-exposed mice showed significantly reduced heat production. In a biologically-relevant model of exposure to polluted air, increased weight gain, impaired glucose homeostasis, and adipose tissue inflammation were observed in mice breathing urban air (27±8 µg $PM_{2.5}/m^3$) versus filtered air (2±1 µg $PM_{2.5}/m^3$), together with altered metabolic gene expression in brown adipose tissue.

Conclusions: Our findings indicate that $PM_{2.5}$ induce a pulmonary and systemic proinflammatory state that blunts metabolic pathways in adipose tissue and promotes obesity.

SS101 / #1024

Topic: *AS04 Clinical Vascular Disease / AS04.15 Other*

EFFECTS OF CARDIAC REHABILITATION ON INFLAMMATORY BIOMARKERS IN UNSTABLE ISCHEMIC HEART DISEASE PATIENTS FOLLOWING PERCUTANEOUS CORONARY INTERVENTION: A RANDOMIZED CONTROLLED STUDY

SAAG SESSION 15: MODULATING INFLAMMATION IN ATHEROSCLEROSIS

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Background and Aims: This study aimed to evaluate these inflammatory markers such as high-sensitivity C-reactive protein (hs-CRP), neutrophil-to-lymphocyte ratio (NLR), and platelet-to-lymphocyte ratio (PLR), after cardiac rehabilitation in patients with unstable ischemic heart disease (UIHD) who underwent successful percutaneous coronary intervention (PCI).

Methods: A cohort of 115 patients with successful PCI due to UIHD enrolled in the study from January 2018 to March 2021. We used a permuted block stratified randomization technique (2:1 ratio). Seventy-seven patients were randomized to the cardiac rehabilitation (CR) group and 38 patients to the control group. The CR group underwent a 12-week pre-specified CR regimen. Blood samples were taken at baseline and follow-up at 12 weeks for both groups. The baseline and follow-up characteristics were evaluated using parametric and non-parametric tests.

Results: Among the 115 patients, 33 patients were female. The mean age was (53±5.55 years) in the control and (53±6.09 years) in the CR group. The two groups were comparable regarding their baseline characteristics and the values of the inflammatory markers. By contrast, at 12 weeks, the inflammatory marker values were significantly lower in the CR group compared to the control group; hs-CRP: 0.11 [0.08,0.14] vs. 0.21 [0.19,0.21], p value<0.001; NLR: 2.17 [1.42,2.43] vs. 2.26 [2.07,2.6] , p-value: 0.016; PLR: 91.2821 [63.3333,103.2000] vs. 92.600 [84.6154,110.0000], p-value: 0.027.

Conclusions: Cardiac rehabilitation after PCI in UIHD patients may attenuate some inflammatory markers, which might benefit cardiovascular health. Further studies are required to evaluate these findings with longer follow-up and the powered to measure major cardiovascular event rates.

SS102 / #1476

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.17 Other*

HYALURONAN-SYNTHASE 3 DERIVED HYLAURONAN PROMOTES LEUKOCYTE INFILTRATION IN A MURINE MODEL OF ABDOMINAL AORTIC ANEURYSM

SAAG SESSION 15: MODULATING INFLAMMATION IN ATHEROSCLEROSIS

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Background and Aims: Abdominal aortic aneurysm (AAA) is accompanied by continuous degradation of the extracellular matrix, apoptosis of smooth muscle cells (SMCs), and infiltration of immune cells leading to progressive dilatation and potential rupture of the aortic wall. Previous work showed HA-synthase (HAS)-3-derived hyaluronan (HA) affects the inflammatory response as well as the SMC phenotype. Therefore, aim of this study was to elucidate the role of HA and HAS3 in AAA formation.

Methods: AAA was induced in *Apoe*-knockout (*Apoe*-KO) and *Apoe/Has3*-double deficient (DKO) mice using Angiotensin-II (Ang-II). Aortae were collected for immunohistochemical stainings of elastin and collagen after 28 days of Ang-II-treatment. Flow cytometry of aortae and blood was performed on day 7 and 28 after pump implantation. Aortic gene expression was assessed by bulk RNA sequencing of aortae on day 3 of Ang-II-treatment.

Results: Our data showed an improved survival of DKO compared to *Apoe*-KO after 28 days of Ang-II treatment. Fewer collagen deposition and fewer elastic breaks were observed within the aortic media of DKO mice. Further, increased levels of circulating myeloid leukocytes, and total monocytes were detected in DKO as compared to *Apoe*-KO. Fewer leukocytes were detected within the aortic wall, suggesting a decreased leukocyte recruitment in DKO mice. Indeed, isolated monocytes showed decreased mRNA expression of inflammatory markers and reduced transmigration *ex vivo*.

Conclusions: In conclusion, *Has3* deficiency attenuates AAA development. Decreased immune cell infiltration of myeloid leukocytes into the aortic wall in *Has3*-KO mice resulting in fewer elastic breaks plays a pivotal role in the progression and mortality of AAA.

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.04 Inflammation, immunity and infection in atherosclerosis*

LEUKEMIA INHIBITORY FACTOR RECEPTOR INHIBITION IN ATHEROSCLEROSIS

SAAG SESSION 16: IMMUNE BIOLOGY IN ATHEROSCLEROSIS

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Background and Aims: Single-cell RNA sequencing data of human carotid artery plaques showed that mast cells highly express Leukemia Inhibitory Factor(LIF). Additionally, the LIF receptor(LIFR) was specifically expressed on endothelial cells. Interaction analysis showed a possible interaction between mast cell specific LIF and LIF receptors expressed on endothelial cells. In this study, LIFR signaling in atherosclerosis will be elucidated by systemically inhibiting LIF receptors in an atherosclerotic mouse model.

Methods: Bone marrow derived mast cells (BMMCs) were sensitized with antiDNP-IgE and activated with DNP to measure LIF production by ELISA. Activated BMMCs (244 ± 58 pg/mL) produced significantly more LIF compared to control BMMCs (75 ± 3.4 pg/mL, $p<0.05$). To study LIFR signaling *in vivo*, female Western-type diet-fed LDLr$^{-/-}$ mice, 9-15 weeks old, were treated with LIFR inhibitor EC359 (5 mg/kg s.c., n=15) or control solvent (n=15) three times per week for eight weeks.

Results: During the experiment, weights of the mice did not differ between groups, whereas cholesterol levels were significantly reduced at week two and five weeks of EC359 treatment. After eight weeks, mice were sacrificed and hearts were isolated to determine atherosclerotic plaque size and composition by histology. LIFR inhibition in LDLr$^{-/-}$ mice ($34.9\pm1.9\%$) reduced atherosclerotic plaque size compared to control mice ($39.8\pm1.4\%$; $p=0.05$), but did not affect collagen and monocyte/macrophage content.

Conclusions: Conclusively, these findings suggest that murine BMMCs are able to produce LIF upon FcɛR-mediated activation *in vitro* and that systemic LIFR inhibition *in vivo* reduces atherosclerotic plaque size, but does not alter plaque composition.

SS104 / #364

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.04 Inflammation, immunity and infection in atherosclerosis*

SINGLE-CELL PROFILING REVEALS AGE-ASSOCIATED IMMUNE CELLS IN ATHEROSCLEROSIS

SAAG SESSION 16: IMMUNE BIOLOGY IN ATHEROSCLEROSIS

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Background and Aims: Aging is a dominant driver of atherosclerosis and induces a series of immunological alterations, called immunosenescence. Given the demographic shift towards elderly, elucidating the unknown impact of aging on the immunological landscape in atherosclerosis is highly relevant. While the young Western diet-fed *Ldlr*-deficient (*Ldlr$^{-/-}$*) mouse is a widely used model to study atherosclerosis, it does not reflect the gradual plaque progression in the context of an aging immune system as occurs in humans.

Methods: We used chow diet-fed and Western diet-fed young *Ldlr$^{-/-}$* mice, and chow diet-fed aged *Ldlr$^{-/-}$* mice to study age-associated immune changes with flow cytometry. By performing histological analysis and single-cell RNA-sequencing (scRNA-seq), we compared plaque morphology and aortic leukocytes in these mice. Lastly, we used flow cytometry to investigate the presence of age-associated immune cells in blood and atherosclerotic plaques of cardiovascular disease patients.

Results: We show that aging promotes advanced atherosclerosis in chow diet-fed *Ldlr$^{-/-}$* mice, with increased incidence of calcification and cholesterol crystals. We observed systemic immunosenescence, including myeloid skewing and T-cells with more extreme effector phenotypes. Using scRNA-seq on aortic leukocytes of young versus aged *Ldlr$^{-/-}$* mice, we identified age-associated cells with pro-inflammatory features, including GzmK$^+$CD8$^+$ T-cells and previously undefined T-bet$^+$ age-associated B-cells, and confirmed their presence in human atherosclerotic plaques. Moreover, we show age-related shifts in atherogenic processes, including phagocytosis, antigen-presentation, cellular activation, and cytokine production.

Conclusions: Collectively, this immune atlas of the aged atherosclerotic plaque enhances our understanding of disease etiology, providing a resource to identify detrimental cellular targets and pathways in atherosclerosis.

SS105 / #469

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.04 Inflammation, immunity and infection in atherosclerosis*

ROLE OF LIGHT/TNFSF14 IN LYMPHOCYTE POPULATIONS IN THE CONTEXT OF ATHEROSCLEROSIS

SAAG SESSION 16: IMMUNE BIOLOGY IN ATHEROSCLEROSIS

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Background and Aims: Previous studies by us have shown that deficiency in the cytokine LIGHT (TNFSF14) in Apolipoprotein e-deficient mice (*Apoe-/-*) aggravates atheroma by modulating immune Th subpopulations and regulatory T cell (Treg) homeostasis in adventitial and circulating blood. The role of LIGHT was further investigated by performing *in vivo* transcriptomics studies in atheromas from *Apoe-/-* and *Apoe-/-Light-/-* mice and *in vitro* studies using human lymphocytes.

Methods: *Apoe-/-* and *Apoe-/-Light-/-* mice were fed on an atherogenic diet for 12 weeks and sacrificed for gene expression analysis by mRNA sequencing (RNAseq) of the adventitia-free aortic arch. To study the role of LIGHT *in vitro*, CD4+ lymphocytes were isolated from human blood, differentiated (during 7 days) and treated with LIGHT for 72 hours for flow cytometry and RT-qPCR gene expression analysis. The effect of LIGHT on T cell proliferation by BrdU incorporation for 24 hours was explored in vehicle/LIGHT-treated cells.

Results: The RNAseq study showed that *Apoe-/-Light-/-* mice under-expressed different genes related to lymphocyte differentiation, activation and proliferation, such as *Cd5l, Tnfrsf1b, Cdkn1c, Tec, Mertk* and *Syk* (**Fig.1**). Treatment of CD4+ lymphocytes with LIGHT increased the percentage of Treg cells, Treg/Th17 cell ratio, the protective/anti-inflammatory Th2 proliferation and Tec, Cd5l (negative regulators of pathogenic Th17 cells) and Cdkn1c (an inhibitor of cell proliferation) gene expression (**Fig.2**).
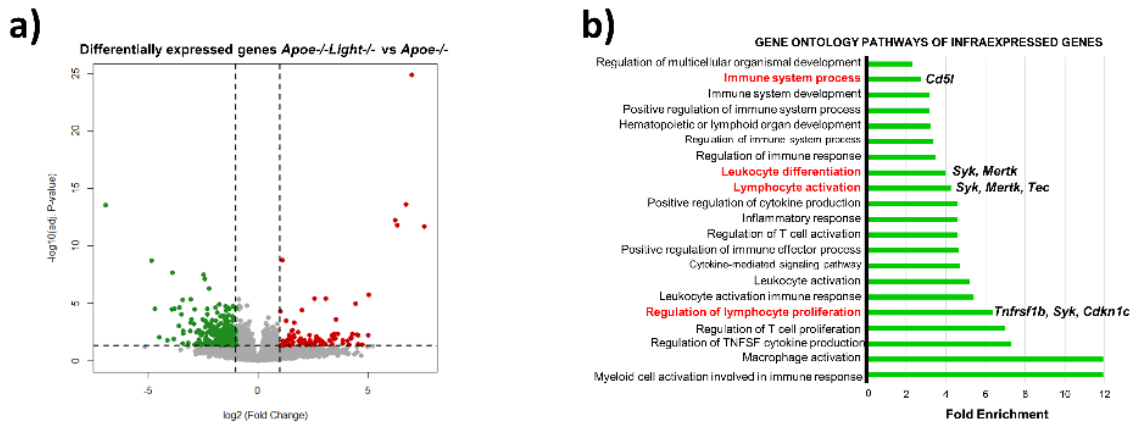
Figure 1. Transcriptomic analysis by RNAseq of atheromas of *Apoe-/-* and *Apoe-/-Light* mice. a) Volcano plot showing differentially expressed genes between *Apoe-/-Light-/-* and *Apoe-/-* mice. Dashed lines indicate the p-value and Fold Change (FC) values used as criteria for the identification of the differentially expressed genes: horizontal: p-value<0.05, vertical: log$_2$FC of -1 and 1. Red dots represent transcripts up-expressed by *Apoe-/-Light-/-* (84 genes), while green dots represent the 308 transcripts with decreased expression. **b)** Biological processes mostly enriched among the under-expressed genes. GO terms (biological processes, vertical axis) have been obtained after performing gene ontology enrichment analysis. The corresponding enrichment levels (horizontal axis) and p-values calculated after FDR correction are shown.
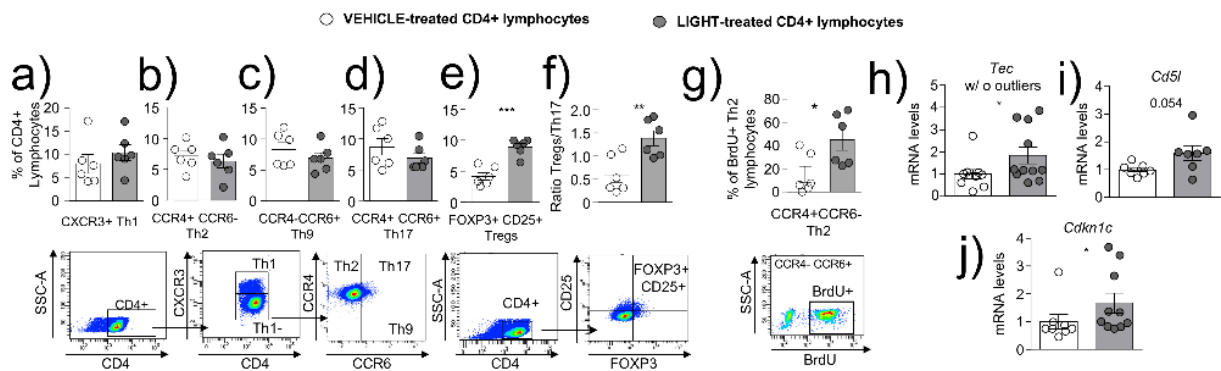
Figure 2. Effect of LIGHT treatment on human CD4+ lymphocytes. Panels **a-e** show percentages of CD4+CXCR3+Th1 (**a**), CD4+CCR4+CCR6 Th2 (**b**), CD4+CCR4-CCR6+ Th9 (**c**), CD4+CCR4+CCR6+Th17 (**d**) and CD4+FOXP3+CD25+ Treg (**e**) cell subsets treated with vehicle (white bars) or LIGHT 50ng/ml (grey bars). T cell ratio Treg/Th17 is represented in panel **f**). **g)** Proliferative BrdU+ CCR4+CCR6 Th2 cells percentage in vehicle/LIGHT-treated lymphocytes. Representative plots are shown for CXCr3, CCR4, CCR6, FOXP3 and CD25 gating strategy below the bar graphs. **h-j.** mRNA levels of Tec (**h**), Cd5l (**i**) and Cdkn1c (**j**) genes in VEHICLE/LIGHT-treated CD4+ lymphocytes. Differences were evaluated with unpaired Student's t test or Mann–Whitney U test (nonparametric test). * p.value<0.05, ** p.value<0.01, *** p.value<0.001.

Conclusions: Results suggest that LIGHT modulate atherosclerosis through regulation of lymphocyte populations, which particularly promotes protective T cells phenotypes, notably Treg cells, through

proliferation and phenotype modulation.

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.04 Inflammation, immunity and infection in atherosclerosis*

IMPACT OF CHANGES IN BILIRUBIN METABOLISM ON ATHEROSCLEROSIS

SAAG SESSION 16: IMMUNE BIOLOGY IN ATHEROSCLEROSIS

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Background and Aims: Bilirubin, a molecule that derives from the breakdown of the heme group, has antioxidant properties and is involved in cardiovascular disease protection with serum levels inversely associated with atherogenesis, although the molecular mechanisms are unknown. Therefore we investigate the effect of bilirubin overload (UGT1KO mice, lacking the enzyme for bilirubin glucuronidation, key step for its elimination) or bilirubine shortage (BvraKO mice, lacking the enzyme that convert biliverdin to bilirubin) during atherosclerosis on ApoE KO background.

Methods: ApoE KO, ApoEKO-BvraKO, ApoEKO-UGT1KO mice were fed on chow diet for 9 months. Blood, liver, spleen and mediastinal lymph nodes were collected and profiled by FACS analysis for immune cell subsets distribution. Atherosclerosis was profiled at the aortic level paralleled by a deep analysis of liver features.

Results: ApoEKO-UGT1KO mice gained less weight compared to the other two groups. Furthermore, cytofluorimetric analysis showed an imbalance of T lymphocytes subclasses in ApoEKO-UGT1KO with a significant increase of CD8+ T effector cells. ApoEKO-UGT1KO mice also presented reduced liver steatosis, together with an increase of neutrophils infiltration. ApoEKO-BvraKO mice, vice versa, shown an increase number of both CD4+ and CD8+ effector cells only in the liver.

Conclusions: Bilirubin increase (UGT1KO mice), in an atherosclerotic background, impact circulating adaptive immune cells and liver steatosis. Further analysis are ongoing to delineate the role of bilirubin on atherosclerosis development.

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.04 Inflammation, immunity and infection in atherosclerosis*

ROLE OF INTESTINAL TRYPTOPHAN METABOLISM IN ATHEROSCLEROSIS

SAAG SESSION 16: IMMUNE BIOLOGY IN ATHEROSCLEROSIS

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Background and Aims: The obesogenic, high fat diet (HFD) is responsible for intestinal dysbiosis. Indoleamine 2, 3-dioxygenase 1 (IDO) is the main enzyme responsible for the degradation of tryptophan (Trp), in the extra-hepatic organs. We have previously shown that under HFD, the global invalidation of IDO impacts gut microbiota and metabolic parameters. **Objective:** Study the specific role of intestinal IDO on atherosclerosis.

Methods: Mice genetically deficient for IDO in intestinal epithelial cell (IEC) and LDLr (low density lipoprotein receptor) were generated. Both male and female (LDLr-/- and LDLr-/- IEC IDOKO) mice were subjected to HC (high cholesterol) or HFD+HC for 8 or 13 weeks. Feces were collected to characterize microbiota. The heart and the aorta were harvested to analyze the development of atheromatous plaques as well as the inflammatory infiltrate.

Results: The invalidation of IDO in IEC contributes to a marked decrease in its expression in the gut (-77%, p=0,004), indicating the importance of its expression in IEC. Moreover, IEC IDOKO under HFD+HC but not HC condition contributes to a significant increase in plaque size (+50%, p=0,0001) as well as lymphocyte accumulation within the plaques in the aortic sinus of males at 8 weeks and females at 13 weeks (+40 %, p=0,02), without any significant changes in plasma cholesterol. IEC IDOKO affects gut microbiota composition, and increases the local intestinal inflammation as well as the systemic inflammation.

Conclusions: under HFD condition, intestinal IDO has a local protective role in the intestine and on the systemic development of atherosclerosis.

SS108 / #1146

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.04 Inflammation, immunity and infection in atherosclerosis*

SCRNA-SEQUENCING OF HUMAN ATHEROSCLEROTIC PLAQUES IDENTIFIES SPECIES-SPECIFIC CELLULAR PHENOTYPES THAT ASSOCIATE WITH CLINICAL DISEASE

SAAG SESSION 16: IMMUNE BIOLOGY IN ATHEROSCLEROSIS

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Background and Aims: The distinct function of immune cells in human atherosclerosis has been mostly defined by preclinical mouse studies. Contrastingly, the immune cell composition of human atherosclerotic plaques and their contribution to disease progression is only poorly understood. It remains uncertain whether genetic animal models allow for valuable translational approaches.

Methods: We performed single cell RNA-sequencing (scRNAseq) to define the immune cell landscape in human carotid atherosclerotic plaques. The human immune cell repertoire demonstrated an unexpected heterogeneity and was dominated by cells of the T cell lineage, a finding confirmed by immunohistochemistry of human plaques. We performed bioinformatical integration with 7 mouse data sets and discovered a total of 51 cellular identities, of which some were not conserved between species and exclusively found in mice or humans.

Results: Locations, frequencies, and transcriptional programs of immune cells in preclinical mouse models did not resemble the immune cell landscape in human atherosclerosis. In contrast to mice, human plaques were not myeloid- and B cell-dominated and instead contained several T cell phenotypes with hallmarks of T cell memory, dysregulation, exhaustion, and activation. Human immune cells were predominantly enriched for transcriptional programs of hypoxia, glucose, and autoimmunity. In a validation cohort of 43 patients, activated immune cell subsets defined by multi-color flow cytometry associated with cerebral ischemia and coronary artery disease.

Conclusions: Here, we uncover yet undefined immune cell types associating with clinical disease. This leukocyte atlas of human atherosclerosis builds the conceptual basis for subsequent identification of cellular targets for clinical immunomodulatory therapies and risk prediction.

SS109 / #1042

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.04 Inflammation, immunity and infection in atherosclerosis*

DENDRITIC CELL IMMUNORECEPTOR 2 (DCIR2) DEFICIENCY IMPACTS IMMUNE CELLS DISTRIBUTION AND ATHEROSCLEROSIS IN LDLR-/- MICE

SAAG SESSION 16: IMMUNE BIOLOGY IN ATHEROSCLEROSIS

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Background and Aims: DCIR2 (Dendritic cell immunoreceptor 2) is an inhibitory receptor mainly expressed by dendritic cells that participates in the modulation of the immune response. Given the strong impact of the immune system in atherogenesis, the aim of this project was to investigate the contribution of DCIR2 in atherosclerosis-related immune response and atherosclerosis itself.

Methods: Male $Dcir2^{-/-} Ldlr^{-/-}$ (DKO) and $Ldlr^{-/-}$ mice were fed a standard or cholesterol-enriched diet for 12 weeks. Subsequently, the profiling of circulating immune cells and bone marrow precursors was performed, paralleled by the characterization of plasma lipid levels and of the atheromatous plaque along ascending aorta.

Results: DCIR2 expression resulted downregulated under hypercholesterolaemia and its deficiency in $Ldlr^{-/-}$ mice was associated with a decreased cholesterol and triglycerides plasma levels (-42%, $p<0.05$; -25%, $p<0.01$ respectively). This reflected a reduced atheromatous plaque formation both at aortic sinus level (-58%, $p<0.01$) and along the first 300 μm of the aortic arch (-64%, $p<0.05$). Of note, while the content of macrophages within the aortic plaque remained unchanged, a significant decrease in circulating neutrophils and monocytes (-47%, $p<0.05$; -38%, $p<0.01$) was observed in the DKO mice compared to $Ldlr^{-/-}$, paralleled to an increased in the number of bone marrow myeloid precursors (+42% $p<0.05$).

Conclusions: Although DCIR2 is known to be expressed by dendritic cells, thus involving the modulation of adaptive immune response, our data suggests that it also affects the lipid metabolism and the development of atherosclerotic plaque. Studies aimed at understanding the underlying molecular mechanisms are still ongoing.

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.06 Vascular biology of the arterial wall: Miscellaneous*

TISSUE FACTOR REGULATES THE CROSSTALK OF CORONARY PERIVASCULAR ADIPOSE-DERIVED STEM CELLS WITH VASCULAR CELLS

SAAG SESSION 17: NOVEL ANTI-ATHEROSCLEROTIC THERAPEUTIC STRATEGIES

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Background and Aims: The quantity of coronary perivascular adipose tissue (CPVAT) has been associated to the underlying vessel atherosclerotic plaque severity. The role of the adipose stem cells (ASCs) reservoir in CPVAT on the underlying arterial smooth muscle cells (VSMCs) function is not known. To determinate the interactions between ASCs present on CPVAT obtained from perivascular niche of non-ischemic or ischemic patients with VSMCs presents on the media, which play a central role in atherosclerosis plaque development.

Methods: ASCs were obtained from the PVAT overlying the left anterior descending coronary arteries (LAD) of patients undergoing heart transplantation. ASCs were phenotypically characterized, and functionally tested by proliferation, differentiation and angiogenic assays. Co-cultures of ASC and VSMCs were used in vitro and in vivo studies to analyze the effect of ASCs on VSMCs.

Results: ASCs expressing typical mesenchymal stem cell markers were detected in the adventitia of the LAD. The differentiation capacity and angiogenic potential of ASCs were evidenced. We identified tissue factor (TF) expressed in ASCs as responsible of ASCs differentiation and recruitment of VSMCs through ERK1/2 /ETS1 signaling. ASCs obtained from non-ischemic or ischemic tissue showed different expression of TF, and consequently different angiogenic capacity. Upregulation of TF in ischemic-ASCs, increased their angiogenic capacity in subcutaneously implanted plugs in mice, whereas silencing TF in ASCs decreased the proangiogenic capacity of non-ischemic ASCs.

Conclusions: Our results indicate for the first time a novel mechanism of regulation of vascular function by stem cells of the perivascular fat. CPVAT-ASCs drive angiogenic and proliferative processes in VSMC,mediated by TF expression in ASCs.

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.10 Clonal Haematopoiesis*

THE VICIOUS CYCLE OF CLONAL HAEMATOPOIESIS AND CVD - DRIVER MUTATIONS IN MIDDLE AGED PATIENTS AND THEIR ROLE AS INDEPENDENT RISK FACTOR

SAAG SESSION 17: NOVEL ANTI-ATHEROSCLEROTIC THERAPEUTIC STRATEGIES

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Background and Aims: CHIP is a mutation-driven clonal hematopoiesis in the absence of overt hematologic disease. In the general population CHIP-carriers are twice as likely to suffer from CHD as non-carriers (Jaiswal et al.). However prevalence of CHIP and its association with risk factors and extent CAD in patients undergoing coronary-angiography are unknown. Aim: To determine whether CHIP mutations are associated with the extend of CAD independent of comorbidities and to investigate possible associations.

Methods: 178 out of 815 screened patients undergoing coronary angiography proved eligible. CHIP-status was determined by whole-blood genome sequencing and correlated with the patients' medical-history, laboratory and coronary-angiography-findings.

Results: 30% of patients carried CHIP mutations, 75% in the DNMT3A and/or TET2-gen. The median age was 69 years, with mutation-carriers being significantly older then non-carriers (72.5 versus 67.1 years, p=0.001). CHIP-carriers showed elevated RDW levels in blood (CHIP positive: 13.3 +/- 0.14, CHIP negative: 13.0 +/- 0.08, p=0.030) with no other laboratory-findings being significantly associated. Among DNMT3A and TET2 mutation carriers, those below median age showed elevated Gensini scores (CHIP positive: 40.1 +/- 10.8, CHIP negative: 16.3 +/- 2.4, p=0.044). In a multivariate-approach, this finding proved to be independent of common cardiovascular risk factors.

Conclusions: Prevalence of CHIP is three times higher in our preselected patient-population compared to the general-population. DNMT3A/TET2-mutations associate with severe CAD particularly in younger patients in which genetic traits are highly-relevant. Given the widely described link between inflammation and CHD as well as hematopoiesis, we recognize this process as a vicious-cycle and will conduct further research in this field.

SS112 / #1263

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.13 Other*

ASTAXANTHIN: AN ANTI-ATHEROSCLEROTIC TREATMENT IN MICE.

SAAG SESSION 17: NOVEL ANTI-ATHEROSCLEROTIC THERAPEUTIC STRATEGIES

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Background and Aims: Beyond the antioxidant ability of astaxanthin (AS), many studies have established that AS can exert preventive actions in cardiovascular protection, reduction of biomarkers against oxidative stress, fat reduction, reduction of inflammation, antihypertensive, reduction of the extent of myocardial infarction, performance improvement in sport. However, the mechanisms underlying AS's bioactivity are still unknown.

Methods: To establish the atherogenesis effect of AS, Ldrl$^{-/-}$ mice, were fed with western diet (WD) for 16 weeks and treated with 70mg/kg of AS/vehicle every second day. A week before the end of feeding period glucose/insulin tolerant test was perform. Lipid content in root and liver was quantified with ORO staining. FACS was used to characterize blood, bone marrow, spleen, and adipose tissue cells, as well for plasmatic cytokines. Plasmatic lipids were measured by ELISA. A second experimental group of Ldlr$^{-/-}$, feed with WD and AS for 10 days, stimulated with TNFα and used for intravital microscopy.

Results: Overall body weight of animals treated with AS was increased. AS induced a reduction of peripheral leukocytes while incrementing splenocytes. In a glucose/insulin tolerant test AS group shown improved glucose metabolism and insulin sensitivity. Lipid accumulation in aortic roots was reduced by AS, however this effect was not correlated with lipid levels in plasma.

Conclusions: We conclude that AS treatment reduces lipid content and macrophage infiltration into atherosclerotic plaque, due to a reduction in circulating inflammatory monocytes, prevents cell activation, and improves splenic functions. In summary, AS is a natural compound that reduce the development of atherosclerotic plaque and contribute to maintain healthy functions during atherosclerosis.

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.13 Other*

A POTENTIAL ROLE OF GUCA1B GENE IN EXPERIMENTAL ATHEROSCLEROSIS

SAAG SESSION 17: NOVEL ANTI-ATHEROSCLEROTIC THERAPEUTIC STRATEGIES

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Background and Aims: Recent mRNA sequencing studies in our laboratory have demonstrated a markedly downregulation of *Guca1b* gene in vascular bed of atherosclerotic *ApolipoproteinE*-deficient (*Apoe-/-*) mice with accelerated atheroma plaque development associated with genetic inactivation of the cytokine LIGHT(TNFSF14). In this study, the possible participation of *Guca1b* in atherosclerosis progression and modulation of its expression by LIGHT in stimulated macrophages were explored.

Methods: Expression of *Guca1b* was investigated by quantitative PCR(qPCR) in bone marrow(BM) and aortic arch(AA) from *Apoe-/-* mice placed on an atherogenic diet(AD) for 4, 8 and 12 weeks. On the other hand, *Guca1b* expression was analysed by qPCR in BM derived-macrophages (BMDM) of *Apoe-/-* and *Apoe-/-Light-/-* mice treated with lipopolysaccharide.

Results: As expected, histopathological characterization showed that lesion increased with the AD (**fig.1**), while *Guca1b* expression was time and tissue-dependent (**fig.2**) with a downregulation expression peak at 8 weeks, compared with both 4 and 12 weeks, in AA and an increase at 8 and 12 weeks compared with 4 weeks of AD in BM. The analysis in BMDM revealed that *Guca1b* is expressed in macrophages but downregulated in stimulated macrophages characterized by an increase in proinflammatory *Il6* and *Mcp1* cytokines. In addition, its expression markedly decreases when *Light* gene is inactivated suggesting that *Guca1b* expression in macrophages is Light-dependent (**fig.3**).
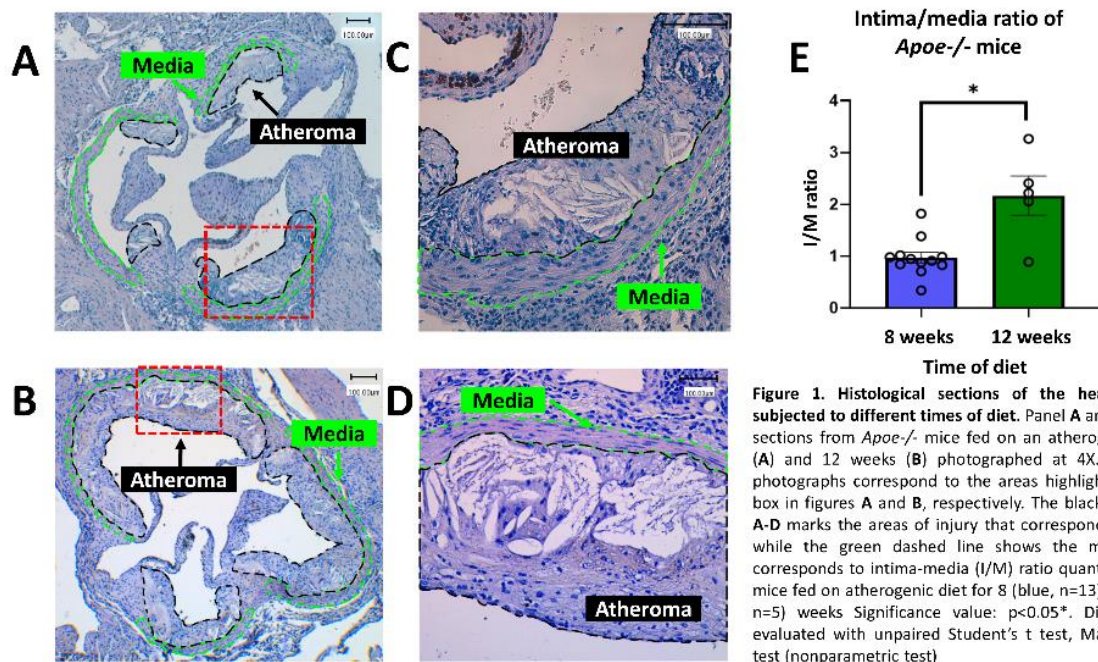
Figure 1. Histological sections of the heart from mice subjected to different times of diet. Panel **A** and **B** show heart sections from *Apoe-/-* mice fed on an atherogenic diet for 8 (**A**) and 12 weeks (**B**) photographed at 4X. **C** and **D** 20X photographs correspond to the areas highlighted with a red box in figures **A** and **B**, respectively. The black dashed line in **A-D** marks the areas of injury that correspond to the intima, while the green dashed line shows the media. Figure **E** corresponds to intima-media (I/M) ratio quantified in *Apoe-/-* mice fed on atherogenic diet for 8 (blue, n=13) and 12 (green, n=5) weeks Significance value: p<0.05*. Differences were evaluated with unpaired Student's t test, Mann–Whitney U test (nonparametric test)
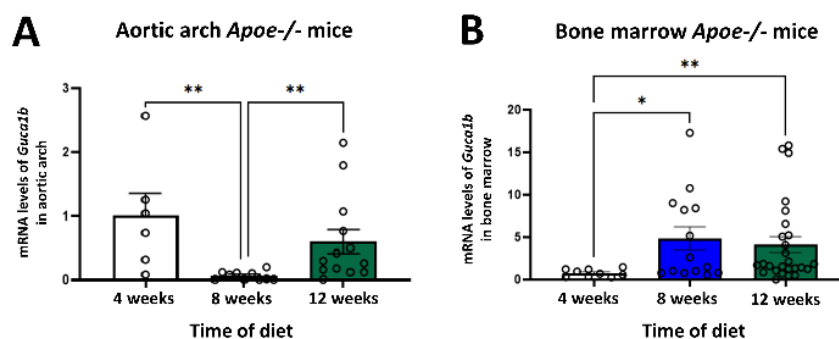
Figure 2. Study of the expression of *Guca1b* in *Apoe-/-* mice fed different times with an atherogenic diet. A) Relative quantification of *Guca1b* mRNA expression levels in the aortic arch at 4 weeks (n=6), 8 weeks (n=10) and 12 weeks (n=13) of an atherogenic diet. **B)** Relative quantification of *Guca1b* mRNA expression levels in bone marrow from *Apoe-/-* mice fed an atherogenic diet for 4 (n=10), 8 (n=14) and 12 (n=27) weeks. For both results, the statistical analysis carried out was a Kruskal-Wallis test, using the Tukey test as a post hoc test. Significance value: *P<0,05 **p<0,01.
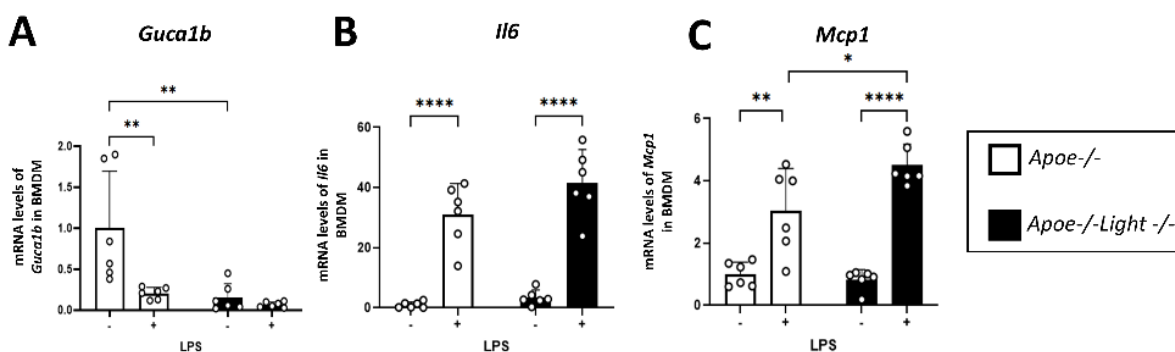
Figure 3. Study of the expression of *Guca1b* and inflammatory cytokines in BMDM derived from *Apoe-/-* and *Apoe-/-Light-/-* mice. Relative mRNA expression levels of **A)** *Guca1b*, **B)** *Il6* and **C)** *Mcp1* for cultured bone marrow derived macrophages (BMDM) of *Apoe-/-* and *Apoe-/-Light-/-* mice treated with LPS (100 ng/ml) for 24h (**+**) (*Apoe-/-*, n=6; *Apoe-/-Light-/-*, n=6) or with saline vehicle (**-**) (*Apoe-/-*, n=6; *Apoe-/-Light-/-*, n=6). Statistical analysis was performed using the two-way ANOVA test followed by Tukey's post hoc test. White bars represent BMDM from *Apoe-/-* mice and black bars from *Apoe-/-Light-/-* mice. *p<0.05, **p<0.01, ****p<0.0001.

Conclusions: Inverse association of *Guca1b* expression with atherosclerosis progression and proinflammatory macrophages suggest a possible protective role of *Guca1b* in atherosclerosis by modulating macrophage polarization. **Funding**: Carlos III Health Institute (PI19/00169, PI22/00062, cofinanced with FEDER funds), SEA/FEA (BIB-07-20) and Proyecto Paula.

SS114 / #1257

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.13 Other*

INVESTIGATING CARDIAC CHANGES CAUSED BY CORONARY ATHEROSCLEROSIS USING SRBI/LDLR KO MICE

SAAG SESSION 17: NOVEL ANTI-ATHEROSCLEROTIC THERAPEUTIC STRATEGIES

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Background and Aims: Cardiovascular disease (CVD) accounts for 31% of all global deaths and is the leading cause of mortality worldwide. The translation of cardioprotective strategies limiting ischaemia-reperfusion (I/R) injury in animal models to the clinic has been disappointing with majority of trials yielding neutral results. This disconnect prompts an urgent re-examination of the experimental models used to study I/R.

Methods: Animal experiments are often conducted in young and healthy animals without co-morbidities and co-medications. The most commonly used atherosclerotic models such as ApoE KO and Ldlr KO mice lack coronary plaques which are present in the majority of MI patients. To characterise a more clinically relevant model, Scarb1;Ldlr KO (DKO) mice were fed a high fat diet (HFD) for 6 weeks to induce plaque formation in their coronary arteries. The presence of coronary plaques was observed by Oil Red-O staining.

Results: Increased ICAM-1 expression was confirmed by immunostaining, Western blot and RNAseq indicating endothelial activation. Basal gene expression in the hearts of DKO mice was significantly different from WT, with 108 increased and 21 decreased transcripts. In DKO mice, gene expression of key components of the pyroptotic pathway; NLRP3 and IL1β were increased, accompanied by an increase in protein levels of Gasdermin D.

Conclusions: The DKO mouse model is exceptional in that it exhibits coronary artery lesions, unlike other rodent models of atherosclerosis making it more suitable than other models to study pathological situations.

SS115 / #1345

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.13 Other*

THE COMMON MARMOSET MONKEY AS A PROMISING ANIMAL MODEL FOR ATHEROSCLEROSIS RESEARCH

SAAG SESSION 17: NOVEL ANTI-ATHEROSCLEROTIC THERAPEUTIC STRATEGIES

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Background and Aims: Background & Objective The common marmoset (*Callithrix jacchus*) monkey is a promising animal model for various research questions, inter alia cardiovascular senescence. Although there are indications in the literature that marmosets naturally develop atherosclerosis, the expression and phenotype of atherosclerotic lesions have not been further characterized. We therefore want to fill this knowledge gap to determine whether this primate is a suitable model for atherosclerosis research compared to more established animal models such as mice and rabbits.

Methods: Methods Thoracic aortas from 5 male common marmosets (mean age 15 years; referred to as aged) were examined using diverse stainings to phenotype and define lesion severity. Animals did not undergo experiments before and were fed a standard diet.

Results: Results En face Oil Red O staining revealed fat deposits in thoracic aortas of all analyzed animals. Plaques were scored according to a human grading system and mean aortic wall thickness was measured in HE staining. The animals used in this study showed a spectrum of plaque progression from intimal thickening over intimal xanthoma to pathological thickening. Mean aortic wall thickness was increased. Further stainings (Masson Goldner Trichrome, Prussian Blue and von Kossa) revealed elevated levels of fibrosis, intraplaque hemorrhage and significant calcification in plaque tissue.

Conclusions: Conclusion & Outlook Results from histological analyses revealed plaque formation in different stages in the here investigated non-human primates with striking similarities to humans. Further characterization e.g., via ncRNA analyses and *in vitro*-experiments will validate and complement these findings and establish the common marmoset as an atherosclerosis model.

SS116 / #128

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.04 Adipose tissue homeostasis*

SERPINA3C ATTENUATES ADIPOSE TISSUE AND HEART DAMAGE DURING OBESITY BY INHIBITING OXIDATIVE STRESS AND ENDOPLASMIC RETICULUM STRESS

SAAG SESSION 17: NOVEL ANTI-ATHEROSCLEROTIC THERAPEUTIC STRATEGIES

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Background and Aims: Adipose tissue (AT) dysfunction is closely associated with obesity-related heart damage. Serpina3c which is highly expressed in mature adipocytes, is a secreted serine protease inhibitor that can regulate AT function. The present study aimed to investigate the effect of Serpina3c on AT and heart during obesity.

Methods: Wild type (WT) and Serpina3c knockout (3cKO) mice were fed with high-fat diet (HFD) for 20 weeks. AAV-mediated overexpression of Serpina3c was injected locally in epididymal white adipose tissue (eWAT) to examine the effect of Serpina3c. Palmitic acid was established to interfere with the differentiated 3T3L1 cells of Serpina3c knockdown (3cKD) or overexpression (3cOV) and corresponding control groups. RNA-seq of 3T3L1 cells was performed to assess the effect of Serpina3c.

Results: The body weight, serum lipids and pro-inflammatory cytokines were increased in 3cKO mice. Increased inflammation, fibrosis and apoptosis of AT and heart were detected, and the impairment of cardiac diastolic function was aggravated in 3cKO mice. Overexpression of Serpina3c in eWAT alleviated these adverse phenotypes. Through RNA-seq, we found endoplasmic reticulum stress (ERS)-related genes were significantly increased in 3cKD group, but significantly down-regulated in 3cOV group. Oxidative stress can trigger ERS, and we detected a significant increase in ROS levels in 3cKO group. Mechanistically, Serpina3c alleviated ERS and production of pro-inflammatory cytokines in adipocytes by inhibiting NOX4 and iNOS mediated oxidative stress.

Conclusions: Our results highlight a protective role for serpina3c as a novel adipocytokine in the obesity-related heart damage, through inhibiting NOX4 and iNOS mediated oxidative stress to negatively regulate

ERS and inflammation in

HFD+Serpina3cKO

HFD+WT

Weight gain

Serpina3c↓

NOX4+iNOS

ROS

endoplasmic reticulum stress

Adiponectin

IL18
CCL2
CCL5
CXCL5
SAA3
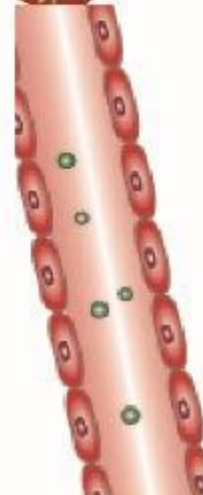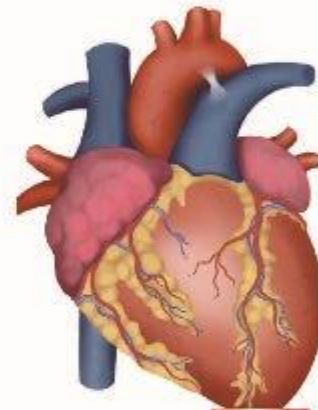
adipocytes.

Topic: *AS02 Lipids and Lipoproteins / AS02.09 Lipid and lipoprotein metabolism*

META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS EVALUATING THE ASSOCIATION BETWEEN MAGNITUDE AND DURATION OF APOLIPOPROTEIN-B LOWERING AND CARDIOVASCULAR RISK REDUCTION AMONG DIFFERENT LIPID-LOWERING THERAPIES

SAAG SESSION 18: THE EFFECTIVENESS OF LDL-LOWERING TREATMENT

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Background and Aims: We sought to compare the association between the proportional risk reduction in major cardiovascular events (MCE) and different classes of lipid-lowering therapies (LLT) for the same magnitude and duration of apolipoprotein-B (apoB) lowering.

Methods: MEDLINE and EMBASE databases were searched (1966-November 2022). Key inclusion criteria were: randomized controlled trials; adjudicated clinical cardiovascular outcomes; enrolled at least 1000 participants; with median follow-up at least one year; and reported absolute achieved difference in plasma apoB levels between the treatment and control groups. Data were analysed using inverse variance-weighted meta-analysis after each year of follow-up.

Results: A total of 254,828 participants (mean age 63 years; 26% female sex) from 20 trials who experienced 30,175 MCE were included. For each 30 mg/dL absolute reduction in plasma apoB levels, statins, ezetimibe, PCSK9-inhibitors, CETP-inhibitors, fibrates, and niacin were each associated with a consistent 10% proportional reduction in MCE after one year of therapy (HR:0.90; 95%CI:0.86-0.94); 15% reduction after two years of therapy (HR:0.85; 95%CI:0.82-0.88); 19% reduction after three years of therapy (HR:0.81; 95%CI:0.78-0.85); and a 20% reduction after four or five years of therapy (HR:0.80; 95%CI:0.77-0.83). There was no evidence of heterogeneity between estimates of the different LLT classes (I^2=6.9%; p-value=0.364).

Conclusions: In this meta-analysis, six different classes of LLT were associated with very similar reductions in the risk of MCE for the same magnitude and duration of apoB lowering, suggesting that the clinical benefit of LLT is determined by the achieved changes in plasma apoB levels, regardless of the corresponding changes in other lipids.

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.01 Epidemiology of cardiovascular diseases and risk factors*

STATIN USE AND REDUCED STEMI RELATIVE TO NON-STEMI: A NATIONWIDE STUDY IN DENMARK

SAAG SESSION 18: THE EFFECTIVENESS OF LDL-LOWERING TREATMENT

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Background and Aims: Myocardial infarction due to atherosclerotic plaque rupture is classified in two subtypes: ST-elevation myocardial infarction (STEMI) and non-ST-elevation myocardial infarction (non-STEMI), with STEMI indicating complete occlusion of a coronary artery, requiring immediate treatment to reduce risk of death or permanent myocardial damage. Lipid-lowering with statin therapy profoundly changes plaque morphology in the coronary arteries, which may affect the relative distribution between STEMI and non-STEMI events. We tested the hypothesis that statin use is associated with reduced STEMI relative to non-STEMI in patients with myocardial infarction.

Methods: This Danish nationwide study included all patients who suffered from a first-time myocardial infarction between 2010 and 2018 (n=72,761) and investigated the odds ratio for STEMI versus non-STEMI events according to prescribed doses of statin treatment.

Results: The odds ratio for STEMI versus non-STEMI was 0.64(0.61-0.68) in current statin users, and 0.95(0.89-1.01) in previous statin compared to never statin users. With higher intensity of daily statin dose, the odds ratio for STEMI versus non-STEMI was 0.81(0.76-0.85) for low statin dose, 0.60(0.57-0.64) for regular statin dose, and 0.47(0.41-0.52) for high statin dose compared to never statin users. The hazard ratio for 60-day mortality after first-time STEMI versus non-STEMI was 1.73(1.64-1.84).

Conclusions: Statin use is associated with reduced STEMI relative to non-STEMI in a dose dependent manner. This indicate that statin therapy, in addition to reducing myocardial infarction event-rates, also results in less severe presentations of myocardial infarction when it occurs.

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.01 Epidemiology of cardiovascular diseases and risk factors*

MANAGEMENT OF PATIENTS WITH VERY HIGH CARDIOVASCULAR RISK ELIGIBLE FOR PCSK9 INHIBITOR TREATMENT: 1-YEAR OUTCOMES OF THE PERI-DYS STUDY

SAAG SESSION 18: THE EFFECTIVENESS OF LDL-LOWERING TREATMENT

Klaus Parhofer[1], David Pittrow[2], Andreas Birkenfeld[3], Uwe Fraass[4], Bernd Hohenstein[5], Carsten Siegert[4], Jens Klotsche[6], Elisabeth Steinhagen-Thiessen[7], Stefan Dexl[4], Volker Schettler[8], Ulrich Laufs[9]
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Background and Aims: The PERI-DYS study compares patients at very high cardiovascular risk treated with PCSK9 inhibitors (PCSK9i alirocumab 89% and evolocumab 11%) with patients qualifying for, but not receiving PCSK9i. Our interim analysis reports changes in LDL cholesterol (LDL-C) and predictors for therapy intensification 12 months after inclusion.

Methods: This prospective observational study collects data from 1713 patients (mean age 63.4±11.5 years, 35% females) at 70 sites in Germany (Study identifier: NCT03110432). Here we report the 1-year results.

Results: At baseline, 810 patients (47.3%) were receiving PCSK9i (31.2% ongoing; 16.1% newly treated). Compared to patients qualifying for but not treated with PCSK9i (n=903), those treated with PCSK9i were younger (62±10 vs 64±12 years) and more likely to have coronary artery disease (74% vs 68%), higher untreated LDL-C (201 vs 179mg/dl), and statin intolerance (67.3% vs 15.3%). At 12 months, patients on PCSK9i had lower median LDL-C (64.4mg/dl; 40.4% with LDL-C <55mg/dl) than patients not on PCSK9i (76.6mg/dl; 21.2% at goal). Patients on PCSK9i+statin showed the highest goal achievement (47.7%). Overall, at 1 year, 80.2% had unchanged LLT and only 19.8% had therapy intensification. Using a multivariate model, significant predictors of LLT intensification were younger age (odds ratio for 60+ years 0.70), no ezetimibe at BL (OR 0.68), LDL-C level >100mg/dl (OR 2.3), and statin intolerance (OR

0.70).

Table 1. LDL-C values and goal achievement rates at 1-year follow-up

Patient group	n	LDL-C at BL mg/dl	LDL-C at 6 months mg/dl	LDL-C at 1 year mg/dl	LDL-C goal achievement* at 1 year %
All Patients	1713	91.9	73.6	71.0	32.0
PCSK9i yes at BL	810	81.2	65.0	64.4	40.4
PCSK9i no at BL	903	99.4	83.0	76.6	21.2
PCSK9i newly treated at BL	275	116.4	65.9	66.5	36.9
PCSK9i newly added after BL	76	124.5	69.5	62.0	38.0
PCSK9i discontinued after BL	42	96.0	70.0	71.2	41.0
PCSK9i yes, statins no at BL	375	87.4	72.3	75.9	31.4
PCSK9i yes, statins yes at BL	435	75.0	55.0	56.2	47.7
PCSK9i no, statins yes at BL	99	145.0	92.0	83.1	22.7
PCSK9i no, statins no at BL	804	97.0	82.0	76.0	21.0
Heterozygote FH	100	126.8	109.0	91.6	21.8
Heterzyg./mixed dyslipidemia	1607	91.0	73.0	69.6	33.0
Statin intolerance yes at BL	709	99.4	77.3	75.4	30.4
Statin intolerance no at BL	1004	88.0	72.0	68.0	33.5

BL = baseline, PCSK9i = PCSK9 inhibitors. Values are medians or percentages. *<55 mg/dl

Conclusions: LLT intensification occurred infrequently and may explain low achievement of the LDL-C goal < 55 mg/dl (40.4% PCSK9i vs. 21.2% without) at 1 year. Lipid lowering in the PCSK9i group occurred faster and was more pronounced.

SS120 / #576

LDL LOWERING EFFECT OF PCSK9 INHIBITION IS REDUCED IN WOMEN

SAAG SESSION 18: THE EFFECTIVENESS OF LDL-LOWERING TREATMENT

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Background and Aims: Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a key regulator of plasma low-density lipoprotein cholesterol (LDL-C) concentration and its inhibition reduces the risk of atherosclerotic cardiovascular diseases. Our aim was to assess the sex-differential effect of either pharmacological or genetic inhibition of PCSK9 on LDL-C levels.

Methods: We meta-analyzed six real-life studies (1'216 men and 641 women) that assess the effects of PCSK9 monoclonal antibodies (mAbs) on LDL-C reduction in men and women. We tested the sex-related association of the loss-of-function variant PCSK9-R46L with LDL-C plasma levels in 382'813 individuals (219'301 women and 163'512 men) free of lipid-lowering drugs from the UK biobank (UKBB) general population cohort.

Results: The meta-analysis revealed that, despite higher basal LDL-C levels in women (mean difference, MD=17.4 mg/dL, $p<0.0001$, women=175 mg/dL vs. men=152 mg/dL), the LDL-C reduction under PCSK9 mAbs treatment was significantly stronger in men (MD=7.6 mg/dL; $p=0.002$) than in women. In the UKBB general population cohort, the magnitude of LDL-C reduction was larger in men than in women (mean LDL-C difference: -35 mg/dL vs. -26 mg/dL, when comparing homozygous carriers with non-carriers in men and women, respectively). The relationship between PCSK9-R46L and LDL-C significantly depended on sex (p-for-interaction = 7.2e-04).

Conclusions: Real-life data confirm a weaker beneficial effect of PCSK9 mAbs on LDL-C reduction in women compared to men. Concordantly, we found that the genetic inhibition of PCSK9 on LDL-C reduction was sex-dependent, further supporting the existence of specific biological processes. These findings are clinically relevant for the management of hypercholesterolemia and cardiovascular care in women.

SS121 / #693

Topic: *AS04 Clinical Vascular Disease / AS04.06 Aneurysms and other non-atherosclerotic arteriopathies*

EVOLOCUMAB, A PCSK9 INHIBITOR, SUPPRESSES ABDOMINAL AORTIC ANEURYSM PROGRESSION IN MICE

SAAG SESSION 18: THE EFFECTIVENESS OF LDL-LOWERING TREATMENT

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Background and Aims: PCSK9 inhibitors have shown the beneficial effect on atherosclerotic cardiovascular disease by lowering LDL cholesterol. However, the possibility that PCSK9 inhibitors affect abdominal aortic aneurysm (AAA) remains unclear. The aim of this study was to determine the effect of evolocumab, a PCSK9 inhibitor, on the development of AAA.

Methods: AAA was induced in C57BL/6 mice by periaortic application of 0.25M $CaCl_2$. After 3 weeks, mice were treated with subcutaneous injection of PBS (Sham, n=10, AAA-control, n=15) or evolocumab (10mg/kg/week) (AAA-evolocumab, n=25) for 5 weeks. Aortic diameter was assessed by echocardiography, masson's trichrome staining by immunohistochemistry, the expression of CD68 and CD31 by immunofluorescent staining, and mRNA levels by RT-PCR.

Results: Aortic diameter was expanded in AAA-control compared with Sham. Treatment with evolocumab decreased the aneurysm size in the aortic wall compared with AAA-control. Masson's trichrome staining showed destruction of the elastic lamellae in AAA-control, while its wavy morphology was maintained in AAA-evolocumab. An increased mRNA expression of transforming growth factor-beta, MR, and Arg-1 and a down-regulation of collagen IV, matrix metalloproteinase (MMP)-9, MMP-2, interleukin 1 beta, chemokine (C-C motif) ligand 2 and tumor necrosis factor-α, and ApoB were observed in AAA-evolocumab compared to AAA-control (all $p<0.05$). The increased CD68- and CD31-positive cell count in AAA-control were also reduced by evolocumab treatment (both $p<0.05$).

Conclusions: Evolocumab treatment inhibited the progression of $CaCl_2$-induced AAA, in association with decreased inflammation, neoangiogenesis, and extracellular matrix disruption. These findings suggest inhibition of PCSK9 appears as a potential therapeutic approach to prevent the AAA formation.

SS122 / #60

Topic: *AS04 Clinical Vascular Disease / AS04.09 Lipid-lowering therapies*

EFFECT OF A FIXED-DOSE COMBINATION OF PITAVASTATIN AND EZETIMIBE, VERSUS MONOTHERAPY ON LIPID PROFILES IN PATIENTS WITH HYPERCHOLESTEROLEMIA: A MULTICENTER, PHASE III STUDY

SAAG SESSION 18: THE EFFECTIVENESS OF LDL-LOWERING TREATMENT

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Background and Aims: The efficacy of 1PC111,a Fixed-Dose Combination of Pitavastatin(P) and Ezetimibe(E) is superior to monotherapy for the treatment of hypercholesterolemia

Methods: A multicenter, randomized, double-blind, Phase III study. Patients(pts) were randomized to receive 1PC111, P 2 mg, or E 10 mg daily for 12 weeks. Primary end point was the difference in LDL-C from baseline to week 12 between the 1PC111 and each monotherapy group(grs). Secondary end points were change in other lipid profiles . All pts. were assessed for adverse events .

Results: Total of 388 pts were assigned to the 1PC111 (n = 128), P (n = 132), or E (n = 128) grs. Baseline were similar among the 3 grs. A significant decrease in the LDL-C level at week 12 was observed in the 1PC111 gr. (–50.50% [14.9%]) compared with either the P (–36.11% [11.4%]; $P < 0.001$) or E (–19.85% [12.4%]; $P < 0.001$) gr. There was a significant difference between 1PC111 and monotherapy gr. in the reduction total cholesterol, non–HDL-C, and apolipoprotein B levels. A trend toward more lowering of LDL-C levels in elderly pts (age ≥65 years) than in younger by 1PC111 . For class I recommendation for atherosclerotic disease prevention, the percentage of pts achieving the LDL-C target of <100 mg/dL at week 12 was higher in the 1PC111 gr. than in monotherapy grs ($P < 0.001$). The incidence of adverse events was similar among 3 grs.

Conclusions: 1PC111 was more effective in improving lipid profiles and achieving the LDL-C goal than P or E alone .

SS123 / #360

Topic: *AS04 Clinical Vascular Disease / AS04.09 Lipid-lowering therapies*

SAFETY OF VERY LOW LDL-C LEVELS WITH EVOLOCUMAB: AN ANALYSIS FROM THE PAN-EUROPEAN OBSERVATIONAL HEYMANS STUDY

SAAG SESSION 18: THE EFFECTIVENESS OF LDL-LOWERING TREATMENT

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Background and Aims: To assess the safety of very low LDL-C levels from an analysis of a cohort study (HEYMANS) that prospectively examined clinical characteristics and LDL-C control among patients initiating evolocumab across 12 European countries (May-2016 to June-2021).

Methods: Patient data were collected for ≤6 months prior to evolocumab initiation (baseline) and up to 30 months post initiation. Patient characteristics, lipid values, adverse drug reactions (ADRs) and fatal adverse events (AEs) were collected from medical records. This analysis examined all safety events occurring after the first instance of low LDL-C (<25mg/dL [<0.65mmol/L] and <40mg/dL [<1.0mmol/L]) to study end. For patients with LDL-C ≥40mg/dL, all events during the study were analysed.

Results: Overall, 1,951 patients were enrolled in HEYMANS (mean study follow-up: 21.7 months). Median (Q1, Q3) baseline LDL-C was 3.98 (3.17, 5.07) mmol/L. Within 3 months of evolocumab initiation median LDL-C fell by 58% to 1.63 (1.03, 2.53) mmol/L. This reduction was maintained over time (30-month LDL-C: 1.63 (1.11, 2.33) mmol/L). During the study, 357 (20%) and 741 (41%) patients had ≥1 LDL-C level <25mg/dL and <40mg/dL, respectively. No increase in ADRs (including nervous system, psychiatric, musculoskeletal and connective tissue disorders, or type 2 diabetes), serious ADRs, nor fatal AEs were observed in patients with progressively lower achieved LDL-C levels (<40mg/dL and <25mg/dL) versus patients with LDL-C ≥40mg/dL

(Table).

Table – Safety according to lowest LDL-C levels achieved

Event, n (%)	Lowest LDL-C level achieved		
	<25mg/dL (N=357)*	<40mg/dL (N=741)*	≥40mg/dL (N=1,083)
Any non-fatal ADR	7 (2.0)	25 (3.4)	74 (6.8)
Nervous system disorders	0	1 (0.1)	11 (1.0)
Psychiatric disorders	0	0	1 (<1.0)
Musculoskeletal and connective tissue disorders	2 (0.6)	12 (1.6)	34 (3.1)
Type 2 diabetes	0	0	1 (<1.0)
Any non-fatal serious ADR	0	4 (0.5)	2 (0.2)
Any fatal adverse event	3 (0.8)	7 (0.9)	11 (1.0)
Exposure time**			
Median time at risk of ADR, months (Q1, Q3)	15.2 (8.8, 26.3)	16.8 (9.7, 26.9)	22.9 (12.0, 30.0)

*Groups not mutually exclusive. Patients with LDL-C <40mg/dL could also have achieved LDLC-C <25mg/dL.
**This analysis examined all safety events occurring after the first instance of low LDL-C. Therefore, a limitation was that exposure time was longer in the patients with LDL-C ≥40mg/dL than the patients with low LDL-C, due to time taken to achieve and record the low LDL-C value.
ADR, adverse drug reaction; Q, quartile

Conclusions: In agreement with previous studies, this analysis shows no evidence of increased ADRs and AEs at very low LDL-C levels achieved with evolocumab in a real-world cohort with long-term follow-up.

SS124 / #1495

Topic: *AS04 Clinical Vascular Disease / AS04.13 New lipid lowering therapies*

ARO-ANG3, AN INVESTIGATIONAL RNAI THERAPEUTIC, DECREASES SERUM LDL-CHOLESTEROL, APOLIPOPROTEIN B, AND ANGIOPOIETIN-LIKE PROTEIN 3 IN PATIENTS WITH HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLAEMIA

SAAG SESSION 19: LIPIDS AND LIPOPROTEINS IN HEALTH AND DISEASE

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Background and Aims: Angiopoietin-like protein 3 (*ANGPTL3*) regulates lipoprotein metabolism by inhibiting lipoprotein and endothelial lipases. *ANGPTL3* loss-of-function variants have decreased circulating LDL-C. ARO-ANG3 is a liver-targeted RNAi therapeutic that inhibits *ANGPTL3* expression and decreases atherogenic lipoproteins through non-LDL receptor mediated mechanisms which may reduce LDL-C in patients with LDL receptor deficiency such as homozygous familial hypercholesterolaemia (HoFH).

Methods: In this open-label Phase 2 study, 18 HoFH patients with a mean fasting baseline LDL-C of 10.0 mmol/L (range: 2.3 to 21.6 mmol/L) on lipid-lowering standard of care (including apheresis), were randomized to receive subcutaneous injections of 200 mg or 300 mg ARO-ANG3 on Day 1 and Week 12. The primary endpoint was fasting LDL-C percent change from baseline to Week 24. The lipid data cut-off date for this interim analysis was 24 March 2023 and includes 16 subjects who have completed Week 20.

Results: At Week 20, 200 mg and 300 mg doses of ARO-ANG3 achieved mean reductions from Baseline (and mean percent change) in LDL-C of -4.4 mmol/L, -5.2 mmol/L (-48.1%, -43.9%), Apolipoprotein B of -1.0 g/L, -1.0 g/L (-40.7%, -31.5%), and *ANGPTL3* of -71.3 ng/mL, -121.4 ng/mL (-81.4%, -83.4%), respectively (Table 1). No drug discontinuations, drug-related SAEs or AEs related to elevated ALTs were reported. The most frequent AEs were injection site pain and erythema (11.1%), and nasopharyngitis

(11.1%).

Table 1: Change from Baseline in Serum Lipid and Lipoprotein Concentrations at Week 20

	Serum Concentrations	
	ARO-ANG3 (N=16)	
	200 mg dose (N=8)	300 mg dose (N=8)
LDL-C (Martin- Hopkins)		
Baseline for Week 20 (mmol/L), Mean (SD)	9.6 (6.2) (n=8)	11.6 (5.8) (n=8)
Week 20 (mmol/L), Mean (SD)	5.3 (4.0) (n=8)	6.4 (3.1) (n=8)
Change from Baseline to Week 20 (mmol/L), Mean (SD)	-4.4 (3.0) (n=8)	-5.2 (3.7) (n=8)
Percent change from Baseline to Week 20, Mean (SD)	-48.1 (13.3) (n=8)	-43.9 (18.5) (n=8)
ApoB		
Baseline for Week 20 (g/L), Mean (SD)	2.5 (1.5) (n=7)	2.9 (0.9) (n=7)
Week 20 (g/L), Mean (SD)	1.5 (0.9) (n=8)	1.7 (0.8) (n=8)
Change from Baseline to Week 20 (g/L), Mean (SD)	-1.0 (0.8) (n=7)	-1.0 (0.6) (n=7)
Percent change from Baseline to Week 20, Mean (SD)	-40.7 (12.8) (n=7)	-31.5 (15.1) (n=7)

Abbreviations: ApoB = apolipoprotein B; LDL-C; low-density lipoprotein cholesterol; SD = standard deviation.
Notes: As of the lipid data cut-off date (24 March 2023) 16 of the 18 patients randomized in the study completed Week 20; N is the total number of patients per dosage group as of the data cut-off date; Baseline is the last measurement available prior to the first dose of study drug; n is the number of patients with measurements at both Baseline and at the Week 20 visit.

Conclusions: These interim data suggest that HoFH patients on lipid-lowering standard care who received ARO-ANG3 achieved additional reductions in LDL-C, similar to *ANGPTL3*-targeted monoclonal antibodies. This supports further investigation of ARO-ANG3 in HoFH patients.

Topic: *AS02 Lipids and Lipoproteins / AS02.02 TG rich lipoproteins metabolism and lipases*

SMALL DENSE LDL CHOLESTEROL AND ISCHEMIC STROKE

SAAG SESSION 19: LIPIDS AND LIPOPROTEINS IN HEALTH AND DISEASE

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Background and Aims: For decades it has been suggested that small dense low-density lipoprotein (sdLDL) may be particularly atherogenic. High levels of sdLDL are associated with an increased risk of ischemic heart disease; however, the association of sdLDL with ischemic stroke has not been explored in a large prospective study on the general population. We tested the hypothesis that high sdLDL cholesterol levels are associated with an increased risk of ischemic stroke.

Methods: This prospective study included 38,319 individuals from the Copenhagen General Population Study with fresh sample measurements of sdLDL cholesterol. Median follow-up time was 3.1 years. We observed 302 and 74 ischemic and haemorrhagic strokes from baseline in 2013-2017 to end of follow-up in 2018. For comparison, we included estimates for large buoyant LDL cholesterol and total LDL cholesterol.

Results: Higher levels of sdLDL cholesterol were log-linearly associated with increased risk of ischemic stroke. Compared to individuals with sdLDL cholesterol in the lowest tertile (≤0.60 mmol/L; ≤23 mg/dL) the multivariable adjusted hazard ratio for ischemic stroke was 1.79 (95%confidence interval: 1.31-2.43) for the highest tertile (≥0.86 mmol/L; ≥33 mg/dL). Multivariable adjusted hazard ratios for ischemic stroke per 1 mmol/L (38.7 mg/dL) higher levels were 1.69 (1.28-2.22) for sdLDL cholesterol, 0.95 (0.78-1.16) for large buoyant LDL cholesterol, and 1.08 (0.93-1.25) for total LDL cholesterol. Hazard ratios were similar

when further adjusting for BMI and diabetes mellitus in the biological pathway in combination with related
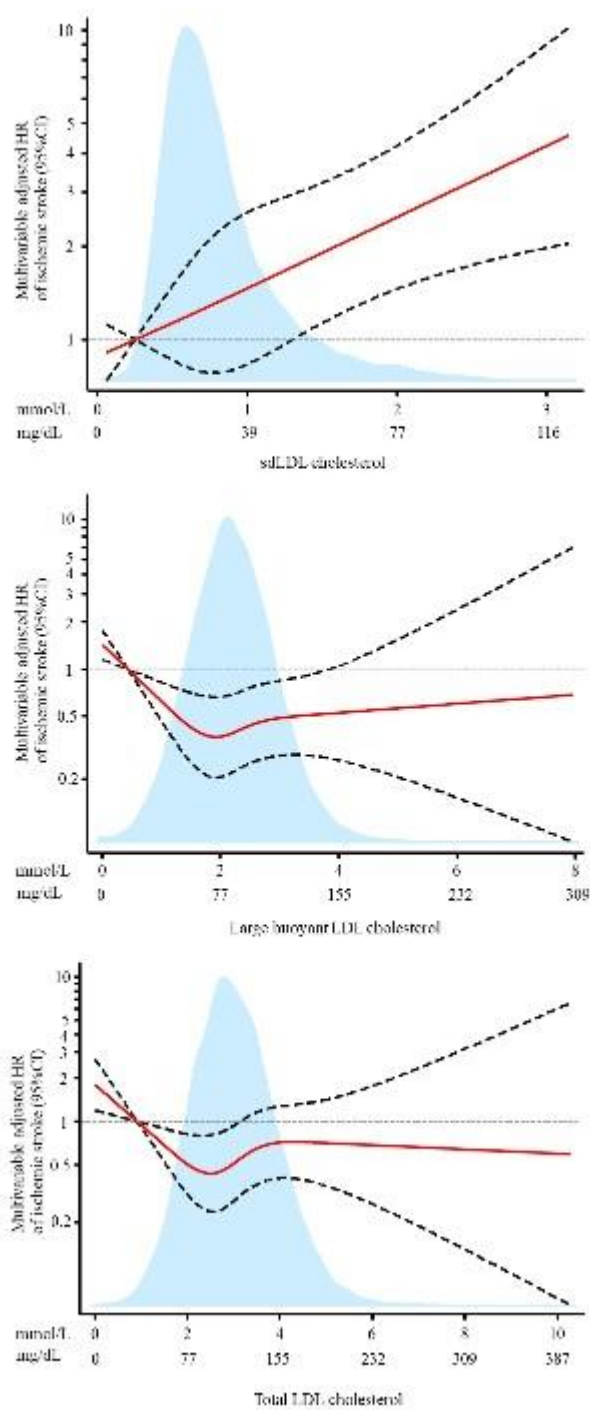
Figure 1.

lipids and lipoproteins.

Conclusions: Higher sdLDL cholesterol levels were robustly associated with increased risk of ischemic stroke.

Topic: *AS02 Lipids and Lipoproteins / AS02.07 Lipidomics*

GENDER DIFFERENCES OF LIPIDOMIC AND BILE ACID PROFILES IN PATIENTS WITH AND WITHOUT CORONARY ARTERY DISEASE: INSIGHTS FROM THE INTERCATH COHORT

SAAG SESSION 19: LIPIDS AND LIPOPROTEINS IN HEALTH AND DISEASE

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Background and Aims: Lipids including phospholipids and bile acids (BA) exert various signalling properties and thus probably contribute to the development of coronary artery disease (CAD). Here we aimed to characterize lipidomic and BA profiles in patients with and without CAD.

Methods: From 2015-2022, 3,012 patients undergoing coronary angiography were recruited in the INTERCATH cohort. Patients with CAD were matched for age, gender, BMI, hypertension, diabetes mellitus, smoking, Mediterranean diet score, intake of statins, triglycerides, tertile of LDL-cholesterol, HDL-cholesterol and hsCrP to patients without CAD in a 1:1 ratio. Lipidomic analyses of stored blood samples using the Lipidyzer platform (SCIEX), and BA analysis using mass spectrometry was carried out.

Results: In the 177 patients included for current analyses, median age was 70.4 years in females (IQR: 61.8, 77.3) and 70.7 years (IQR: 61.3, 77.1) in males. Further baseline characteristics including cardiovascular risk factors were balanced in-between groups *(Table 1)*. Females with CAD had distinct changes in phospholipid levels, whilst no differences in BA profiles were detected in comparison to female patients without CAD. In contrast, in male patients with CAD, decreased concentrations of the secondary BA species glycolithocholic and lithocholic acid, as well as increased levels of specific phospholipids were determined compared to male patients without CAD (*Figure 1*).

	Females (N=88)	Males (N=89)	p-value
Age	70.4 (61.8, 77.3)	70.7 (61.3, 77.1)	0.81
BMI (kg/m^2)	25.7 (22.9, 29.4)	26.3 (24.2, 29.1)	0.25
Diabetes mellitus (%)	6 (6.8)	7 (7.9)	1.00
Active smoking (%)	46 (52.3)	50 (56.2)	0.71
Intake of statins (%)	22 (25.0)	16 (18.0)	0.34
Arterial hypertension (%)	84 (95.5)	82 (92.1)	0.55
MDS (points)	12.99 (11.0, 14.3)	13.3 (11.5, 14.7)	0.44
Triglycerides (mg/dl)	96.5 (75.8, 118.8)	84.0 (69.0, 117.0)	0.12
HDL-c (mg/dl)	54.5 (45.8, 62.6)	51.0 (41.0, 61.0)	0.20
hsCrP (mg/dl)	0.3 (0.1, 0.8)	0.3 (0.1, 0.7)	0.73

Table 1: Baseline characteristics of the female and male subgroup. Categorical variables are shown as absolute numbers and percentages. Continuous variables are described by mean ± standard deviation or median and the 25th percentile and 75th percentile. BMI = body-mass index; CAD = coronary artery disease; HDL-c = high-density lipoprotein cholesterol; hsCrP = high-sensitivity C-reactive protein; MDS = Mediterranean diet score.
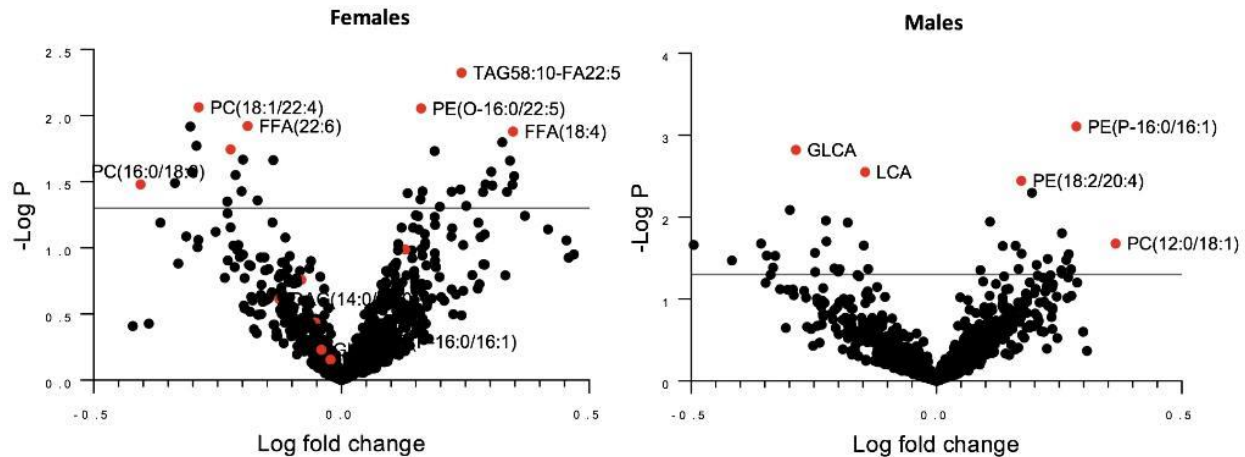
Figure 1: Volcano plot for plasma lipid and bile acid species according to gender and in comparison between patients with and without CAD. The x-axis represents log fold change, and the y-axis represents −log10 p- value. *CAD* Coronary artery disease; *DAG* Diacylglycerol; *FFA* Free fatty acid; *GLCA* Glycolithocholic acid; *LCA* Lithocholic acid; *PC* Phosphatidylcholines; *PE* Phosphatidylethanolamine; *TAG* Triacylglycerol.

Conclusions: We describe a sex-specific lipidomic pattern as well as BA profile in patients with CAD. The data suggest that altered phospholipid and bile acid composition contribute to CAD development and/or progression.

Topic: *AS02 Lipids and Lipoproteins / AS02.09 Lipid and lipoprotein metabolism*

EVIDENCE SUPPORTING A CAUSAL RELATIONSHIP BETWEEN AN APOB-INDEPENDENT 1H-NMR METABOLOMICS PROFILE AND ATHEROSCLEROTIC CARDIOVASCULAR DISEASE

SAAG SESSION 19: LIPIDS AND LIPOPROTEINS IN HEALTH AND DISEASE

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Background and Aims: In-depth lipoprotein profiling by ^{1}H-NMR has yielded significant insight into the pathophysiology of atherosclerotic cardiovascular disease, but the interrelated nature of lipoproteins complicates causality inference. We aimed to define unrelated dimensions of metabolomic measures and investigate their associations with coronary artery disease (CAD).

Methods: Principal component analysis was performed on 168 ^{1}H-NMR-based metabolomic measures in 56,712 European participants (57% women) from UK Biobank (UKB) to retrieve independent metabolomic principal components (PCs), which were subjected to multivariable-adjusted Cox-proportional hazard models to assess association with CAD and genome-wide association analyses. Two-sample mendelian randomisation (MR) analyses were conducted in three outcome databases with a combined sample size of 755,481 (128,728 cases), which were subsequently meta-analysed.

Results: Six PCs collectively explained 88% of the total variance. For the risk of CAD, results from the Cox and the MR analyses were mostly aligned. The pooled ORs [95% CI] for per one-SD increase in genetically-influenced PC1 and PC3 were 1.04 [1.03, 1.05] and 0.94 [0.93,0.96], in agreement with PC1 and PC3 being mainly characterized by increased and decreased ApoB-associated lipoproteins, respectively. Surprisingly, the pooled OR for CAD of PC4 was 1.05 [1.03, 1.07], with PC4 being characterized by simultaneously decreased small HDL and increased large HDL, independent of ApoB. No associations were found between PC5 (amino acids) and PC6 (ketone bodies) with CAD risk. Additional analyses revealed no evidence for bias due to directional pleiotropy.

Conclusions: A metabolomic feature involving multiple lipoprotein particles, independent of ApoB, may be a driver of CAD risk.

SS128 / #1268

Topic: *AS02 Lipids and Lipoproteins / AS02.07 Lipidomics*

LONGITUDINAL ASSOCIATION BETWEEN NMR METABOLOMIC LIPID PROFILES AND CAROTID INTIMA-MEDIA THICKNESS AMONG YOUTH WITH SEVERE OBESITY

SAAG SESSION 19: LIPIDS AND LIPOPROTEINS IN HEALTH AND DISEASE

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Background and Aims: Obesity in youth is associated with cardiometabolic risk factors, which contribute to adult premature cardiovascular disease (CVD). Blood metabolomic lipid profiles are associated with CVD in adults with and without obesity, but data on youth with obesity are scarce. This study investigated the association of nuclear mass resonance (NMR) lipid profiles at two time-points with carotid intima-media thickness (cIMT) in youth with severe obesity.

Methods: Data were from 66 participants (aged 3-16 years at baseline) from the Childhood Overweight BioRepository of Australia (COBRA) who were reassessed at follow-up 5.7 [SD 2.1] years later when mean cIMT (in micrometer) was measured. Generalized linear models were applied using a life-course epidemiology framework to model the patterns of associations between 30 lipids and lipoproteins at two time-points and cIMT from normalized data. We tested competing models of association between the lipids at both timepoints and follow-up cIMT: *accumulation*; *lifetime growth*; *sensitive and critical periods*; and *no association*.

Results: Cross-sectional associations at both timepoints revealed no/limited associations with cIMT. Longitudinal analyses prioritized a lifetime growth model, meaning that change over time for the following lipids provided the best estimate of the longitudinal association with cIMT (beta-coefficient, 95%CI per 1-SD higher lipid measure): Apolipoprotein B 34.7 (15.7-53.6); VLDL cholesterol 31.9 (15.0-48.8); non-HDL cholesterol 19.7 (3.5-35.8), total triglycerides 37.9 (18.8-57.9), total fatty acids 25.7 (9.5-41.8), triglycerides in various-sized VLDLs and IDLs (all $p < 0.005$).

Conclusions: Longitudinal assessment of in-depth lipid and lipoprotein measures show patterns of associations with cIMT among severely obese youth that are superior to cross-sectional assessments.

SS129 / #662

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.08 Novel risk factors and biomarkers*

THE ASSOCIATION OF APPENDICULAR LEAN MASS AND GRIP STRENGTH WITH LDL, VLDL AND HDL PARTICLE DIAMETER: A MENDELIAN RANDOMIZATION STUDY OF THE UK BIOBANK COHORT

SAAG SESSION 19: LIPIDS AND LIPOPROTEINS IN HEALTH AND DISEASE

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Background and Aims: Reduced muscle mass and strength is frequently associated with alterations in blood lipids and poorer cardiometabolic outcomes in epidemiological studies, however, a causal association cannot be determined from such observations. Mendelian randomization (MR) was applied to assess the association of genetically determined appendicular lean mass (ALM) and handgrip strength (HGS) with serum lipid particle diameter.

Methods: MR was implemented using summary-level data from the largest genome-wide association studies (GWAS) on ALM (n=450,243), HGS (n=461,089) and LDL, VLDL and HDL particle diameters, (n=115,078). Inverse variance weighted method (IVW) was used to estimate the causal estimates. Weighted median (WM)-based method, and MR-Egger, leave-one-out were applied as sensitivity analysis.

Results: Increased ALM had a statistically significant positive effect on HDL particle diameter (MR Egger= β:0.055, p=0.081 and IVW= β:0.068, p=6.15x10-7; respectively), and a negative and statistically significant effect on VLDL particle diameter (MR Egger= β:-0.114, p=0.003 and IVW= β:-0.081, p=1.57x10-6, respectively). Increased HGS, had a statistically significant positive effect on HDL particle diameter (MR Egger= β:0.433, SE:0.184, p=0.019 and IVW= β:0.121, SE:0.052, p=0.021), and a negative and statistically significant effect on VLDL particle diameter (MR Egger= β:-0.416, SE:0.163, p=0.011 and IVW= β:-0.122, SE:0.046, p=0.009). There was no significant effect of ALM or HGS on LDL particle diameter.

Conclusions: Evidence for a potentially causal association of both increasing ALM and HGS, with both increasing HDL particle size and decreasing VLDL particle size was found, highlighting their potential for improving CVD risk profile.

SS130 / #1571

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.13 Genomics, GWAS and population genetics; Mendelian randomization*

EXPRESSIVITY OF FAMILIAL HYPERCHOLESTEROLEMIA VARIANTS IN 1 MILLION INDIVIDUALS.

SAAG SESSION 19: LIPIDS AND LIPOPROTEINS IN HEALTH AND DISEASE

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Background and Aims: Familial hypercholesterolemia (FH) is a genetic condition caused by a single penetrant mutation in the hallmark genes for lipid metabolism. Pathogenicity assessments rely on inferred penetrance often from clinically referred probands and families. Population-scale genetic studies are now of sufficient size to provide unbiased effect estimates of single putative Mendelian variants, specifically applied to FH here.

Methods: Using a large-scale genetic cohort in the Million Veterans Program and UK Biobank (N_{Total} = 1,057,913), we tested the association between variants of FH-associated genes and low-density lipoprotein (LDL) cholesterol levels. We tested 1,742 coding variants with a minor allele frequency < 1% on *PCSK9*, *APOB*, and *LDLR*. We intersected these findings with 1,167 variants in the ClinVar database (100 pathogenic, 738 uncertain significance, 329 benign).

Results: We observed a strong enrichment of variants with exome-wide significance ($P < 1\times10^{-9}$) in ClinVar Pathogenic/Likely Pathogenic variants (Odds ratio 7.5 [4.4–12.5]) relative to variants with Benign/Uncertain significance. ClinVar Pathogenic/Likely Pathogenic variants generally showed large effect sizes (median 1.16 SD per allele, IQR [0.65-1.70]). However, we identified four penetrant variants (effect size > 1.16 SD per allele) with exome-wide significance and seven with Bonferroni-adjusted significance ($P < 2.8\times10^{-5}$) in the variants previously considered as Benign/Uncertain significance. We also identified two variants previously considered pathogenic but negatively affecting blood LDLC

levels.
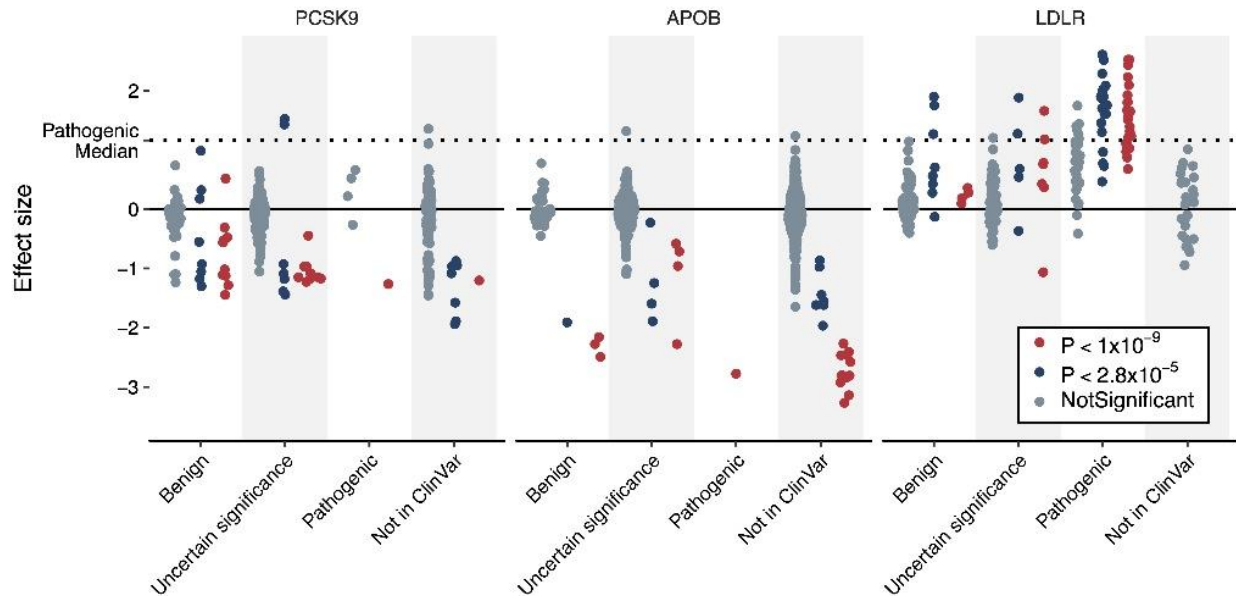
Conclusions: A population-scale association analysis provides stable effect estimates for rare FH-associated variants and potentially reclassifies the pathogenicity of many variants. This approach provides complementary data for current frameworks of pathogenicity assessments with implications beyond FH.

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.01 Epidemiology of cardiovascular diseases and risk factors*

CARDIOVASCULAR MORTALITY ATTRIBUTABLE TO DIETARY RISK FACTORS IN 54 COUNTRIES IN THE WHO EUROPEAN REGION FROM 1990-2019: A SYSTEMATIC ANALYSIS OF THE GBD STUDY

SAAG SESSION 20: TOOLS TO DEFINE CVD RISK

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Background and Aims: Cardiovascular diseases (CVDs) are a leading cause of death in Europe, and dietary risks are one of the most important behavioural risk factors. The aim of this study was to estimate the contribution of dietary risk factors to CVDs in the WHO European Region (WHO ER).

Methods: To estimate the contribution, 13 dietary risks and 13 CVDs were examined and the comparative risk assessment framework of the Global Burden of Disease Study 2019 was used. The data were analysed by age and sex and calculated for 54 countries and their related regions.

Results: In 2019, 1.55 million people in the WHO ER died from diet-related CVD deaths (DRCDs; 16.6% of total deaths and 36.7% of CVD deaths). Between 1990 and 2019, there was a reduction in DRCDs. Simultaneously, the percentage of DRCDs and the age-standardised death rate decreased. The numbers of DRCDs are almost equally distributed between women and men. The largest percentage is found in the group 85+ years (32.1%). Most DRCDs were caused by a diet low in whole grains (21.1%), followed by a diet low in legumes (15.0%), and a diet high in sodium (12.3%). 80.3% of the DRCDs could be

attributed to ischemic heart disease, which was the main cause of DRCDs in all countries.

Year	Number of deaths	Deaths per 100.000 people*	% of CVD deaths	% of total deaths
1990	1.686.540	212	41.5 %	19.8 %
1995	1.897.793	236	41.8 %	20.1 %
2000	1.802.287	221	40.7 %	19.5 %
2005	1.763.761	213	39.3 %	18.7 %
2010	1.582.521	180	37.9 %	17.4 %
2015	1.529.240	161	37.1 %	16.5 %
2019	1.550.233	150	36.7 %	16.4 %

* age-standardised

Conclusions: In terms of CVD deaths in the WHO ER, more than every third death is attributable to an unbalanced diet, making the diet one of the most important factors in preventing premature CVD death in the WHO ER.

Topic: *AS04 Clinical Vascular Disease / AS04.03 NASH and other ectopic lipid diseases*

A SIGNIFICANT ASSOCIATION OF ROUTINE CHEST COMPUTED TOMOGRAPHY-MEASURED PERICARDIAL FAT AREA WITH CORONARY RISK FACTORS AND CORONARY ARTERY DISEASE

SAAG SESSION 20: TOOLS TO DEFINE CVD RISK

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Background and Aims: Pericardial fat has been shown to be associated with the development of coronary arterial disease (CAD). So far, to quantify pericardial fat, the method to measure pericardial fat volume (PFV) by using contrast-enhanced cardiac computed tomography (CT) was used. We determined pericardial fat area (PFA) in the cross section with the height of sternal angle by using routine chest CT.

Methods: We picked up patients who underwent routine chest and abdominal CT from April 2017 to October 2018, and we selected patients whose coronary arteries were evaluated by coronary angiography (CAG) or coronary CT within 3 years. CAD was defined as more than 75% lumen stenosis in coronary arteries evaluated by CAG or coronary CT. PFA was defined as any pixel with CT attenuation of -150 to -30 Hounsfield Unit (HU) within the pericardial sac at the sternal angle level.

Results: Fifty-three patients were eligible. PFA was significantly larger in men than in women. Serum HDL-C level was significantly and negatively correlated with PFA. HbA1c and carotid arterial intima-media thickness tended to be positively correlated with PFA. PFA was significantly and nearly 50% larger in patients with CAD than in patients without CAD.

Conclusions: To our knowledge, present study is the first to show a significant association of PFA determined by routine chest CT with gender and CAD. PFA measurement is simpler and more reproducible and more available in a greater number of medical institutes as compared with PFV.

SS133 / #806

Topic: *AS04 Clinical Vascular Disease / AS04.12 Prevention and treatment of cardiovasculardisease; miscellaneous*

LOW PLASMA TRANSTHYRETIN IS ASSOCIATED WITH ALL-CAUSE AND CARDIOVASCULAR MORTALITY IN THE GENERAL POPULATION

SAAG SESSION 20: TOOLS TO DEFINE CVD RISK

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Background and Aims: Transthyretin tetramer destabilization is the rate-limiting step in transthyretin cardiac amyloidosis, which is an underrecognized contributor to heart failure and mortality in older adults. Evidence suggests that low plasma transthyretin is an *in vivo* marker of transthyretin tetramer instability. We tested the hypothesis that low plasma transthyretin genetically and observationally associates with all-cause and cardiovascular mortality.

Methods: We genotyped 102,204 individuals and measured plasma transthyretin concentrations in 19,619 individuals from two studies of the Danish general population, the Copenhagen City Heart Study and the Copenhagen General Population Study. We first tested whether genetic variants in *TTR*, which associated with increasing transthyretin tetramer instability, also associated with lower plasma transthyretin and higher risk of all-cause and cardiovascular mortality. Second, we tested whether low plasma transthyretin associated with higher risk of all-cause and cardiovascular mortality.

Results: Compared to p.T139M, a well-known transthyretin stabilizing mutation, *TTR* genotype was associated with stepwise lower transthyretin concentrations of -20% for wild-type and -30% for heterozygotes for "Other mutations" (p.V142I, p.H110N, p.D119N). The corresponding hazard ratios (HR) for all-cause and cardiovascular mortality were 1.37(95% CI: 1.06-1.77) and 1.63(0.92-2.89) in wild-types, and 1.66(0.95-2.88) and 2.23(0.78-6.34) in carriers of "Other mutations", respectively (Figure). In adjusted observational analysis individuals with plasma transthyretin ≤5th percentile (<19.1 mg/dL) versus 6-

95th percentile (reference) had HRs of 1.39(1.17-1.64) and 1.66(1.17-2.38) for all-cause and
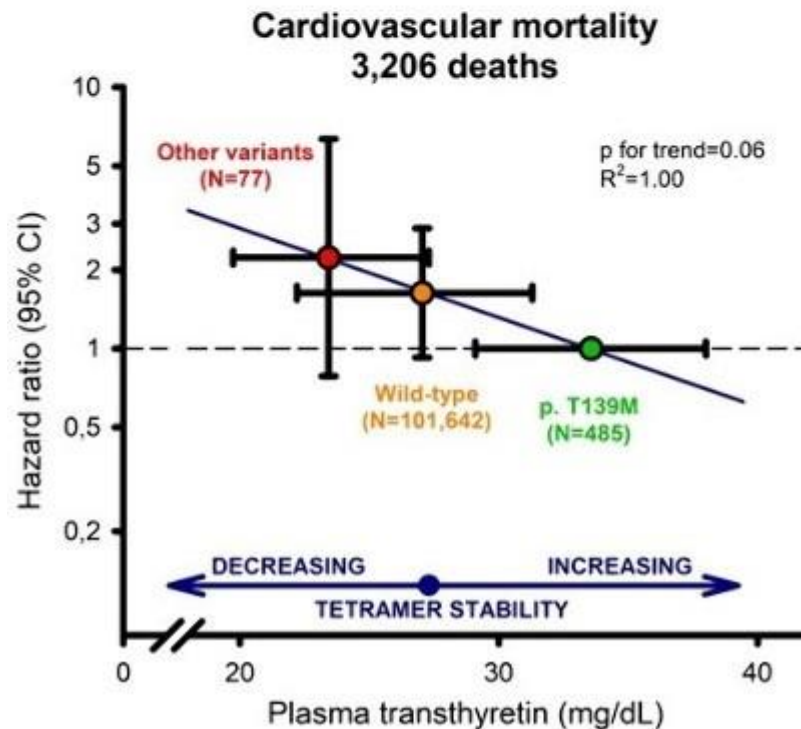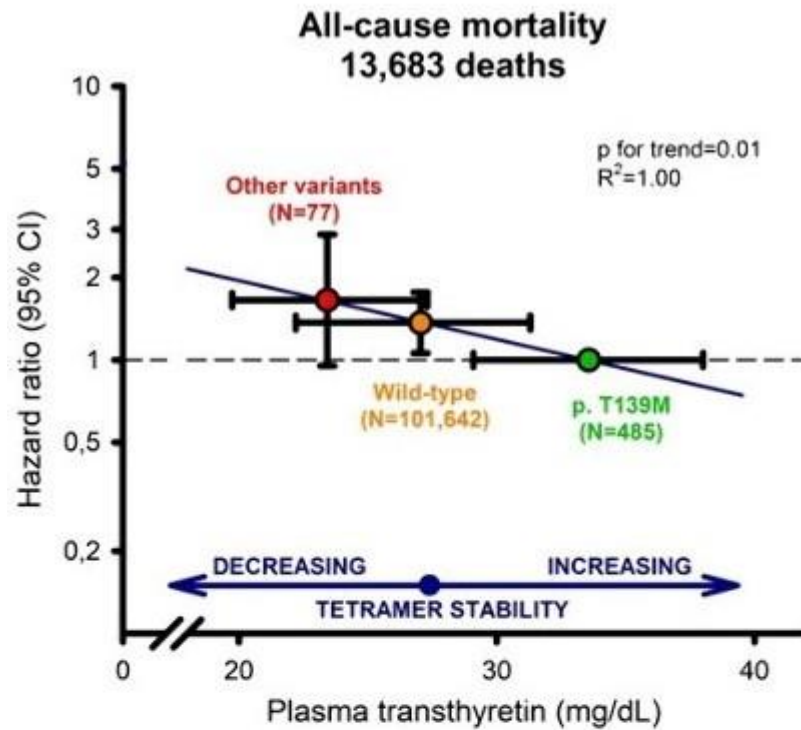
cardiovascular mortality.

Conclusions: Genetically and observationally low transthyretin concentrations are associated with higher risk of all-cause and cardiovascular mortality.

SS134 / #960

Topic: *AS04 Clinical Vascular Disease / AS04.12 Prevention and treatment of cardiovasculardisease; miscellaneous*

BURDEN AND PREDICTORS OF MAJOR ADVERSE CARDIAC AND CEREBROVASCULAR EVENTS IN PREMATURE [18-49 YEARS] PERIPHERAL VASCULAR DISEASE PATIENTS: A NATIONWIDE ANALYSIS

SAAG SESSION 20: TOOLS TO DEFINE CVD RISK

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Background and Aims: Premature peripheral vascular disease (P-PVD) remains underdiagnosed and is increasingly being reported in the US. We aimed to study the burden and predictors of major adverse cardiac and cerebrovascular events (MACCE) in P-PVD.

Methods: We identified admissions with P-PVD [18-49 years] complicated by MACCE using 2019's National Inpatient Sample and ICD-10 CM codes. Primary outcomes were burden and predictors of MACCE. The multivariable analysis was adjusted for confounders to assess the independent predictors of MACCE in P-PVD. After excluding missing data (n=66), the P-PVD cohort (unweighted n=21934) was randomly split into training data (n=17457, 80%) and testing data (n=4477, 20%). Training data were used to calibrate Artificial Neural Network (ANN), while testing data were used to evaluate the algorithm's accuracy in predicting MACCE (all-cause mortality, acute myocardial infarction, cardiac arrest, and stroke).

Results:

Figure 1a. Normalized Importance of MACCE Predictors in Premature PVD
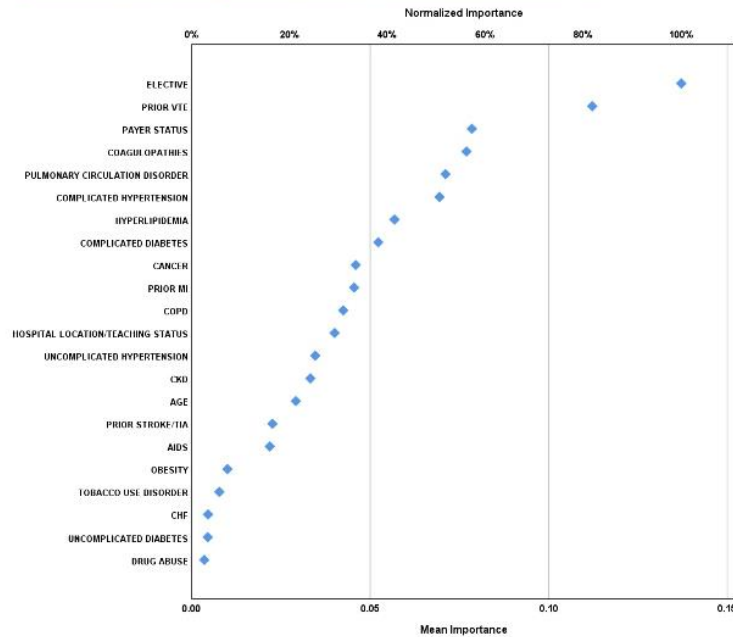
Figure 1b. Area Under the Curve for Performed Methods
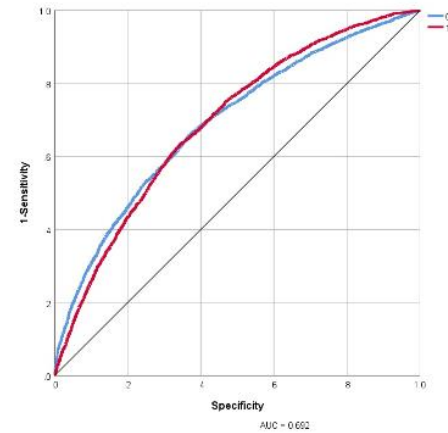
P-PVD cohort consisted of 110,000 admissions with a mean age of 42± 5 years (52.9% males, 56.1% whites, 36.2% low-income quartile). 14.3% of P-PVD patients had MACCE. Training data showed a prediction rate of 85.7% with an error rate of 14.3% in training/testing groups. Normalized predictors of MACCE are displayed in Fig 1a. Major predictors (aOR) were non-elective admission (3.99), coagulopathy (2.11) complicated (1.91) and uncomplicated hypertension (1.29), hyperlipidemia (1.45), prior MI/PCI (1.40/2.00), and pulmonary circulation disorder (1.50). Our ANN model had an AUC of 0.7 (Fig 1b).

Conclusions: Our ANN model successfully identified the prevalent predictors for MACCE in P-PVD and may enable clinicians to screen high-risk patients, improve their survival, and reduce costs.

SS135 / #778

Topic: *AS04 Clinical Vascular Disease / AS04.12 Prevention and treatment of cardiovasculardisease; miscellaneous*

CUMULATIVE EFFECT OF ACCELEROMETER-BASED MODERATE-TO-VIGOROUS PHYSICAL ACTIVITY FROM CHILDHOOD THROUGH YOUNG ADULTHOOD ON CAROTID INTIMA-MEDIA THICKNESS PROGRESSION: THE ALSPAC BIRTH COHORT STUDY

SAAG SESSION 20: TOOLS TO DEFINE CVD RISK

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Background and Aims: Recent guidelines have recommended at least 60 mins/day of moderate-to-vigorous physical activity (MVPA) in youth. However, longitudinal evidence on the cumulative effect of exposure to MVPA from childhood on carotid intima-media thickness (cIMT) progression, a measure of subclinical atherosclerosis, is limited. This study examined whether persistent exposure to 60 mins/day of MVPA from childhood associates with cIMT progression.

Methods: This study included 1339 British 11-year-olds (755 females) followed up for 13 years. cIMT was repeatedly measured by ultrasound device at ages 17 and 24 years. MVPA was measured by ActiGraphTM accelerometer at ages 11, 15, and 24 years. MVPA times were categorized as <40 mins/day (reference), 40 – <60mins/day, and ≥60mins/day. Generalized linear mixed-effect model analyses were adjusted for repeated measures of covariates viz; age, low-density lipoprotein cholesterol, insulin, triglyceride, high sensitivity C-reactive protein, high-density lipoprotein cholesterol, heart rate, systolic blood pressure, glucose, fat mass, lean mass, smoking status, family history of hypertension/diabetes/high cholesterol/vascular disease, and socioeconomic status, sedentary time and light PA.

Results: Persistent exposure to ≥60mins/day of MVPA from ages 11 – 24 years was negatively associated with the 7-year cIMT progression in females, effect estimate -0.017 mm; [95% CI -0.026 to -0.009; p <0.001] but not in males 0.008 mm; [-0.005 to 0.022; p = 0.238]. Persistent exposure to MVPA 40 - <60 mins/day had no statistically significant associations with cIMT progression in both males and females.

Conclusions: Cumulative exposure to ≥60mins/day of MVPA from childhood through young adulthood seems protective against sub-clinical atherosclerosis progression, particularly in females.

SS137 / #1545

Topic: *AS02 Lipids and Lipoproteins / AS02.01 Lp(a)*

LIPOPROTEIN(A) IS ASSOCIATED WITH LONG-TERM PLAQUE PROGRESSION ON SERIAL CORONARY CT ANGIOGRAPHY

SAAG SESSION 20: TOOLS TO DEFINE CVD RISK

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Background and Aims: Lipoprotein(a) (Lp[a]) is causally associated with atherosclerotic cardiovascular disease (ASCVD), however, data on the relationship between Lp(a) and coronary plaque burden are conflicting. This study investigated the effect of Lp(a) on long-term plaque progression in patients suspected of coronary artery disease (CAD).

Methods: Per-protocol, patients from a coronary CT angiography (CCTA) cohort (Diemen et al., 2021) were invited for repeat CCTA imaging, regardless of symptoms. A total of 299 patients underwent follow-up CCTA imaging with a median scan interval of 10.2 [IQR 8.7-11.2] years. Patients who underwent coronary artery bypass grafting were excluded. Scans were analyzed using artificial intelligence-guided quantitative CCTA (AI-QCT; Cleerly Inc.). Quantitative plaque volumes (total and non-calcified and calcified plaque subsets) were adjusted for vessel volume (percent atheroma volume; PAV). The association between Lp(a), baseline and follow-up PAV as well as PAV change was evaluated in a multivariate linear regression model adjusted for clinical risk factors.

Results: In total, 272 patients were included, mean age was 57±7 years, 42% were women. At baseline, median PAV was 2.52% [IQR 0.69-8.05], which increased to 6.14% [IQR 1.18-12.88] at follow-up. An interquartile range (IQR; 103 nmol/l) higher plasma Lp(a) concentration was associated with an 1.17% higher baseline PAV, resulted in a 0.65% higher increase in PAV during follow-up, and led to an 1.83% higher PAV at follow-up imaging (Figure). Similar associations were found for calcified and non-calcified

plaque volumes.

Figure 1. Associations between Lp(a) and coronary plaque burden

Outcome variable	Beta per IQR Lp(a) increase	(95% CI)	P-value
Baseline			
Percent atheroma volume		1.82 (0.66 to 2.99)	0.002
Percent non-calcified plaque volume		0.60 (0.15 to 1.05)	0.009
Percent calcified plaque volume		0.57 (0.14 to 1.00)	0.009
Follow-up (10 years)			
Percent atheroma volume		1.82 (0.66 to 2.99)	0.002
Percent non-calcified plaque volume		0.53 (0.15 to 0.91)	0.006
Percent calcified plaque volume		1.27 (0.37 to 2.17)	0.006
Change (delta)			
Percent atheroma volume		0.64 (0.05 to 1.24)	0.036
Percent non-calcified plaque volume		-0.07 (-0.33 to 0.20)	0.629
Percent calcified plaque volume		0.70 (0.08 to 1.32)	0.028

(x-axis: -1, 0, 1, 2, 3)

Shown are beta coefficients with 95% CI from linear regression models with Lp(a) and clinical risk factors as independent variables and the plaque volumes as dependent/outcome variable. Change was calculated subtracting baseline from follow-up values.

Conclusions: Patients with high Lp(a) levels have an up to 50% increased coronary plaque burden and markedly increased coronary artery progression throughout 10-year follow-up.

Topic: *AS02 Lipids and Lipoproteins / AS02.09 Lipid and lipoprotein metabolism*

EFFICACY AND SAFETY OF LOMITAPIDE IN PEDIATRIC PATIENTS WITH HOMOZYGOUS FAMILIAL HYPERCOLESTEROLEMIA: IS DISCONTINUATION OF LIPOPROTEIN AFHERESIS POSSIBLE?

SAAG SESSION 21: EFFICIENT MANAGEMENT OF FH

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Background and Aims: Lomitapide is a microsomal transfer protein inhibitor approved for the treatment of adults with homozygous familial hypercholesterolemia (HoFH). The use of lomitapide in HoFH pediatric subjects is described only by few case-reports. In this study, as part of the first multicenter trial in the word, we evaluated the efficacy and the safety of lomitapide on top of conventional therapy with statin, ezetimibe and lipoprotein apheresis (LA) in pediatric subjects with HoFH.

Methods: 2 males and 2 females, aged between 6 and 10 years, with HoFH (3 out of 4 with genetic diagnosis) were included in the study. Lomitapide was initiated at a dose of 2 mg/day and escalated up to 20 mg/day. The efficacy of lomitapide was defined by the LDL cholesterol change, hepatic function and liver ultrasound were assessed.

Results: Untreated LDL cholesterol (LDL-C) was 794±53 mg/dl (mean±SD). With rosuvastatin 5-20 mg plus ezetimibe 10 mg plus weekly LA, LDL-C was 324±27 mg/dl. The addition of lomitapide 20 mg/day, resulted in a robust LDL-C decrease (221±52 mg/dl after 28-40 weeks of treatment), allowing the reduction of LA frequency in 3 children (every two weeks) and the LA suspension in 1. Three out of 4 patients showed a transient mild elevation of AST and ALT (<2xULN), and the mild hepatic steatosis remained stable during the therapy. At present, none of the children has discontinued lomitapide treatment.

Conclusions: Our findings suggest that lomitapide is effective and safe in children with HoFH, leading to a significant reduction in LDL-C and allowing a reduction/suspension of LA.

CLINICAL PREDICTORS OF POSITIVE GENETIC TESTING IN HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA

SAAG SESSION 21: EFFICIENT MANAGEMENT OF FH

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Background and Aims: The diagnostic pathway of familial hypercholesterolaemia (FH) involves a step whereby patients clinically suspected to have FH should have the diagnosis confirmed through genetic testing (GT) (gold standard for FH diagnosis). However, availability and accessibility to GT is resource-dependent and usually restricted to specialised lipid clinics. We aimed to identify what clinical factors may be associated with a positive GT among subjects with a clinical suspicion of FH.

Methods: Cross-sectional study including all subjects who were conducted a GT for FH within the Dyslipidaemia Registry of the Spanish Atherosclerosis Society. A positive GT was defined as having a pathogenic/likely pathogenic variant related to FH (*LDLR/APOB/PCSK9* genes). Homozygous FH cases were excluded. Demographics, clinical characteristics and lipid levels were compared between those with and without a positive GT. The association of different variables with a positive GT was assessed through multivariate logistic regression.

Results: From the 2939 patients who were performed a GT, 2113 (71.9%) had a positive GT. These patients (compared with those with a negative GT) were younger (47.1±15.4 vs. 53.8±11.4 years), with a lower frequency of cardiovascular risk factors, and higher untreated LDL-C (256 [IQR 214-307] vs. 233 [206-264] mg/dl). ORs for the association of variables with a positive GT are presented in the

table.

Table. Odds ratios for the association of different variables with a positive genetic testing result for FH

	Univariate analysis OR (95% CI)		Multivariate analysis OR (95% CI)	
Age (years)	0.96 (0.96-0.97)	<0.001	0.95 (0.94-0.96)	<0.001
Men (vs. women)	1.03 (0.87-1.21)	0.698	-	-
Xanthomas	1.51 (1.24-1.84)	<0.001	1.50 (1.19-1.90)	0.001
Arcus cornealis at age <45 years.	1.01 (0.84-1.22)	0.857	-	-
Current Smoker (vs. non-smoker or former smoker)	0.79 (0.65-0.95)	0.015	0.65 (0.51-0.82)	<0.001
BMI (Kg/m²)	0.95 (0.94-0.97)	<0.001	0.99 (0.96-1.01)	0.487
Hypertension	0.59 (0.48-0.72)	<0.001	1.17 (0.88-1.55)	0.260
Diabetes mellitus	0.86 (0.62-1.21)	0.404	-	-
Cardiovascular disease	1.07 (0.84-1.36)	0.567	-	-
Premature CAD	0.94 (0.72-1.23)	0.694	-	-
Total cholesterol (mg/dL)	1.00 (1.00-1.00)	<0.001	-	-
HDL cholesterol (mg/dL)	0.98 (0.97-0.98)	<0.001	0.97 (0.97-0.98)	<0.001
LDL cholesterol (mg/dL)	1.00 (1.00-1.01)	<0.001	1.01 (1.00-1.01)	<0.001
Triglycerides (mg/dL)	0.99 (0.99-0.99)	<0.001	0.99 (0.98-0.99)	<0.001

ORs for quantitative variables are shown per 1-unit increase. Premature CAD: age of CAD onset <55 years in men <60 in women. CAD: coronary artery disease; LDL: low-density lipoprotein; HDL: high-density lipoprotein; 95% CI: 95% confidence interval; OR: odds ratio.

Conclusions: A younger age, presence of xanthomas, higher LDL-C, and lower HDL-C and triglycerides were independently associated with a positive GT for heterozygous FH. Our findings may help inform the adequacy of current clinical criteria for the diagnosis of (genetically confirmed) FH.

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.02 Epidemiology of dyslipidemias*

APPROACHES TO LDL-C MANAGEMENT IN CHILDREN AND ADOLESCENTS WITH FAMILIAL HYPERCHOLESTEROLAEMIA: ANALYSIS ON OVER 3000 INDIVIDUALS RECEIVING LIPID-LOWERING MEDICATION IN THE FHSC REGISTRY

SAAG SESSION 21: EFFICIENT MANAGEMENT OF FH

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Background and Aims: Initiation lipid-lowering medications (LLM), early, in children/adolescents with heterozygous familial hypercholesterolaemia (HeFH) enables a life-course resembling the general population (without FH). We assessed approaches to use of LLM in children/adolescents with HeFH in the FHSC Registry.

Methods: Cross-sectional analysis at registry entry of children/adolescents (age <18 years) with HeFH and receiving LLM. Homozygous FH and apheresis cases were excluded. Attainment of recommended LDL-C goals (<3.4 mmol/L) was assessed through logistic regression.

Results:

Figure. Adjusted odds ratio for attaining an LDL <3.4 mmol/l (<130 mg/dl).

(A)

(B)
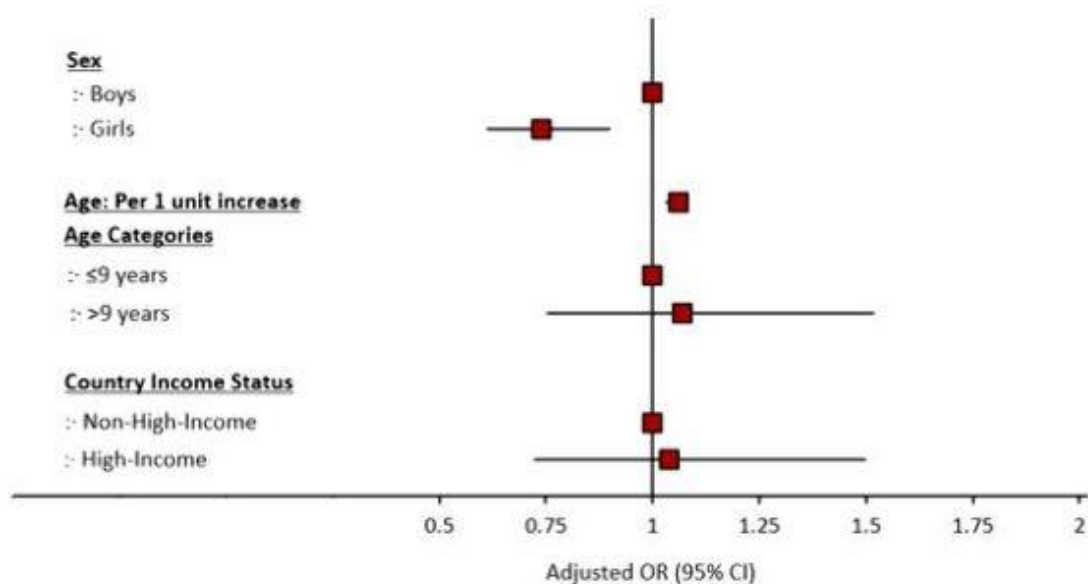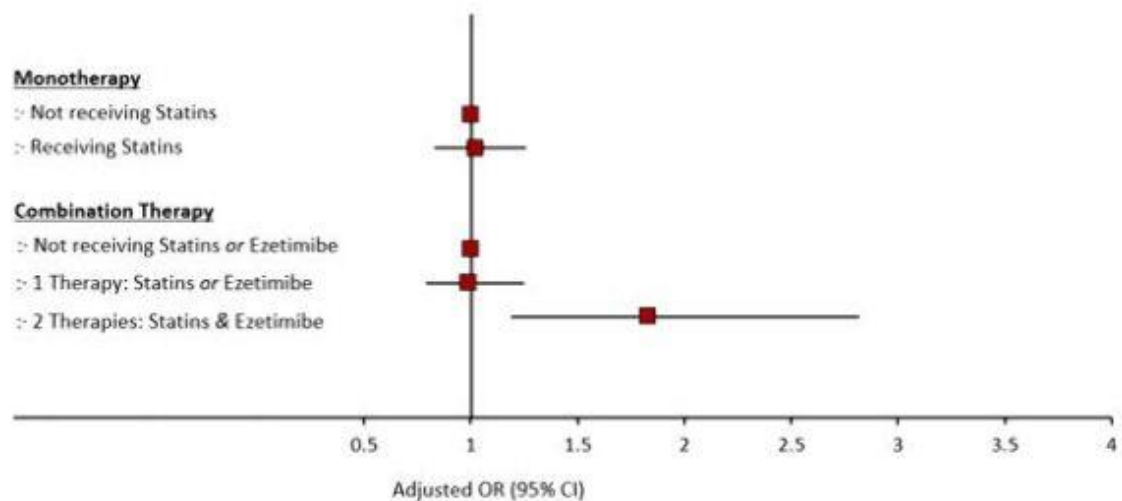
From 11,848 HeFH children/adolescents in the FHSC Registry, 3143 (28.5%) were receiving LLM (50.5% girls; 63.5% age >9 years; 89.9% with genetic diagnosis). Percentage of children/adolescents on LLM increased by age to 33.4% among those >15 years old. Use of statins ranged from 10.0% to 41.0% among participants aged <5 and >15 years, respectively (corresponding figures for ezetimibe: 4.3%–7.8%). 10 cases were receiving PCSK9i. Median LDL-C was 4.35 mmol/L (IQR 3.44–5.34) (13% lower than children/adolescents not on LLM). Attainment of LDL-C <3.4 mmol/L was more frequent in boys, in those aged ≥9 years, or with combination therapy (all, p<0.01). Figure shows adjusted odds ratios for LDL-C goal attainment.

Conclusions: Although statins and ezetimibe are approved for use in childhood, their use is low resulting in low attainment of recommended LDL-C goals. Achieving lipid goals will require greater use of combination therapies to prevent the future burden of ASCVD. Like observations of sex differences in adults, girls had lower odds of achieving recommended LDL-C levels. Further work is needed to understand barriers to better use LLM in children/adolescents with FH.

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.13 Genomics, GWAS and population genetics; Mendelian randomization*

COMPARING HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA PHENOTYPE TO GENOTYPE IN AN IRISH COHORT

SAAG SESSION 21: EFFICIENT MANAGEMENT OF FH

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Background and Aims: Currently, diagnostic criteria schemes such as the Dutch Lipid Clinic Network (DLCN) criteria are used to establish a clinical diagnosis of hFH. Subsequent genetic testing via Next-Generation Sequencing (NGS) or multiplex ligation-dependent probe amplification (MLPA) confirms the genetic diagnosis. The aim of this study was to establish the FH phenotype of patients attending a lipid clinic in Ireland as per the DLCN criteria and compare these diagnoses with subsequent genetic testing results.

Methods: An ethically approved retrospective chart review was performed of consecutive adult patients attending a specialist Lipid Clinic over a six-month period in 2021. Patient data recorded included demographics, clinical characteristics, biochemical profiles, DLCN score and genetic testing results.

Results: A total of 370 patients were included. 192 patients (52%) had genetic testing results available. 70 patients (36%) had a genetically confirmed diagnosis of hFH. An LDL-R mutation was the most common mutation detected in the study population (83%) followed by ApoB (14%) and a positive exon deletion on MLPA (3%). No mutations in PCSK9 were detected. 58% of patients with a definite hFH phenotype subsequently had a genetic defect identified. A genetic mutation was identified in 21% of those tested with probable hFH and 8% of those with possible FH (8%).

Conclusions: This study demonstrates a discrepancy between clinical FH phenotypes and resulting hFH genotypes. This is the first such study in an Irish cohort and is broadly consistent with international studies. We will now expand this cohort and contribute to the EAS FH Studies Collaboration.

SS142 / #600

FREQUENCY OF VARIANTS CAUSING FAMILIAL HYPERCHOLESTEROLAEMIA (FH) AND IMPACT ON LDL-C CONCENTRATION IN INDIVIDUALS OF SOUTH ASIAN AND AFRICAN ANCESTRY IN THE UK BIOBANK

SAAG SESSION 21: EFFICIENT MANAGEMENT OF FH

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Background and Aims: Background: It is thought that, in the UK, FH individuals from non-European ancestry backgrounds are underrepresented in lipid clinics, and likely missing out on LDL-C-lowering treatment. While this could be due to socioeconomic factors, it is possible that the prevalence of FH-causing variants differs between these ancestries. We examined this in the UK Biobank study.

Methods: Methods and Results: Using genetically confirmed ancestry (PCA) in UK Biobank, there were 140,439 European, 3,906 African and 4,067 South Asian individuals with whole exome sequencing data. LDL-C concentration was adjusted for self-reported statin use.

Results: Mean (SD) LDL-C concentrations were respectively 3.73(0.85)mmol/l, 3.44(0.89)mmol/l and 3.60(0.85)mmol/l (overall difference $p<2.2\times10^{-16}$). FH-causing variants were determined using ACMG criteria. There was no significant difference in the prevalence of an FH-causing variant between the three groups, being respectively 1/288 (95%CI: 1/316-1/264), 1/260 (95%CI: 1/526-1/173) and 1/226 (95% CI: 1/419-1/155). Carriers of an FH-causing variant had significantly higher LDL-C concentration than non-carriers in all ancestry groups, with the largest percentage difference seen in Africans (Europeans=4.43mmol/l (20.6%): Africans=4.87mmol/l (45.0%) and South Asians=4.39mmol/l (24.0%)). In the non-FH groups statin use was highest in South Asians compared to Europeans and Africans (20.3% vs 13.1% vs 12.5%) with similar ranking in those carrying an FH-causing variant (55.6% vs 33.8% vs 40%).

Conclusions: Conclusions: The prevalence of FH-causing variants in the UK Biobank is similar in all three ancestry groups studied. In all ancestry groups, the proportion of FH-variant carriers being treated with lipid lowering therapy should be improved to reduce future risk of premature cardiovascular disease.

SS143 / #764

Topic: *AS04 Clinical Vascular Disease / AS04.12 Prevention and treatment of cardiovasculardisease; miscellaneous*

FAMILIAL HYPERCHOLESTEROLEMIA IN PRIMARY CARE IN THE NETHERLANDS: WHAT ARE THE GAPS?

SAAG SESSION 21: EFFICIENT MANAGEMENT OF FH

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Background and Aims: Familial hypercholesterolemia (FH) is a prevalent monogenic disorder that warrants early diagnosis to prevent premature cardiovascular disease (CVD). Nonetheless, FH is hallmarked by profound underdiagnosis. General practitioners (GPs) are well-positioned to enhance FH identification; however, routine screening for FH in primary care is suboptimal. The aim of the present study is to explore gaps in knowledge, awareness, and practice of FH screening and treatment among GPs in the Netherlands.

Methods: A formal questionnaire assessing physicians' knowledge, awareness and practice of FH was anonymously completed by 221 Dutch GPs between February 2021 and July 2022. In addition to demographic data, the survey contained 19 questions on FH, including general familiarity with the disorder, awareness of management guidelines, hereditary pattern, prevalence, CVD risk, and practiced care for and screening of FH patients.

Results: Of the participating GPs, 62.4% rated their familiarity with FH as above average (score >4; scale 1-7) and 91.4% considered themselves familiar with treatment and referral guidelines. Of the respondents, 83.7%, 87.8%, 55.7%, 19.5%, and 13.6% identified the correct definition of FH, typical lipid profile, inheritance pattern, prevalence, and CVD risk, respectively. Only 40% of respondents answered more than half of the knowledge questions correctly. Combination therapy of statin and ezetimibe was considered an appropriate treatment by 85.5% of the GPs.

Conclusions: Important gaps exist in knowledge and awareness of FH among Dutch GPs. Enhancing identification of FH patients in the Netherlands requires extensive work in FH education and awareness programs in primary care.

SS144 / #1175

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.09 Aortic valve stenosis*

VALPROIC ACID PROMOTES OSTEOGENIC DIFFERENTIATION OF VALVE INTERSTITIAL CELLS

SAAG SESSION 22: TREATMENT OF AORTIC VALVE AND CAROTID STENOSIS

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Background and Aims: Calcific aortic valve disease (CAVD) is the most common valvulopathy in the Western world, but the pathophysiological processes are still poorly understood. Valproic acid (VPA), an inhibitor of histone deacetylases (HDAC), promotes osteogenic differentiation of different cell types, but evidence is lacking on its potential involvement in CAVD. We aimed to determine the effects of VPA in valve interstitial cells (VICs), the main cell population of the aortic valve.

Methods: Different subclones of bovine VICs were treated with VPA (2.5 or 5 mM). Isolated human VICs were treated with an osteogenic medium (OM) for 12 days to induce VICs calcification and osteogenic differentiation. These cells were also exposed to VPA (5 mM) alone or supplemented with OM. Alkaline phosphatase (ALP) activity was determined with a kinetic assay, calcium deposition with Alizarin red staining and colorimetric assay. Proteins and RNA were extracted for western blot and gene expression analyses.

Results: VPA induced a significant overexpression of inflammatory cytokines (IL6) and osteogenic markers (such as ALP and BMP2) and an increase in ALP activity in both bovine and human VICs. Moreover, VPA induced calcium deposition and had a synergistic pro-calcific effect when supplemented to OM. The exposure of VICs to VPA increased the levels of acetylated histone H3 due to HDAC inhibition.

Conclusions: Valproic acid (VPA) promotes pro-inflammatory activation, osteogenic differentiation, and calcium deposition in isolated VICs, probably through the inhibition of HDAC. These preliminary findings need to be confirmed but may suggest the pharmacological modulation of HDAC as a novel therapeutic strategy for CAVD.

SS145 / #609

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.06 Gender and cardiovascular risk*

FIBRO-CALCIFIC REMODELING OF STENOTIC AORTIC VALVE LEAFLETS EVOLVES DIFFERENTLY IN MEN AND WOMEN.

SAAG SESSION 22: TREATMENT OF AORTIC VALVE AND CAROTID STENOSIS

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Background and Aims: Aortic stenosis (AS) is the most common valve disorder, characterized by fibro-calcific remodeling of valve leaflets. Progressive aortic valve calcification (AVC) occurs in both sexes. However, sex-specific fibro-calcific aging of the AS leaflets has not yet been studied.

Methods: We included 200 patients matched for age and sex with severe AS who underwent cardiac contrast-enhanced computed tomography (CT) before intervention. The calcium and fibrosis volumes were indexed dividing the volume by the aortic annular area. Fibro-calcific ratio, which indicates the predominance of valve fibrosis if >1.0, was calculated.

Results: The mean age of our cohort is 78.4±8 years (men: 78.3±8 and female: 78.6±8) and the CT quantifications showed that women had significantly lower AVC content compared to men (529.5±43.8 vs. 913.9±56.9, respectively; p<0.0001), while the fibro-calcific ratio was significantly higher in women compared to men (2.08±0.3 vs. 0.83±0.1, respectively; p<0.0001). To assess the fibro-calcific changes of the leaflets according to age, we divided our cohort into three groups (<75, 75-80, >80 years) and observed that AVC in men was stable, while in women AVC grew steadily reaching values similar to those in men. Indeed, above the age of 80 years, no significant difference was noticeable in the AVC content between the sexes. However, when analyzing the fibro-calcific ratio, we found a significant predominance of fibrosis in female valves in all groups (all p<0.01).

Conclusions: With advancing age, differences in AVC between men and women disappear, while the fibrotic component of the stenotic aortic valve remains significantly higher in women.

SS146 / #232

Topic: *AS04 Clinical Vascular Disease / AS04.15 Other*

CURRENT GERMAN RESULTS OF SURGICAL BAILOUTS ASSOCIATED WITH TRANSCATHETER AORTIC VALVE REPLACEMENT 2018-2020

SAAG SESSION 22: TREATMENT OF AORTIC VALVE AND CAROTID STENOSIS

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Background and Aims: Complications associated with transcatheter aortic valve replacement (TAVR) have decreased substantially. We analyze the current results of surgical bailout in Germany.

Methods: All TAVR procedures in 2018-2020 in Germany have been identified by ICD and OPS codes and compared to 2007-2017. Outcomes were overall in-hospital mortality, surgical bailout, and in-hospital mortality after bailout.

Results: 64,511 patients receiving TAVR in 2018-2020 had a mean age of 80.8 years, a logistic EuroSCORE of 13.51%, and 48.9% were female. Baseline characteristics of TAVR cases with surgical bailout were comparable for 2018-2020 and 2007-2017, with a similar logistic EuroSCORE of 15.63 versus 15.02%. The overall in-hospital mortality between 2018 and 2020 was lower compared to 2007-2017 with 2.49% versus 4.77%, as was the rate of surgical bailout at 0.69% versus 3.38%. In-hospital mortality after surgical bailout was higher with 25.95% versus 15.20%. In 2018-2020, the risk adjusted odds ratios of both overall in-hospital mortality and surgical bailout for balloon-expandable and self-expanding transfemoral TAVR have been significantly lower than for patients receiving transapical TAVR (odds ratios for transfemoral balloon-expandable and self-expanding TAVR: in-hospital mortality: OR=0.38, p<0.001 and OR=0.38, p<0.001; surgical bailout: OR=0.33, p<0.001 and OR=0.17, p<0.001).

Conclusions: The overall in-hospital mortality and rate of surgical bailout after TAVR between 2018 and 2020 were substantially lower compared to 2007-2017. On the other hand, in-hospital mortality after surgical bailout even increased.

SS147 / #541

Topic: *AS04 Clinical Vascular Disease / AS04.15 Other*

PRE-CRANIAL ARTERY CALCIFICATION BURDEN: A PREDICTOR OF REPERFUSION OUTCOME IN ACUTE LARGE ARTERY OCCLUSION PATIENTS

SAAG SESSION 22: TREATMENT OF AORTIC VALVE AND CAROTID STENOSIS

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Background and Aims: Background: Calcification is used widely as an imaging indicator of atherosclerotic burden and cerebrovascular function. We aimed to estimate the predictive value of aortic arch calcification (AoAC) and carotid sinus calcification (CaSC) for symptomatic intracerebral hemorrhage (sICH) and poor outcome in acute large artery occlusion (LAO) after mechanical thrombectomy (MT).

Methods: Methods: Consecutive patients with LAO who received MT at a single comprehensive stroke center between 3/2018-3/2021 were included. Pre-cranial Artery Calcification Burden (PACB) score, consisting of the burden score of AoAC and CaSC, was calculated by AoAC grading scale (AGS) score plus Woodcock visual score. sICH was defined according to the European Cooperative Acute Stroke Study III (ECASS III) definition. Three-month modified rankin scale score of 3-6 was designated as poor outcome.

(A)
0 point: no visual calcification
1 point: spotty calcification
2 point: lamellar calcification
3 point: circular calcification

(B)
1 point: thin discontinuous calcification
2 point: thin continuous calcification
2 point: thick discontinuous calcification
3 point: thick continuous calcification
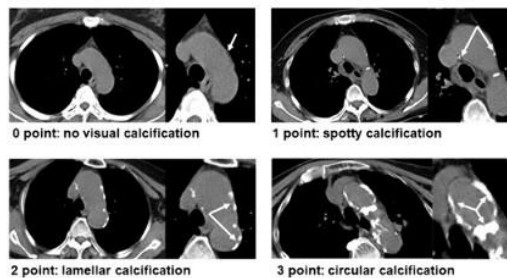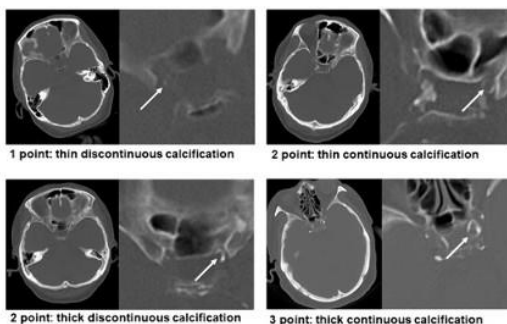
Results: Results: After reperfusion therapy, 17.2% (32/186) of patients developed sICH, and 60.2% (112/186) had poor clinical outcomes. Patients with PACB score≥3 had a significantly increased risk of sICH (OR=2.567, 95%CI=1.187-5.550, *P*=0.017) and poor outcome (OR=4.777, 95%CI=1.659-13.756, *P*=0.004) when compared to those with PACB<3. ROC analysis also showed that, compared with the regression model without PACB, including PACB improved the predictive value for poor outcome (AUC: 0.718 vs 0.519, Z=2.340, *P*=0.019) and in patients who received MT (AUC: 0.714 vs 0.584, Z=2.021, *P*=0.043), separately.

Conclusions: Conclusions: PACB was effective in predicting sICH and 3-months poor outcome in patients with LAO after MT.

SS148 / #473

Topic: *AS04 Clinical Vascular Disease / AS04.05 Diabetes; macro- and microangiopathies*

CALCIFICATION AND COAGULATION RELATED PATHWAYS ARE ENRICHED IN ATHEROSCLEROTIC PLAQUES OF DIABETIC PATIENTS

SAAG SESSION 22: TREATMENT OF AORTIC VALVE AND CAROTID STENOSIS

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Background and Aims: The pathophysiology behind aggravated atherosclerosis in diabetes is still incompletely understood. We performed transcriptomic analysis of plaques from type 2 diabetic (T2D) patients with an aim to reveal underlying molecular mechanisms common between diabetes and atherosclerosis.

Methods: The Biobank of Karolinska Endarterectomies comprises plaques and clinical data from patients undergoing endarterectomy for carotid stenosis, profiled with whole-genome transcriptomic arrays. Multilevel bioinformatic analyses of differentially expressed genes were performed comparing plaques from patients with T2D (n=30, HbA1c>4.9) vs. non-diabetics (n=39, HbA1c<4.9) and further stratified according to stroke symptoms.

Results: IIn microarrays from diabetic vs. non-diabetic patients, the most affected pathways were related to metabolic, coagulation, calcification and cell trans-differentiation processes. In the asymptomatic group, cholesterol storage genes were supressed, while proinflammatory, calcification and cell phenotypic transition genes were upregulated. In the symptomatic group, the dominating overexpressed genes were related to cell trans-differentiation. CHIT1 protease was strongly and specifically repressed in plaques from diabetic patients (p<0.0001). CHIT1 expression was associated with macrophages and markers of ossification. Immunohistochemical staining revealed co-localization of CHIT1 and CD68 in multinucleated cells resembling osteoclasts. Whole transcriptome Nanostring analysis revealed 10-fold increase in CHIT1 expression in multinucleated cells in the plaques compared to other regions of interest.

Conclusions: Our findings reveal induction of stabilization processes related to ossification in plaques from all diabetic patients, but also cell trans-differentiation was relatively enriched in diabetic plaques from both symptomatic and asymptomatic patients. CHIT1 was identified as a novel gene inversely associated with atherosclerotic plaques from T2D patients that is currently being investigated mechanistically.

SS149 / #954

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.07 Environmental risk factors*

THE INFLAMMATORY POTENTIAL OF DIET IS ASSOCIATED WITH FUTURE DEVELOPMENT OF PRE-CLINICAL CAROTID ATHEROSCLEROSIS

SAAG SESSION 22: TREATMENT OF AORTIC VALVE AND CAROTID STENOSIS

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Background and Aims: Inflammatory and atherogenic effect of nutrients has been proposed but epidemiological studies provide conflicting results on its relevance for Cardiovascular Disease (CVDs) risk. The biological significance has been postulated by screening of few, general inflammatory biomarkers. To unveil these relations, we harnessed a targeted panel of proteomics and metabolomics biomarkers, that we previously related to CVDs.

Methods: During the basal visit of the "PLIC" Study in Milan ('99-'01), dietary habits of 474 subjects without pre-clinical carotid atherosclerosis ("SCA"), determined by carotid ultrasonography, were collected. Dietary records were analyzed to (i) derive the percentage of energy intake from nutrients (En%) and (ii) estimate the pro-/anti-inflammatory potential of diet via the Dietary Inflammatory Index (DII). We measured 368 proteins plasma expression (Olink[TM]) and the entire panel of Nightingale[TM] metabolomics to validate the biological relevance of the estimated pro-/anti-inflammatory potential of diet (DII below/above the cohort median). Next, all subjects were re-evaluated after 11 years (10-11, 25th-75th percentiles) to assess the development of SCA.

Results: At the basal visit, pro-inflammatory potential of diet was associated with increased En%SFA decreased En%PUFA and fiber. Machine Learning analysis (*XgBoostClassifier*) underscored metabolomics inflammatory proteins (19 in total) that predicted the pro-inflammatory potential of diet (AUC[CI 95%]:0.741[0.591-0.941],p=0.024). Higher DII values at basal visit predicted the SCA developement at follow-up: subjects that developed SCA (n=203) presented with higher basal DII (1.80[0.84.-2.49]) as compared to subjects that did not (1.48[0.76-2.17]),p=0.016).

Conclusions: We support a plausible inflammatory potential of diet, predicting the development of pre-clinical atherosclerosis. Larger studies are warranted to confirm this possibility.

SS150 / #1195

Topic: *AS04 Clinical Vascular Disease / AS04.06 Aneurysms and other non-atherosclerotic arteriopathies*

BICUSPID AORTIC VALVE AORTOPATHY IS CHARACTERIZED BY EMBRYONIC ENDOTHELIAL TO MESENCHYMAL TRANSITION AND ENDOTHELIAL INSTABILITY

SAAG SESSION 22: TREATMENT OF AORTIC VALVE AND CAROTID STENOSIS

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Background and Aims: Bicuspid aortic valve (BAV) is the most common congenital heart malformation, frequently associated with ascending aortic aneurysm (AscAA). Endothelial to mesenchymal transition (EndMT) has been suggested to play a role in BAV-associated AscAA. The aim of the present study was to investigate the type of EndMT associated with BAV aortopathy using patients with a normal tricuspid aortic valve (TAV), with and without AscAA, and as a reference. The state of the endothelium was further evaluated.

Methods: Aortic biopsies were taken from patients undergoing open-heart surgery. Aortic intima/media miRNA and gene expression analysis was performed using Affymetrix Human Transcriptomic Array. Histological staining assessed structural, endothelial and basement integrity, as well as localization and degree of protein expression. Migration/proliferation was assessed using ORIS migration assay.

Results: We show differential EndMT types associated with BAV and TAV AscAA. Specifically, signs of embryonic EndMT were observed in the dilated BAV ascending aorta, with enrichment of EndMT genes related to endocardial cushion formation, less proliferative and migratory vascular smooth muscle cells, and lack of a fibrotic proteoglycan-rich extracellular matrix. In contrast, TAV aneurysmal aortas displayed a clear fibrotic EndMT phenotype with disorganized media, elastin fragmentation and proteoglycan deposition. Furthermore, non-dilated aortas of BAV-patients showed a lower miRNA-200c-associated endothelial basement membrane LAMC1 expression and a lower expression of CD31. This was accompanied by increased endothelial permeability, indicated by increased albumin infiltration in the BAV aortic media.

Conclusions: Our findings indicate embryonic EndMT as a characteristic of BAV-AscAA, as well as endothelial instability and vascular permeability prior to aortic dilatation.

SS151 / #1527

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.05 Inherited dyslipidemias*

LIFETIME EXPOSURE TO HIGH LDL CHOLESTEROL IN FAMILIAL HYPERCHOLESTEROLEMIA CAUSES CORONARY ATHEROSCLEROSIS IN YOUNG ADULTS: THE CHEETAH TRIAL

SAAG SESSION 23: RISK PROFILE FOR MACE, MORTALITY AND RENAL FAILURE

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Background and Aims: Familial Hypercholesterolemia (FH) patients are exposed to elevated low-density-lipoprotein cholesterol (LDL-C) levels from birth onwards. Early and intensive lipid-lowering therapy (LLT) is advised, but guideline-based treatment is often not implemented. Here, we assessed the impact of LDL-C packyears on coronary plaque burden in young FH patients.

Methods: This single-center prospective study (NCT05352386) compared plaque burden between FH patients and matched healthy controls using coronary CT angiography (CCTA). CCTA output was analyzed using artificial intelligence-guided quantitative analysis (AI-QCT). The primary outcome was defined as total plaque volume adjusted for total vessel volume (percent atheroma volume; PAV).

Results: Ninety genetically diagnosed FH patients and 45 healthy controls (mean age 41±3 years, 51 (38%) female), were included. Eighty-three percent of FH patients was on LLT, initiated at a mean age of 24±11 years. Nonetheless, only 13 (14%) patients achieved LDL-C target levels (<1.8 mmol/l). FH patients had higher cumulative LDL-C exposure compared with controls (181±54 vs 105±33 mmol/l*years). Forty-six (51%) FH patients versus 10 (22%) controls had plaque on CCTA (OR3.66 [95%CI 1.62-8.27]). FH patients had significantly higher PAV than controls (0.53 [IQR 0.17-1.35] vs 0.19 [IQR 0.08-0.48], respectively; p<0.001). In an adjusted Cox regression model, every 75 mmol/l*years of cumulative LDL-C exposure was associated with a doubling in

PAV.

Figure 1. Association between lifetime LDL cholesterol exposure and coronary plaque burden
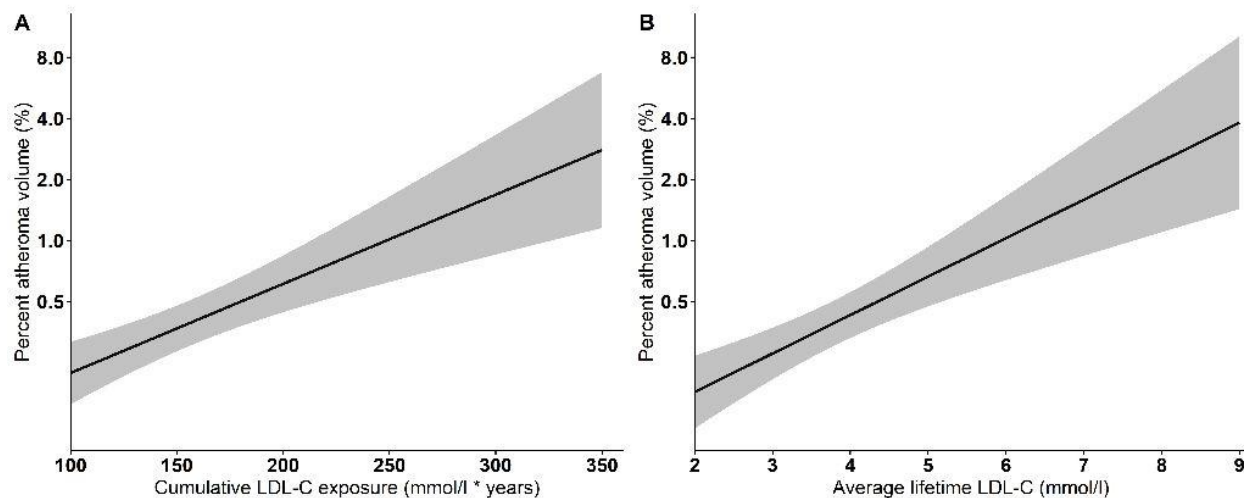
Shown are the associations between cumulative LDL-C exposure (A) as well as average lifetime LDL-C (B) and percent atheroma volume. Estimates with 95% CI were derived from Cox regression.

Conclusions: Cumulative LDL-C exposure was associated with coronary PAV. Despite LLT, FH patients had a twofold higher cumulative LDL-C exposure compared with controls, with half of the FH patients already displaying coronary atherosclerosis. These data emphasize the importance of early and potent LLT initiation in FH.

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.07 Environmental risk factors*

NON-TRADITIONAL SOCIAL, ECONOMIC AND ENVIRONMENTAL EXPOSURES AS MAJOR DRIVERS OF CARDIORENAL RISK IN THE UNITED STATES

SAAG SESSION 23: RISK PROFILE FOR MACE, MORTALITY AND RENAL FAILURE

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Background and Aims: Cardiorenal diseases such as atherosclerotic cardiovascular disease (ASCVD) and chronic kidney disease (CKD) are leading causes of global morbidity and mortality. Non-traditional factors in the social and natural environment are increasingly implicated in the genesis of these conditions with significant geographic variation in prevalence, that is only partially explained by the traditional risk factors. We sought to use census tract-level environmental, social and economic factors to understand the prevalence of cardiorenal disease in the United States.

Methods: We aggregated various US-wide data sources that provided 153 attributes of health, social, demographic, built-environment, environmental exposures with 3 major health outcomes, ASCVD, stroke, and CKD. Machine learning models (Random Forest and Gradient Boost) were employed to identify the best social and environmental predictors of health outcomes.

Results: Random Forest provided the best prediction for prevalence of health outcomes with R^2 values of 0.875 and a root mean squared error below 1%. Socioeconomic factors such as vulnerability indices, income and education, dominated the top predictors along other environmental and health exposures such as air pollution, smoking and oral hygiene. A Random Forest model exclusively relying on socioeconomic factors without environmental or other health determinants showed improved prediction with R^2 values of 0.92.
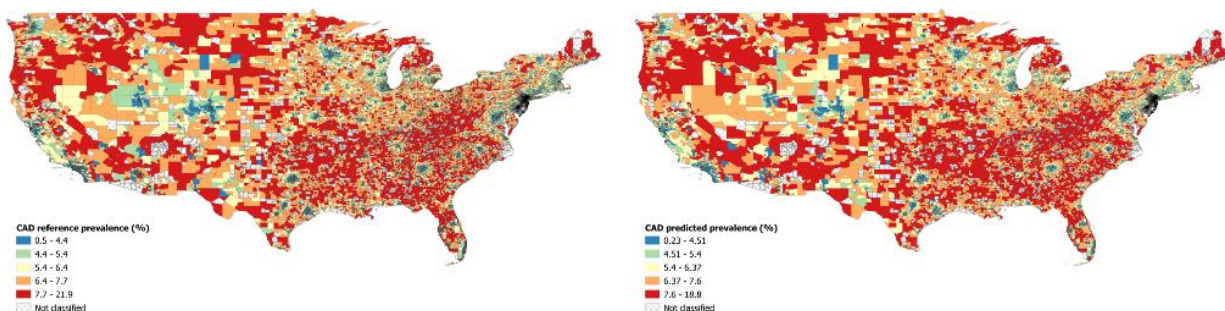
(a) Ground truth prevalence of CAD

(b) Predicted prevalence of CAD using Random Forest ($R^2 = 0.876 \pm 0.14$)

Conclusions: Social and environmental factors can explain a significant proportion of the regional variability in prevalence of cardiometabolic diseases with socioeconomic determinants dominating the top predictors. A sparser analysis should be conducted to explore the impact of social and environmental determinants on individual cardiometabolic risk.

SS154 / #993

Topic: *AS04 Clinical Vascular Disease / AS04.15 Other*

THE SERUM PROTEOME OF VA-ECMO PATIENTS CHANGES OVER TIME AND AND DIFFERENTIATES SURVIVORS AND NON-SURVIVORS ON DAY 3

SAAG SESSION 23: RISK PROFILE FOR MACE, MORTALITY AND RENAL FAILURE

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Background and Aims: Veno-arterial extracorporeal membrane oxygenation (VA-ECMO) is applied in patients with refractory hemodynamic failure. Exposure of blood components to high shear stress and the large extracorporeal surfaces in the ECMO circuit trigger a complex ECMO-associated inflammatory response syndrome and coagulopathy which are believed to worsen the already poor prognosis of these patients. Mass spectrometry-based proteomics allow a detailed characterization of the serum proteome. In this study, we aimed to characterize the serum proteome of VA-ECMO patients over time and compared to controls.

Methods: Serum samples were collected on day 1 and 3 from VA-ECMO patients. Samples underwent albumin and immunoglobulin depletion, in-solution digestion and PreOmics clean-up. Individual samples were measured in data independent acquisition (DIA) mode. Differential expression analysis was conducted with the LIMMA–R-package. EnrichROAST was applied to generate gene ontology enrichment analyses.

Results: Fifteen patients receiving VA-ECMO were recruited. Eight patients survived. 351 unique proteins were identified. 139 proteins were differentially expressed between ECMO patients and controls. Many of these proteins were involved in stress response, coagulation and the inflammatory response. 144 proteins were differentially expressed on day 3 compared to day 1. Many of these proteins could be attributed to the complement and coagulation system. On day 3, 50 proteins were differentially expressed between survivors and non-survivors (Figure 1). Many of these proteins have been ascribed to processes in coagulation and

inflammation.

A

B
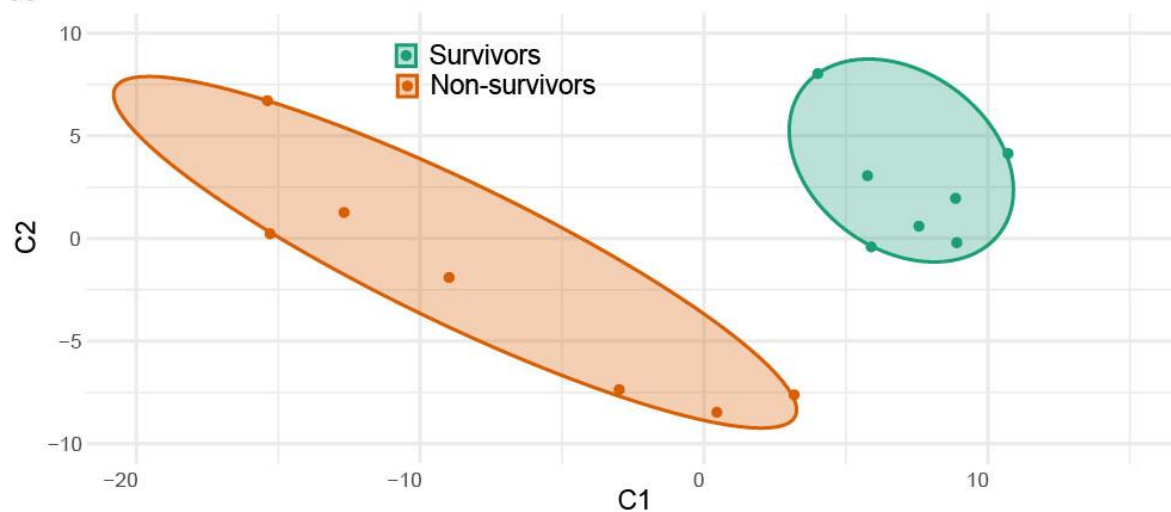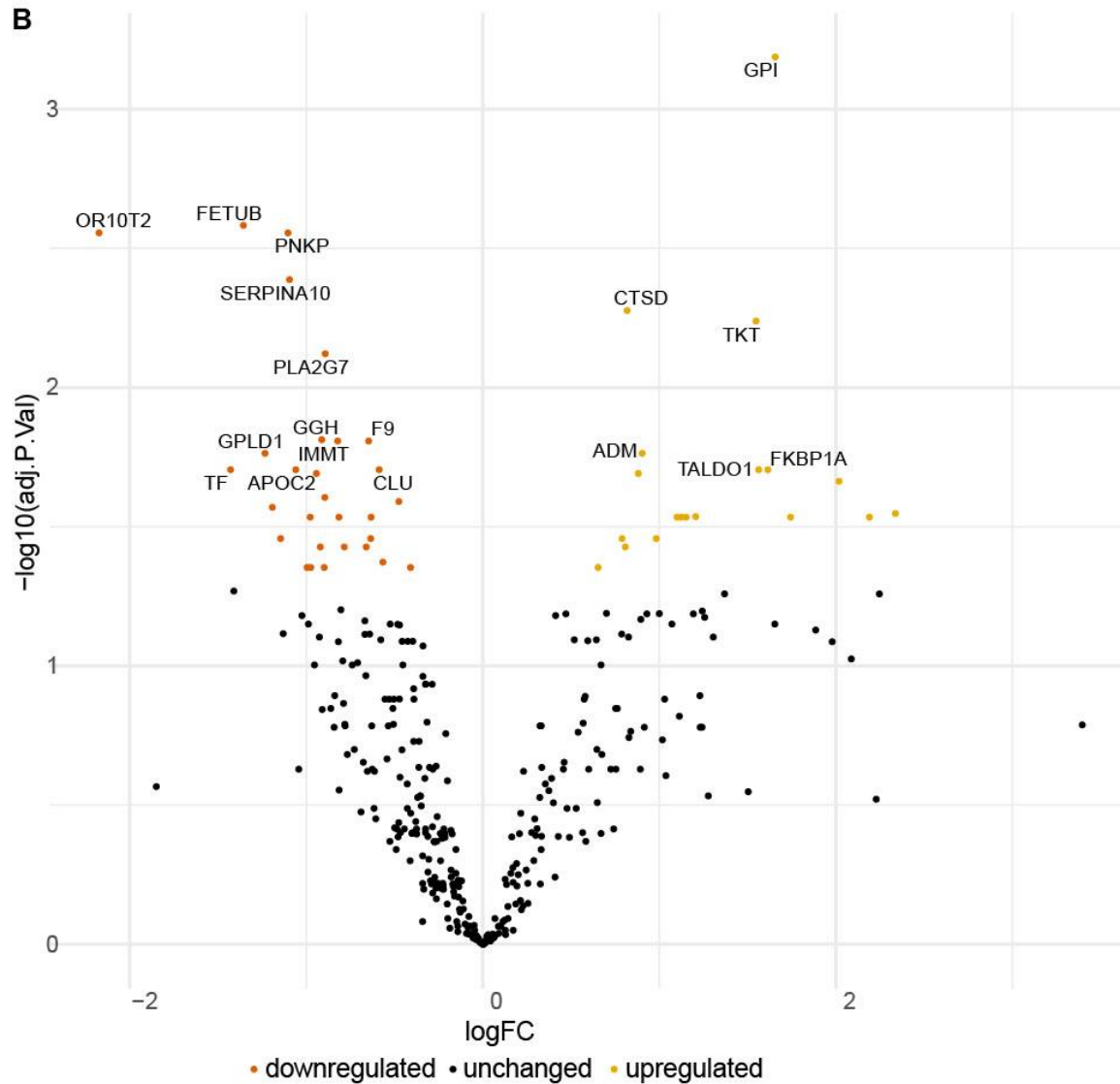
Conclusions: The serum proteome of VA-ECMO patients displays major differences compared to controls. It changes during VA-ECMO therapy and allows differentiation of survivors and non-survivors on day 3.

SS155 / #340

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.08 Novel risk factors and biomarkers*

REMNANT CHOLESTEROL PREDICTS MAJOR CARDIOVASCULAR EVENTS IN PATIENTS WITH CORONARY ARTERY DISEASE BOTH AMONG PATIENTS WITH TYPE 2 DIABETES AND IN NON-DIABETIC INDIVIDUALS

SAAG SESSION 23: RISK PROFILE FOR MACE, MORTALITY AND RENAL FAILURE

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Background and Aims: Remnant cholesterol, which is calculated as total cholesterol minus LDL cholesterol minus HDL cholesterol has attracted interest as a marker of cardiovascular event risk. The power of remnant cholesterol to predict major cardiovascular events (MACE) in patients with established coronary artery disease is unclear and is addressed in the present study.

Methods: We enrolled 1472 consecutive patients with established coronary artery disease. Prospectively, cardiovascular events were recorded over a mean follow-up period of 8.0±5.03 years.

Results: At baseline, remnant cholesterol was significantly higher in patients with T2DM (n=446) than in non-diabetic subjects (27±24 vs. 21±23 mg/dl; p<0.001). During follow-up, 493 of our patients suffered MACE; the event rate was significantly higher in patients with T2DM than in non-diabetic subjects (62.5 vs. 37.5%; p<0.001). Remnant cholesterol in Cox regression models adjusting for age, sex, hypertension, smoking, body mass index and LDL cholesterol independently predicted MACE in the total study population (standardized adjusted HR 1.17 [1.09-1.28], p<0.001), and in patients with T2DM as well as in non-diabetic subjects (standardized adjusted HRs 1.24 [1.07-1.44], p=0.005 and 1.14 [1.02-1.26], p=0.017, respectively).

Conclusions: From our data we conclude that remnant cholesterol in patients with established coronary artery disease predicts MACE both among patients with T2DM and among non-diabetic subjects.

SS156 / #532

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.02 Epidemiology of dyslipidemias*

ELEVATED REMNANT CHOLESTEROL IN ATHEROSCLEROTIC CARDIOVASCULAR DISEASE IN DIABETES: A POPULATION-BASED PROSPECTIVE COHORT STUDY

SAAG SESSION 23: RISK PROFILE FOR MACE, MORTALITY AND RENAL FAILURE

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Background and Aims: Elevated remnant cholesterol is causally associated with increased risk of atherosclerotic cardiovascular disease (ASCVD) in the general population. We tested the hypothesis that elevated remnant cholesterol is associated with increased risk of peripheral artery disease, myocardial infarction, ischemic stroke, and any ASCVD in individuals with diabetes.

Methods: We studied 4,569 individuals with diabetes and 102,674 individuals without diabetes from the Copenhagen General Population Study (2003-2015). In those with diabetes, during up to 15 years of follow-up, 236 were diagnosed with peripheral artery disease, 234 with myocardial infarction, 226 with ischemic stroke, and 498 with any ASCVD in national Danish health registries. Remnant cholesterol was calculated from a standard lipid-profile.

Results: Multivariable adjusted hazard ratios (95% confidence interval) per doubling of remnant cholesterol and LDL cholesterol were 1.8 (1.2-2.5) and 0.9 (0.6-1.2) for peripheral artery disease, 1.8 (1.3-2.6) and 1.0 (0.7-1.4) for myocardial infarction, 1.6 (1.1-2.3) and 1.1 (0.8-1.6) for ischemic stroke, and 1.7 (1.4-2.2) and 0.9 (0.7-1.1) for any ASCVD, respectively. Excess risk conferred by diabetes was 2.5-fold for peripheral artery disease, 1.6-fold for myocardial infarction, 1.4-fold for ischemic stroke, and 1.6-fold for any ASCVD. Elevated remnant cholesterol explained 24% excess risk of any ASCVD in diabetes, while elevated LDL cholesterol did not explain excess risk.

Conclusions: Unlike elevated LDL cholesterol, elevated remnant cholesterol was associated with increased risk of ASCVD in individuals with diabetes. Elevated remnant cholesterol explained a large fraction of excess risk of ASCVD in diabetes.

Topic: *AS04 Clinical Vascular Disease / AS04.09 Lipid-lowering therapies*

ASSOCIATION BETWEEN NON–HIGH-DENSITY LIPOPROTEIN CHOLESTEROL CHANGE AND MAJOR ADVERSE OUTCOMES AFTER MYOCARDIAL INFARCTION

SAAG SESSION 23: RISK PROFILE FOR MACE, MORTALITY AND RENAL FAILURE

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Background and Aims: To determine if reductions in non-HDL cholesterol (non–HDL-C) after MI provide information on lipid associated atherogenic risk beyond that provided by LDL-C.

Methods: Patients (n=64,684) with an MI (2004–2021) in the SWEDEHEART registry were included. Cox regression, adjusted for cardiovascular risk factors and statin treatment, assessed the association between changes in non–HDL-C after index event and all-cause mortality and MACE (all-cause mortality, ischaemic stroke, and MI), and the impact of timing of non–HDL-C reduction.

Results: During a median follow-up of 5.3 years, 8040 patients died and 13,287 had a MACE. A greater reduction in non–HDL-C at 1 year was associated with lower mortality and MACE (Figure 1). Mortality and MACE decreased consistently across the quartiles of reduction in non–HDL-C between index event and 1 year. Patients with ≥50% reduction in non–HDL-C at 1 year had lower risks of mortality (hazard ratio [HR] 0.89, 95% CI 0.81–0.97) and MACE (HR 0.83, 95% CI 0.78–0.89). When also adjusting for LDL-C reduction, the HR for mortality was 0.86 (95% CI 0.77–0.97) and for MACE was 0.87 (95% CI 0.79–0.94). The risk of death and MACE was lower with early and maintained reduction in non–HDL-C of ≥50% compared with only late reduction or no reduction (Figure 2).

Figure 1. Waterfall plot for change in non–HDL-C level and event rates. Data are shown for an increase or no reduction in non–HDL-C (red), >0% and <50% reduction in non–HDL-C (blue), and ≥50% reduction in non–HDL-C (green) between index event and 1 year. MACE is the composite outcome of all-cause mortality, myocardial infarction, and ischaemic stroke
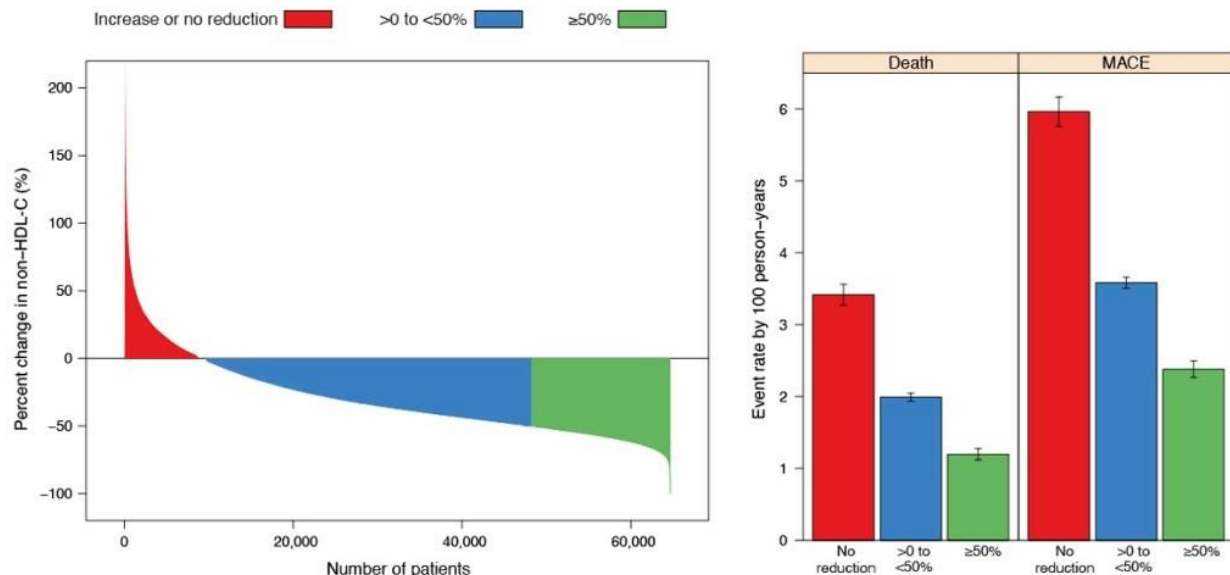
Figure 2. HRs and forest plot comparing reduction in non–HDL-C of ≥50% vs <50% at different stages of follow up

	Magnitude of reduction in non–HDL-C from index event	Events (%/year)	HR (CI 95%)	HR (CI 95%)	
All-cause mortality	<50% reduction first year after MI	2.22	Reference	Reference	
	≥50% reduction after 6–10 weeks and then <50% reduction after 1 year	1.42	0.84 (0.76–0.94)	0.90 (0.79–1.03)	
	<50% reduction after 6–10 weeks and then ≥50% reduction after 1 year	1.26	0.84 (0.75–0.94)	0.98 (0.86–1.11)	
	≥50% reduction after 6–10 weeks and then ≥50% reduction after 1 year	1.01	0.74 (0.66–0.83)	0.77 (0.67–0.88)	
MACE	<50% reduction first year after MI	4.02	Reference	Reference	
	≥50% reduction after 6–10 weeks and then <50% reduction after 1 year	2.88	0.83 (0.76–0.89)	0.93 (0.85–1.02)	
	<50% reduction after 6–10 weeks and then ≥50% reduction after 1 year	2.53	0.76 (0.70–0.82)	0.89 (0.81–0.98)	
	≥50% reduction after 6–10 weeks and then ≥50% reduction after 1 year	2.08	0.66 (0.61–0.71)	0.75 (0.68–0.83)	

Adjusted for non–HDL-C at index event
Adjusted for cardiovascular risk factors and statin treatment

Conclusions: An earlier and larger decrease in non–HDL-C after MI was associated with lower risks of death and MACE, regardless of LDL-C change.

SS158 / #244

Topic: *AS04 Clinical Vascular Disease / AS04.07 Nutrition, nutraceuticals*

EFFECTS OF TOTUM-070, A POLYPHENOL-RICH COMPOUND, ON LDL-CHOLESTEROL IN SUBJECTS WITH MODERATE HYPERCHOLESTEROLEMIA (THE HEART STUDY): A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL

SAAG SESSION 24: NOVELTIES IN LIPID-LOWERING TREATMENT

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Background and Aims: TOTUM-070 is a patented blend of five plant extracts. This clinical study aims to assess the efficacy of TOTUM-070 on plasma lipid concentration in subjects with moderate hypercholesterolemia.

Methods: This randomized, double-blind, placebo-controlled trial included 120 subjects (men and women, aged 18-70 years), with moderate hypercholesterolemia (between 130 and 190 mg/dL), who were not receiving cholesterol-lowering medication. Subjects were randomly assigned to consume a 5-g daily dose of TOTUM-070 or placebo for 24 weeks. The primary outcome was the fasting blood LDL-cholesterol concentration after 24 weeks. Safety markers were also measured. Analysis was performed by intention to treat. The HEART study is registered at ClinicalTrials.gov: NCT04760951.

Results: Blood LDL-cholesterol concentration was reduced by -13% ($p<0.001$) and -9% ($p<0.001$) with TOTUM-070 compared to placebo after 12 and 24 weeks of supplementation, respectively. In addition, TOTUM-070 consumption reduced blood levels of total cholesterol (-15% at 12 weeks $p<0.05$; -16% at 24 weeks, $p<0.01$), non-HDL-cholesterol (-14% at 12 weeks, $p<0.01$; and -17% at 24 weeks, $p<0.0001$), triglycerides (-5% vs placebo at both 12 and 14 weeks, $p<0.05$), apolipoprotein B (-7% at V2 and V3, $p<0.01$) and apolipoprotein B / apolipoprotein A1 ratio (-3% after 12 weeks, $p=0.06$; -4% after 24 weeks, $p<0.05$) when compared to placebo. Moreover, the atherogenic index decreased -6% after 12 and 24 weeks when compared to placebo ($p<0.05$). In addition, safety markers showed that TOTUM-070 was well tolerated.

Conclusions: In conclusion, this Phase II clinical trial showed that daily intake of TOTUM-070 lowers LDL-cholesterol in subjects with moderate hypercholesterolemia

Topic: *AS04 Clinical Vascular Disease / AS04.09 Lipid-lowering therapies*

IMPACT OF HIGH NEUTROPHIL-TO-LYMPHOCYTE RATIO ON THE CARDIOVASCULAR BENEFIT OF PCSK9 INHIBITORS IN FAMILIAL HYPERCHOLESTEROLEMIA SUBJECTS WITH ATHEROSCLEROTIC CARDIOVASCULAR DISEASE

SAAG SESSION 24: NOVELTIES IN LIPID-LOWERING TREATMENT

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Background and Aims: Neutrophil-to-lymphocyte ratio (NLR) is an intriguing inflammatory biomarker strongly associated with atherosclerotic cardiovascular disease (ASCVD). Our aim was to evaluate the role of NLR on pulse wave velocity (PWV) after adding-on proprotein convertase subtilisin/kexin type 9 inhibitor (PCSK9-i) in familial hypercholesterolemia (FH) subjects with ASCVD.

Methods: In this prospective observational study, we evaluated 45 FH subjects with ASCVD on high-intensity statins plus ezetimibe and with an off-target LDL-C. Study population was divided into two groups according to the mean value of NLR. All patients received PCSK9-i therapy and obtained biochemical analysis as well as PWV evaluation at baseline and after six months of PCSK9-i.

Results: After six months of add-on PCSK9-i therapy, a significant reduction of TC, LDL-C, Non-HDL-C, Lp(a) and ApoB plasma levels was observed in the two groups; while low-NLR group exhibited a significant PWV reduction after six-month therapy with PCSK9-i (D   16.2%, $p < 0.05$), no significant changes in PWV were observed in the high-NLR group.

Conclusions: Only FH subjects with low-NLR experienced a significant reduction of PWV after PCSK9-i. Our findings suggest a role of NLR in predicting PCSK9-i effect in FH subjects with ASCVD.

SS160 / #70

Topic: *AS04 Clinical Vascular Disease / AS04.09 Lipid-lowering therapies*

EFFECT OF ROSUVASTATIN ON CAROTID INTIMA-MEDIA THICKNESS; A SYSTEMATIC REVIEW AND META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS.

SAAG SESSION 24: NOVELTIES IN LIPID-LOWERING TREATMENT

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Background and Aims: Although atherosclerosis is a leading cause of cardiovascular diseases (CVD) such as coronary artery diseases, its symptoms typically don't manifest until later in the disease's progression. Carotid intima-media thickness (CIMT) is an early predictor of atherosclerotic changes, as increased CIMT can predict the future risk of major cardiovascular events. Therefore, this systematic review and meta-analysis aimed to investigate the effect of Rosuvastatin on the reduction of CIMT.

Methods: We conducted a PRISMA-compliant systematic review and meta-analysis. We ran an electronic search of PubMed, Scopus, Web of Science, and Cochrane CENTRAL to identify relevant published studies. Data were extracted and analyzed using the Review Manager software (version 3.5 for Windows).

Results: Nine RCTs (n=1727) that used Rosuvastatin as an intervention and compared it with either placebo or other statins, and included patients with low or moderate risk of CVD, hyperlipidemia, carotid atherosclerosis, HIV, SLE, and Rheumatoid arthritis were included in this meta-analysis. The Rosuvastatin group was superior to the control group regarding the change of mean CIMT at the end of the follow-up period (SMD [95%CI], -0.35 [-0.48, -0.22], P<0.00001), and the change of mean CIMT after only 1 year (SMD [95%CI], -0.31 [-0.48, -0.14], P=0.0003). But there was no significant difference regarding the change of maximum CIMT by the end of the follow-up (SMD [95%CI], -0.23 [-0.52, 0.06], P=0.11).

Study or Subgroup	Experimental			Control			Weight	Std. Mean Difference IV, Random, 95% CI	Std. Mean Difference IV, Random, 95% CI
	Mean	SD	Total	Mean	SD	Total			
crouse2007	0.0004	0.01912	702	0.0088	0.019438	282	31.5%	-0.44 [-0.58, -0.30]	
Igasa2012	-0.06	0.15621	26	0.01	0.170294	25	5.2%	-0.42 [-0.98, 0.13]	
Liu2014	0.01121	0.0292	39	0.037	0.0296	40	7.2%	-0.87 [-1.33, -0.41]	
Liu2016	-0.32	0.67676	60	-0.2	0.731095	60	10.9%	-0.17 [-0.53, 0.19]	
Longenecker2016	0.003	0.07576	72	0.028	0.086161	75	12.6%	-0.31 [-0.63, 0.02]	
Mok2011	-0.01	0.23854	36	0.04	0.228035	36	7.2%	-0.21 [-0.68, 0.25]	
Nohara2012	-0.003	0.103	67	0.045	0.131	73	12.0%	-0.40 [-0.74, -0.07]	
Tam2011	0.01	0.19799	24	0.01	0.240832	26	5.2%	0.00 [-0.55, 0.55]	
Trevillyan2021	0.004	0.0239	44	0.0061	0.0247	40	8.2%	-0.09 [-0.51, 0.34]	
Total (95% CI)			1070			657	100.0%	-0.35 [-0.48, -0.22]	

Heterogeneity: Tau² = 0.01; Chi² = 10.61, df = 8 (P = 0.23); I² = 25%
Test for overall effect: Z = 5.10 (P < 0.00001)

Favours Rosuvastatin   Favours control

Study or Subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Igasa2012	-0.06	0.156205	26	0.01	0.170294	25	9.4%	-0.42 [-0.98, 0.13]
Longenecker2016	-0.004	0.049786	72	0.017	0.061869	75	27.3%	-0.37 [-0.70, -0.04]
Nohara2012	0.012	0.093	145	0.042	0.094	144	53.9%	-0.32 [-0.55, -0.09]
Tam2011	0.01	0.19799	24	0.01	0.240832	26	9.4%	0.00 [-0.55, 0.55]
Total (95% CI)			267			270	100.0%	-0.31 [-0.48, -0.14]

Heterogeneity: Tau² = 0.00; Chi² = 1.50, df = 3 (P = 0.68); I² = 0%
Test for overall effect: Z = 3.61 (P = 0.0003)

Favours rosuvastatin   Favours control

Study or Subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
crouse2007	-0.0014	0.035048	624	0.0131	0.035232	252	48.9%	-0.41 [-0.56, -0.27]
Nohara2012	0.02	0.35	72	0.03	0.32	76	32.9%	-0.03 [-0.35, 0.29]
Tam2011	-0.02	0.282843	24	0.02	0.390512	26	18.1%	-0.11 [-0.67, 0.44]
Total (95% CI)			720			354	100.0%	-0.23 [-0.52, 0.06]

Heterogeneity: Tau² = 0.04; Chi² = 5.12, df = 2 (P = 0.08); I² = 61%
Test for overall effect: Z = 1.58 (P = 0.11)

Favours [Rosuvastatin]   Favours [control]

Conclusions: Among participants of the included studies, Rosuvastatin has a clear effect on the reduction of mean CIMT, but not on the maximum CIMT. Therefore, we suggest further trials to investigate this measurement.

SS161 / #275

Topic: *AS04 Clinical Vascular Disease / AS04.09 Lipid-lowering therapies*

REAL-LIFE EXPERIENCE OF THE FIRST PATIENTS TREATED WITH BEMPEDOIC ACID IN 2 LIPID CLINICS OF BELGIUM

SAAG SESSION 24: NOVELTIES IN LIPID-LOWERING TREATMENT

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Background and Aims: Bempedoic acid (BA), a new oral first-in-class adenosine triphosphate-citrate lyase (ACLY) inhibitor, was marketed since February 2022 in Belgium. The CLEAR study program showed a 17.4 to 28.5% reduction in LDL cholesterol (LDL-C) compared to placebo respectively in patients with or without statin and a good safety and tolerability profile, except with a slight increase in uric acid (UA) and gout events as well as in blood creatinine levels.The objective of this study is to present a real-life experience of the first patients treated with BA in 2 lipid clinics of Belgium.

Methods: We collected the data on the levels of lipids, UA and creatinine before and after (at least 3 months) initiation of BA as well previous lipid-lowering treatment and treatment tolerance.

Results: We identified 83 patients (57 female) of whom 54 (65%) were statin intolerant. Before BA treatment, average levels were: LDL-C 141±51 mg/dl, UA 5,4±1,4 mg/dl and creatinine 0,86±0,19 mg/dl. Eighteen (22%) patients stopped treatment with BA, two patients had a gout attack and one had elevated liver enzymes. At follow-up, average levels were LDL-C 102±49 mg/dl, UA 6,4±2,1 mg/dl and creatinine 0,93±0,23 mg/dl. This represented a 27% decrease of LDL-C, a 1 mg/dl increase of UA and a 0,07 mg/dl increase of creatinine. The percentage of LDL-C reduction between statin-treated and statin-intolerant patients was almost identical.

Conclusions: In conclusion, the results on the decrease of LDL cholesterol, the elevation of uric acid and creatinine are similar to the values found in the studies. However, the discontinuation rate is higher when used in daily life.

SS162 / #1602

Topic: *AS04 Clinical Vascular Disease / AS04.13 New lipid lowering therapies*

EFFECTS OF PCSK9 GENE SILENCING BY SMALL INTERFERING RNA INCLISIRAN ON CORONARY ATHEROSCLEROTIC PLAQUE LIPID CONTENT

SAAG SESSION 24: NOVELTIES IN LIPID-LOWERING TREATMENT

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Background and Aims: Lipid burden quantification by near-infrared spectroscopy (NIRS) is associated with adverse cardiovascular outcomes. Aim of this study was to assess the effect of inclisiran on plaque lipid content in patients not reaching therapeutic target by statin/ezetimibe therapy.

Methods: Study was conducted among stable CAD patients having angiographic evidence of a nonobstructive atherosclerotic plaque 20-50% in the proximal/middle segment of a coronary artery, referred to as the region of interest (ROI). NIRS imaging of the ROI was performed at baseline and 450 days later. All participants received maximally tolerated statin/ezetimibe therapy for 4 to 6 weeks before inclusion. In patients with low-density lipoprotein cholesterol (LDL-C) >1.8 mmol/l, inclisiran add-on therapy was started and continued for 450 days.

Results: A total of 33 patients were enrolled in the study. After 90 days of inclisiran therapy, 13 out of 17 patients reached LDL-C target <1.8 mmol/l. In inclisiran group the mean change of maximum lipid-core burden index within 4 mm (maxLCBI4 mm) of the ROI was -82.91 (95%CI -149.79 to -16.04, P=0.018). Among statin/ezetimibe users, maxLCBI4 demonstrated a change of -145.75 (95%CI -239.26 to -52.24, P=0.005). Based on the change in plaque burden index, 52.9% of patients in inclisiran group and 68.8% in statin/ezetimibe group are classified as regressors (P=0.353).

	LDL-C and maxLCBI4 mm changes among inclisiran and statin/ezetimibe users										
	Inclisiran group (N=17)			Statin/ezetimibe group (N=16)			Absolute change, mean (95%CI)		P	Difference, % (95%CI)		P
	Baseline, mean (±SD)	Follow-up, mean (±SD)	P	Baseline, mean (±SD)	Follow-up, mean (±SD)	P	Inclisiran group (N=17)	Statin/ezetimibe group (N=16)		Inclisiran group (N=17)	Statin/ezetimibe group (N=16)	
LDL-C, mmol/l	2.69 (0.83)	1.62 (0.64)	<0.001	1.50 (0.46)	1.71 (0.63)	0.119	-1.06 (-1.44 to -0.70)	0.21 (-0.16 to 0.58)	0.891	-38.3% (-50.15 to -26.56)	23.74% (-3.97 to 51.46)	0.006
maxLCBI4 mm	172.41 (150.70)	89.50 (134.91)	0.018	210.19 (166.21)	64.44 (99.79)	0.005	-82.91 (-149.79 to -16.04)	-145.75 (-239.26 to -52.24)	0.242	-54.93% (-94.04 to -15.82)	-75.79% (-99.26 to -52.33)	0.075

Conclusions: Add-on inclisiran effectively reduces plaque lipid content and promotes plaque regression in patients with suboptimal response to statin/ezetimibe lipid-lowering therapy.

SS163 / #925

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.02 Epidemiology of dyslipidemias*

LDL- AND NON-HDL-CHOLESTEROL IN TYPE 1 AND TYPE 2 DIABETES: LIPID GOAL ATTAINMENT IN A LARGE GERMAN DIABETES REGISTRY

SAAG SESSION 24: NOVELTIES IN LIPID-LOWERING TREATMENT

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Background and Aims: We aim to assess the implementation of 2019 European Society of Cardiology (ESC)/European Atherosclerosis Society (EAS) guidelines on dyslipidaemia according to respective lipid goal achievements in years 2020/2021 in a large registry from Germany and Austria across more than 30.000 patients with type 1 (T1DM) and type 2 diabetes mellitus (T2DM).

Methods: Registry data from March 2022, containing the last complete lipid profile for 2020/2021 included 32,170 patients (8,314 patients with T1DM and 23,856 with T2DM), was analysed. Patients were stratified according to ESC 2019 risk categories for diabetes, and guideline-based low-density lipoprotein (LDL)-C and non-high-density lipoprotein (HDL)-C goal attainment.

Results: Among patients with T1DM, 6.16% reached their risk-based recommended LDL-C goal of <55 mg/dl (very high risk), 10.97% of <70 mg/dl (high risk), and 69.50% of <100 mg/dL (moderate risk), respectively. In patients with T2DM 11.81% reached their risk-based goal of LDL-C <55 mg/dL, 16.25% of <70 mg/dL, and 51.33%% <100 mg/dL. Non-HDL-C goals were reached more often, with 15.3%, 25.52% and 91.61% in patients with T1DM and 18.56%, 17.96% and 82.30% in T2DM for very high, high and moderate risk, respectively.

Conclusions: Approximately two years after guideline publication, LDL-C and non-HDL-C goal attainment according to cardiovascular risk category was rarely achieved in patients with T1DM and T2DM with high or very high cardiovascular risk.

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.05 Inherited dyslipidemias*

PARTICIPATION OF LRPPRC GENE IN FAMILIAL HYPERTRIGLYCERIDEMIA: STUDY IN THE UNDERLYING PHYSIOPATHOLOGICAL MECHANISMS

SAAG SESSION 26: THE FRONTIERS IN DYSLIPIDEMIAS

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Background and Aims: The cause of familial hypertriglyceridemia (FHTG) is unknown. A new potential gene LRPPRC identified by exome sequencing could explain the severity of the phenotype in FHTG , the main objective is toa ssess the pathophysiological mechanisms between carriers and non-carriers of risk haplotype in LRPPRC gene.

Methods: This was a comparative study. Non related FHTG cases and controls carriers and non carriers of risk haplotype in LRPRRC gene, matched by age, gender, BMI and A1 were evaluated. Clinical and biochemical measurements was done, absorciometry dual X-ray (DXA), mixed meal tolerance test, subcutaneous adipose tissue biopsy, PWV and frequent sampling Intravenous glucose tolerance test

(FSIVGTT) were realized.

Design Study

- Unrelated cases with FHTG with and without risk haplotype
- Unrelated Controls with normal levels of Tg with and withoutrisk haplotype

Insulin secretion:
FSIVGTT/ Minimal model / METS-IR

NAFLD; NASH
FIBROSCAN

LPL activity
LPL mass

Subfractions and LDL-p
NMRI-spectroscopy of lipoproteins
LDL-particle number
Remnants

Visceral fat content/Adi posity:
DXA
METS-VF

Adipose tissue and squeletal muscle biopsies

Cqrdiovascular risk
Pulse wave velocity y CIMT

Triglyceride clearance
Mixed meal tolerance test (posprandial lipemia

Potential Biochemical markers associated :
Leptin, adiponectin, FGF-21, ANGPTL3, ANGPTL4

Results: We recruited 120 subjects, the carriers present higher levels of triglyceride and apo B48 concentrations at 4 and 8 hours of MMTT and they also have a higher visceral fat content despite not having differences in BMI .In addition they have a higher liver steatosis .The size and area of adipocytes was higher and they presented a higher maximum IMT ($p=0.03$). Large VLDL-p and also small HDL-p and LDL-p being more abundant in carriers. (all $p<0.05$). There was no difference in PVW and in insulin sensitivity but greater insulin iAUC was observed ($p<0.05$)..

Table 3. Triglyceride tAUC and iACT and apo B 48 concentrations in MMTT.

	Cases			controls		
Variable	Non-carrier n= 32	carrier n=20	p	Non-carrier n=51	carrier n=17	p
iAUIC TG	3825 (2820-6619)	4788 (2395-6784)	0.06	1274 (988-1700)	1925 (1235-2269)	0.05
iAUC TG	1042 (623-1291)	1290 (712-2370)	0.04	389 (256-615)	558 (352-608)	0.038
Apo B 48 mg/dl 0'	21.9 (3.9-71)	29 (4 .2-122)	0.120	2.5 (1.3-4.35)	3.2 (0.89-6.	0.101
Apo B 48 4 hr	16.5 (4.7-97)	40 (7-144)	0.031	2.6 (1.1-5.7)	4 (1.1-6.5)	0.0
Apo B 48 8hr	12.1 (3.8-65)	29 (5.4-141)	0.025	2.3(0.99- 4.1)	2.8 (1.1- 3.7)	0.431

Figure 1.Adipocytes area: carriers and non-carries, n= 250 cels per subject, Mann-Whitney Rank Sum Test
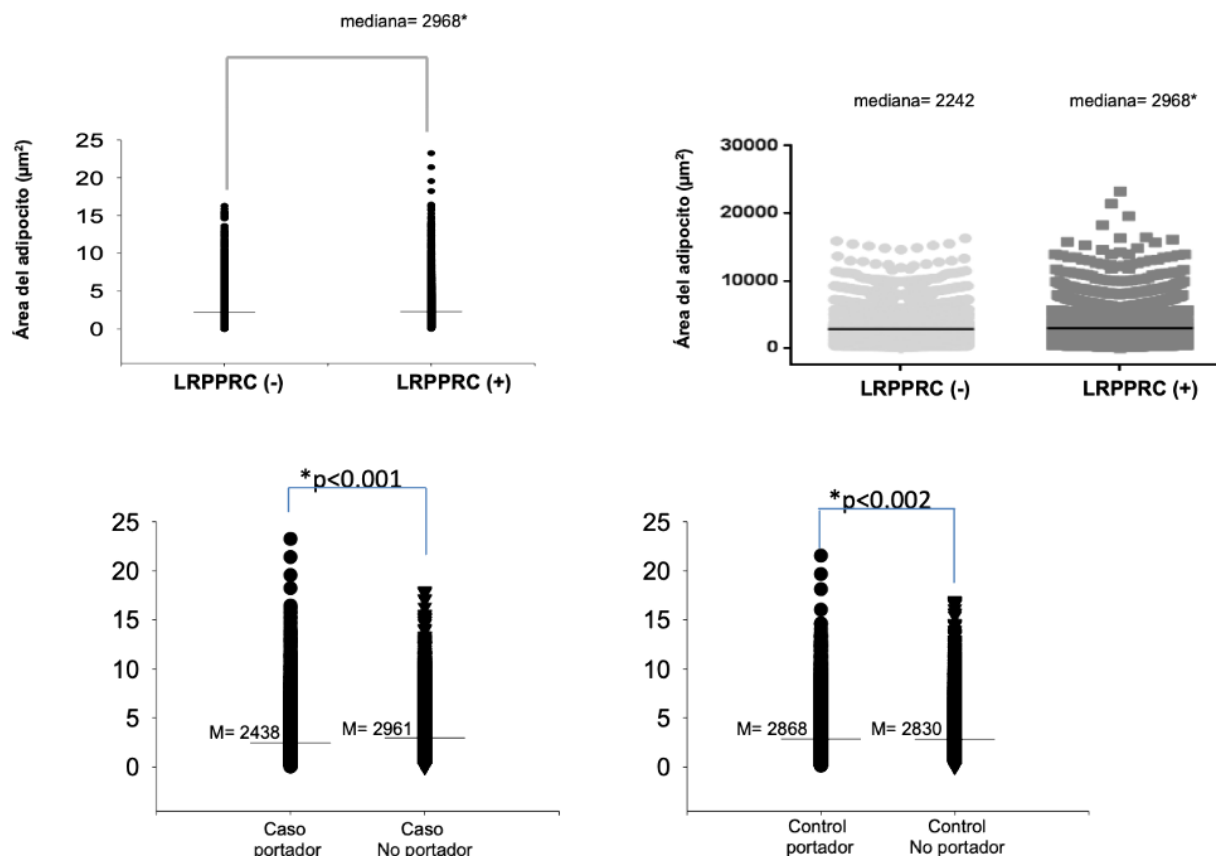
Conclusions: In subjects with FHTG, the risk haplotype confer an OR 6.2 (2.7-14.3) p =9.86 $^{E-06}$ of having severe HTG ,carriers have a higher visceral fat volume, a larger area and size of adipocytes, a higher postprandial lipemia, a greater number of larger VLDL particles. No differences are observed in insulin sensitivity

SS165 / #1275

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.13 Genomics, GWAS and population genetics; Mendelian randomization*

META-GWAS IDENTIFIES FADS2 A NOVEL LOCUS FOR PCSK9 CONCENTRATIONS

SAAG SESSION 26: THE FRONTIERS IN DYSLIPIDEMIAS

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Background and Aims: Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a key regulator of lipid homeostasis. Genome-wide association studies (GWAS) aimed to investigate the underlying genetic variants associated with circulating PCSK9 concentrations. Due to their low power, an uncertainty remains about some of the genetic loci discovered beyond the *PCSK9* locus. By conducting the largest PCSK9 meta-analysis of GWAS, we have the power to identify novel loci and validate the previously reported loci that regulate PCSK9 levels.

Methods: PCSK9 concentrations were measured in two large independent studies (GCKD (n=4963) and KORA F3 (n=2895)), followed by GWAS on PCSK9 concentrations in both studies. The present meta-analysis of GWAS reaches a sample size of 20,579 individuals of European ancestry by additionally including data from a meta-GWAS by Pott et al.*(Hum.Mol.Gen 31, 2022)* to GCKD and KORA F3. The main model was adjusted for age, sex, current smokers, statin treatment and principal components. We further conducted the meta-analyses in statin-free individuals (n=15,390).

Results: Eight loci were genome-wide significantly associated with PCSK9 levels. We successfully replicated the *PCSK9* (chr1), *APOB* (chr2)*, KCNA1/KCNA5* (chr12)*, and *TM6SF2/SUGP1* (chr19) loci. We further identified *FADS2* (chr11) as a novel locus that was also found in statin-free participants. Additionally, three further loci (*RPS17P2, SDK1 and SPATA16*) were genome-wide significant in either the main model or the statin-free subset.

Conclusions: Our study identified a novel locus (*FADS2*). Additionally, we confirm the genome-wide significant hits that were previously detected. Further loci were also detected in single models or subsets of the meta-analysis and require additional research.

SS166 / #1199

Topic: *AS02 Lipids and Lipoproteins / AS02.14 Other*

PRIMARY HYPOCHOLESTEROLEMIA AND RISK OF NEW-ONSET DIABETES IN GENERAL POPULATION

SAAG SESSION 26: THE FRONTIERS IN DYSLIPIDEMIAS

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Background and Aims: Mendelian randomisation studies and randomised statin trials have established a potential link between low LDL cholesterol (LDL-C) levels and diabetes risk. Hypobetalipoproteinemia (HBL) is defined as a spontaneous LDL-C level < 5th percentile of the population adjusted for age and sex. In this study, we analyse the relationship between HBL and the risk of diabetes in the general population.

Methods: From the nationwide CONSTANCES cohort, we selected subjects with HBL to compare with those with normal LDL-C levels (40th<LDL-C≤60th percentile=CTRL). Individuals on lipid-lowering therapy and those who were vegan were excluded. The history of diabetes was collected via the CONSTANCES questionnaire and French Health Insurance Database (SNDS) data. New cases of diabetes were identified via the SNDS. These findings were replicated in the UK-Biobank (UKBB) cohort.

Results: In CONSTANCES cohort, 6,978 HBL (mean LDL-C: 71 mg/dl) and 27,863 CTRL (LDL-C: 129 mg/dl), of the same age (45 years) and sex (54.3% women) were compared. A history of type 2 diabetes was more frequent in HBL (3.17% vs 1.61%; p<0.0001). However, the incidence of new onset diabetes during follow-up was comparable between the 2 groups: incidence density ratio (IDR)=0.79 [0.60-1.02] for HBL vs CTRL. Similarly in the UKBB cohort (18,914 HBL (mean LDL-C: 86 mg/dl) and 75,752 CTRL (LDL-C: 142mg/dl)), the IDR for new onset diabetes did not differ between the two groups (1.07 [0.97-1.17]).

Conclusions: The risk of new-onset diabetes is not increased in people with HBL, challenging the hypothesis of a mechanistic link between low LDL-C and diabetes risk.

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.02 Epidemiology of dyslipidemias*

DIFFERENCES IN SUBCLINICAL ATHEROSCLEROSIS BASED ON THE GENOTYPE OF PATIENTS WITH SEVERE HYPERCHOLESTEROLEMIA

SAAG SESSION 26: THE FRONTIERS IN DYSLIPIDEMIAS

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Background and Aims: Familial hypercholesterolemia (FH) is an inherited disorder that confers an increased risk of premature cardiovascular disease (CVD). However, CVD risk is highly heterogeneous in these subjects and up to 60% of clinically diagnosed patients do not harbor a mutation in FH genes. Our objective was to evaluate the effect of the genotype on the burden of atherosclerosis in a series of hypercholesterolemic individuals.

Methods: A cohort of 324 Spanish individuals with LDL>190mg/dl and <65 years old at diagnosis was studied. Clinical data, coronary artery calcium (CAC) score, and whole genome sequencing data were obtained. Characteristics between patient groups (monogenic, polygenic, negative) were compared. Statistical analyses were performed using R.

Results: 37% of patients were monogenic, 25% polygenic and 38% negative. As expected, male and older age were significantly associated with higher CAC score (P<0.05). 53% of the patients had CAC=0, 25% CAC=1-99 and 22% CAC>100. In agreement with prior studies, mean CAC score was greater in monogenic ($\bar{x}$=170, 95%CI: 85-255) compared to polygenic ($\bar{x}$=82, 95%CI: 26-138) and negatives ($\bar{x}$=117, 95%CI: 54-179). Among subjects with CAC>100, 48.6% were monogenic, 19.4% polygenic and 31.9% negative. Carriers of *LPA* variants did not significantly differ across CAC categories and genotypes, with the exception of individuals in the CAC=1-99 category (P=0.006). Also, in the monogenic group, the mean CAC score of patients carrying *ANGPTL3* or *PCSK9* loss-of-function variants was lower ($\bar{x}$=38.8).

Conclusions: Monogenic FH patients have higher preclinical atherosclerosis than polygenic and negative ones. Deeper understanding of the genetics of hypercholesterolemia will improve CVD risk assessment.

SS168 / #1052

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.05 Inherited dyslipidemias*

ASSESSMENT OF GENETIC BACKGROUND OF HYPOCHOLESTEROLEMIA AND CORRELATIONS WITH CLINICAL CHARACTERISTICS IN CHILDREN AND ADOLESCENTS: A PRELIMINARY REPORT

SAAG SESSION 26: THE FRONTIERS IN DYSLIPIDEMIAS

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Background and Aims: Hypocholesterolemia is defined by total cholesterol levels below 5th percentile adjusted for age and gender. Hereditary forms of hypocholesterolemia include familial hypobetalipoproteinemia (FHBL), abetalipoproteinemia (ABL), Chylomicron retention disease and familial combined hypolipidemias. Common symptoms are atypical retinitis pigmentosa, fat malabsorption, and fat-soluble vitamin deficiency. We report genetic analysis results of a group of 44 patients aged from 3 to 17 years with measured TC (total cholesterol) less or equal to 3mmol/L.

Methods: DNA was isolated from the peripheral blood of the patients. Next-generation sequencing was performed. A panel of genes associated with hypocholesterolemia was used to filter changes. The identified variants were confirmed by Sanger sequencing. The detected variants were classified according to the American College of Medical Genetics and Genetics and the Association for Molecular Pathology (ACMG AMP) classification criteria as (likely) benign, variants of uncertain significance (VUS) and (likely) pathogenic.

Results: According to ACMG classification, genetic investigation revealed the presence of Likely pathogenic and Pathogenic variants in 11 patients. All of them were heterozygous. Observed variants were present in *ANGPTL3*, *APOB*, *ABCA1*, and *DHCR7* genes. Among them, three frameshift variants *APOB* (NM_000384.3) c.2786dupC, *APOB* (NM_000384.3) c.908del and *APOB* (NM_000384.3) c.1276del haven't been described in literature yet.

Conclusions: In this preliminary study, we report 11 subjects, from a group of 44 patients with lower TC levels, with Likely pathogenic and Pathogenic variants in genes associated with hypocholesterolemia. Early detection and treatment of patients with primarily hypocholesterolemia are of great importance since symptoms can be alleviated by regular follow-ups and sufficient vitamin supplementation.

SS169 / #637

Topic: *AS02 Lipids and Lipoproteins / AS02.02 TG rich lipoproteins metabolism and lipases*

FAMILIAL CHYLOMICRONAEMIA SYNDROME (FCS) SCORE VALIDATION IN UNITED KINGDOM FCS REGISTRY: CAN ADDITIONAL VARIABLES IMPROVE THE FCS SCORE PERFORMANCE?

SAAG SESSION 26: THE FRONTIERS IN DYSLIPIDEMIAS

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Background and Aims: FCS is a rare autosomal recessive disorder. It remains a challenge to differentiate between FCS and MCS clinically. Moulin *et al* (2018) proposed 8 item FCS score with sensitivity and specificity of 88% and 85% respectively. We aim to validate the score in UK population and to identify additional variables that can increase its sensitivity.

Methods: While the data collection is ongoing, we conducted a validation study using data for 103 patients (50 FCS and 53 MCS patients). All patients underwent genetic testing for FCS. Logistic regression analysis was done to establish if the clinical/biological items proposed in FCS score remain valid and to assess if adding additional variables increase the performance of the score.

Results: In addition to FCS predictors proposed in FCS score, we found ethnicity, parental consanguinity, BMI <25 kg/m^2, recurrent pancreatitis as positive predictors and BMI>30 kg/m^2 as a negative predictor for FCS. At the FCS score of $\geq$10 sensitivity of the score in UK population is 92% (95% CI 0.82 – 0.97) and specificity of 71.7% (95% CI 0.59 – 0.83) that was reproducible when cohort was

divided into 2 parts based on geographical location. ROC curve area was 0.86 (0.79 – 0.93), p= <0.001. Additional FCS predictors had no impact on sensitivity or specificity and AUC was comparable. (Table 1,2 & Figure 1).

Table 1: Additional Predictors of FCS

Predictor Variable		Regression Coefficient	Score Assigned
Ethnicity	Asian	1.7	2
	Caucasian		0
Parental Consanguinity	Yes	2.3	2
	No		0
BMI <25kg/m^2		1.5	2
BMI >30 kg/m^2		-1.9	-2
Recurrent Pancreatitis		1.3	1

Table 2: Models to predict FCS

Model	Variables
1	8 item clinical FCS Score
2	Model 1 + BMI
3	Model 1 + Recurrent Pancreatitis
4	Model 1 + Ethnicity
5	Model 1 + Parental Consanguinity
6	Model 1 – Trough TG < 2 mmol/L
7	Model 1 +BMI + Recurrent Pancreatitis + Ethnicity + Parental Consanguinity

Figure 1: ROC Curves for different models
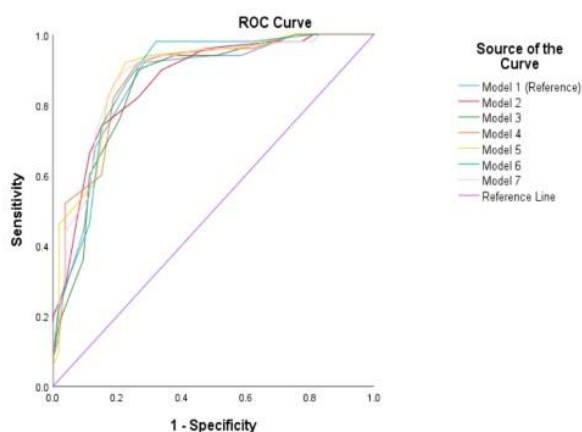
Conclusions: Moulin *et al* (2018) FCS score performs well in UK population and provides a useful tool to differentiate between FCS and MCS. Additional FCS predictors did not improve the FCS score.

SS170 / #413

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.01 Epidemiology of cardiovascular diseases and risk factors*

OVERWEIGHT/OBESITY AND PREVALENT ATHEROSCLEROTIC CARDIOVASCULAR DISEASE IN PATIENTS WITH HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA: AN ANALYSIS FROM HELLAS-FH REGISTRY

SAAG SESSION 26: THE FRONTIERS IN DYSLIPIDEMIAS

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Background and Aims: Although obesity is considered a major modifiable cardiovascular risk factor in the general population, relevant data is limited in patients with familial hypercholesterolemia (FH). We aimed to investigate the effect of overweight and obesity on the cardiovascular phenotype of patients with heterozygous FH enrolled in the Hellenic Familial Hypercholesterolemia Registry (HELLAS-FH).

Methods: FH diagnosis was based on the Dutch Lipid Clinic Network (DLCN) criteria. Adult patients with at least a possible FH diagnosis (DLCN score ≥3) and available body mass index (BMI) values were included in the present cross-sectional analysis.

Results: A total of 1655 FH patients (mean age 51 ± 14.4 years, 48.6% female) were included. Of those, 378 (22.8%) and 430 (26.0%) were diagnosed with probable and definite FH. Moreover, 371 (22.4%) were obese and 761 (46.0%) were overweight. Prevalence of cardiovascular risk factors increased

progressively with BMI. Prevalence of coronary artery disease (CAD) was 23.4% (3.2% for stroke and 2.7% for peripheral artery disease), and increased progressively across BMI groups. After adjusting for traditional cardiovascular risk factors and lipid-lowering therapy, obese patients had higher odds of CAD or premature CAD compared with normal BMI, but no effect on stroke or peripheral artery disease (PAD) risk was found
(Figure).
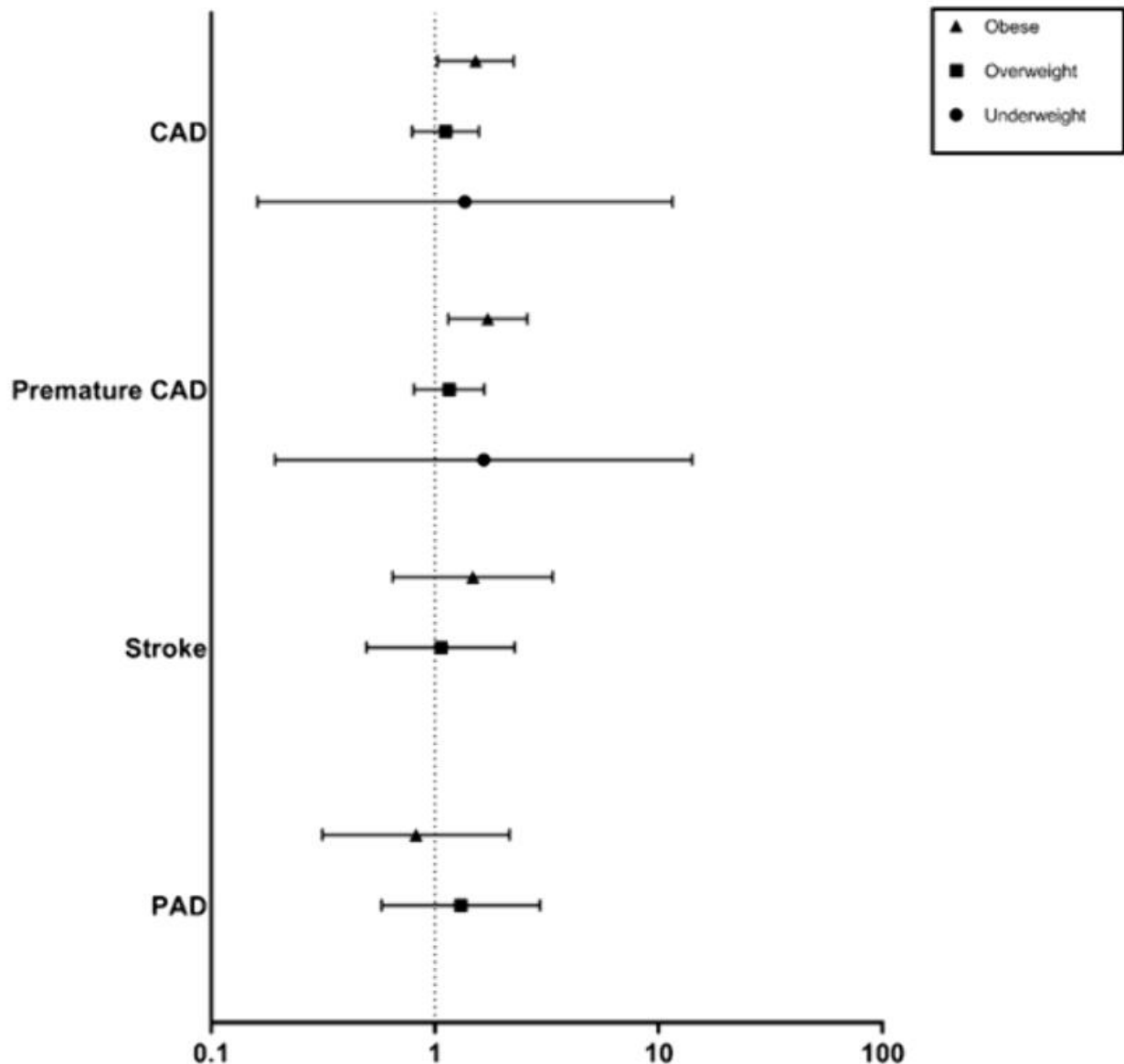
Conclusions: Over half of patients with heterozygous FH are overweight or obese. Obesity was associated with increased prevalence of CAD in this population, but no effect on stroke or peripheral artery disease was evident.

Topic: *AS02 Lipids and Lipoproteins / AS02.08 Cellular lipid metabolism and lipid droplets*

MOLECULAR REGULATION OF ENDOTHELIAL AND VASCULAR DYSFUNCTION BY LIPID LACTONE MEDIATORS

SAAG SESSION 27: IN THE FOREFRONT OF VASCULAR BIOLOGY

Meitar Asulin[1], Rotem Fridman[1], Nadav Gorodetzer[1], Andreas Beyer[2], Dimitry Chuyun[3], David Gutterman[2], Andrea Szuchman-Sapir[1]
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Background and Aims: Microvascular endothelial dysfunction is a known contributor to and predictor of major adverse cardiovascular events. The loss of nitric oxide (NO)-mediated dilation in the dysfunctional endothelium may be compensated by CYP450 epoxygenase-generated endothelial-derived hyperpolarizing factors (EDHFs) that regulate vascular tone. Our recent data point to a novel family of lactone metabolites of polyunsaturated fatty acids (PUFA-Ls), which are potential EDHFs. Unlike known epoxy-metabolites, their structure is chemically stable and is a poor substrate for the epoxide hydrolase enzyme. Our study aimed to reveal their potential mechanism and physiological function in microvascular dilation.

Methods: Human adipose arterioles were extracted from adipose tissues from HTN and normotensive (NT) subjects and detected for their dilation response to EPA-L. Hypertensive rats were administrated with EPA-L and measured for their blood pressure, blood and urine chemistry, and kidney function. Human endothelial cells were used to investigate the EPA-L signaling mechanism by calcium and potassium efflux with antagonists for GPCRs and the PLC-IP$_3$ pathway.

Results: Lactone metabolites, derived from arachidonic acid (AA-L) and eicosapentaenoic acid (EPA-L), were shown to mediate endothelial-dependent vasodilation in isolated human microvessels. In hypertensive arterioles, EPA-L-induced dilation was not affected by eNOS inhibitors. In hypertensive rats, EPA-L reduced blood pressure *in vivo* and restored the microvascular dilation capacity. The mechanism of action revealed to initiate G-protein coupled receptors that activate the PLC-IP$_3$ pathway and mediate calcium flux from the endoplasmic reticulum, resulting in potassium efflux and hyperpolarization of endothelial cells.

Conclusions: These results demonstrate that lactone-derived PUFA are potentially EDHFs that may regulate endothelial dysfunction.

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.08 Novel risk factors and biomarkers*

CIRCULATING GLYCOME AND PROTEOME SIGNATURE ON ASGR1 AND MRC1 DEFICIENT MICE

SAAG SESSION 27: IN THE FOREFRONT OF VASCULAR BIOLOGY

Monika Svecla[1], Jasmine Nour[1], Marco Bladergroen[2], Simone Nicolardi[2], Manfred Wuhrer[2], David Falck[2], Giuseppe Norata[1]
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Background and Aims: Recent studies showed that deficiency of asialoglycoprotein receptor (ASGPR) and mannose receptor C-type 1 (MRC1) improve the cardiometabolic status. Those two receptors carry out the clearance of circulating glycoproteins based on the selective affinity for the glycan moiety. Here we investigated the ability of glycome and proteome to predict cardiometabolic status.

Methods: ASGR1$^{-/-}$, MRC1$^{-/-}$ and their WTlittermate mice, were fed on a high-fat diet (45% Kcal fat) for 20 weeks. Plasma was collected and processed for high-resolution shotgun proteomics and glycomics, using orbitrap Fusion™ Tribrid™ mass spectrometer, and MALDI-FTICR-MS respectively.

Results: In plasma from ASGR1$^{-/-}$ and MRC1$^{-/-}$ mice, more than 5000 proteins were label free quantified, and 79 glycan compositions were assigned. No change is observed in the galactose in ASGR1$^{-/-}$ mice nor in mannose in MRC1$^{-/-}$. ASGR1$^{-/-}$ presented a 32% increase in O-acetylation compared to the WT mice (WT 8.2%±0.2%, ASGR1$^{-/-}$ 10.8%±0.4%, P-value<0.001). MRC1 deficient mice did present a 24% reduction in core fucosylation (WT 34.3%±2.2%, MRC1$^{-/-}$ 26.2%±1.1%, P-value=0.002). Interestingly, the reduction in fucosylation was in line with pathways related to inflammation such as reduced chemokine signaling, leukocyte transendothelial migration, ECM-cell interaction (FDR <0.05)

Conclusions: This study suggests that tight control of the glycome is so important for an organism that significant redundancy exists in terms of plasma glycoprotein clearance receptors with glycan-epitope specificity. Whether those glycan changes can predict the cardiometabolic state of the disease is under investigation through proteomics.

SS173 / #627

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.06 Vascular biology of the arterial wall: Miscellaneous*

OLFACTORY RECEPTOR 2 DEFICIENCY PROTECTS FROM EXPERIMENTAL ABDOMINAL AORTIC ANEURYSM FORMATION

SAAG SESSION 27: IN THE FOREFRONT OF VASCULAR BIOLOGY

Patrik Schelemei[1], Felix Picard[1], Harshal Nemade[1], Dennis Mehrkens[1], Simon Grimm[1], Katharina Tinaz[1], Elena Wagner[1], Wiebke Kreuzberg[1], Marco Orecchioni[2], Markus Wagenhäuser[3], Hubert Schelzig[3], Joy Roy[4], Moritz Liljeqvist[4], Martin Mollenhauer[1], Stephan Baldus[1], Holger Winkels[1]
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Background and Aims: Abdominal aortic aneurysm (AAA) is defined as permanent dilation of the abdominal aorta by >50% or >3cm and particularly affects the elderly. It AAA is accompanied by vascular inflammation and macrophage infiltration. Recent data has shown extra-nasal expression of olfactory receptor 2 (OLFR2) on vascular macrophages and their activation. Here, we questioned whether OLFR2 also contributes to AAA formation.

Methods: Expression of the OLFR2 orthologue OR6A2 was determined in human AAA tissue histologically and transcriptomically. AAA was induced by porcine pancrease elastase infusion in mice. OLFR2 expression in AAA was analyzed by flow cytometry (FACS) and histology at baseline, day7 and day 28 post AAA induction. The aortic diameter in OLFR2 KO and WT mice was measured weekly by ultrasound analysis. AAA tissue was collected at day 28 for histological analysis. Lastly, we performed bulk transcriptome analysis of aortic tissue collected from KO and WT mice at day 7.

Results: OR6A2 co-localized with macrophages in human AAA sections. OR6A2 expression increased in human AAA tissue compared to controls. Spectral FACS revealed OLFR2 expression in 3 out of 4 aortic myeloid clusters peaking at day 7. Functionally, OLFR2 deficiency attenuated aneurysm formation and preserved vessel integrity. AAA of KO mice showed reduced macrophage and increased smooth muscle cell content. Transcriptional profiling showed enrichment for vascular smooth muscle cell contractility, while pathways involved in inflammation and leukocyte activation were downregulated in KO compared to WT AAA tissue.

Conclusions: We show that OLFR2 deficiency attenuates AAA development, aortic remodeling, and inflammation within the vessel wall.

Topic: *AS04 Clinical Vascular Disease / AS04.06 Aneurysms and other non-atherosclerotic arteriopathies*

SCRNA- AND CITE-SEQ-BASED IDENTIFICATION AND CHARACTERIZATION OF IMMUNE CELL TYPES INVOLVED IN ELASTASE-INDUCED ANEURYSM PROGRESSION

SAAG SESSION 27: IN THE FOREFRONT OF VASCULAR BIOLOGY

Christin Elster, Miriam Ommer-Bläsius, Marius Böttenberg, Sarah Verheyen, Tanja Vajen, Susanne Pfeiler, Malte Kelm, Alexander Lang, Norbert Gerdes
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Background and Aims: Abdominal aortic aneurysm (AAA) is a progressive disease defined by inflammation and extracellular matrix degeneration. The pathogenesis is still poorly understood and no drug-based therapies are available. Single-cell sequencing approaches enable a comprehensive and unbiased characterization of cell types and genes involved in AAA development. We aim to characterize cell types and their specific function in AAA and identify potential targets for therapeutic approaches to prevent AAA progression.

Methods: We performed single-cell RNA sequencing (scRNA-seq) and Cellular Indexing of Transcriptomes and Epitopes by Sequencing (CITE-seq) of isolated cells from infrarenal aortae of C57BL/6J mice 3, 7, 14, and 28 days after AAA induction via elastase perfusion. NaCl-perfused mice (d3, d28) and non-operated mice served as controls. In total, we analyzed 23,516 cells with scRNA-seq and 7,291 cells with CITE-seq.

Results: We identified the cell types present in AAA by RNA expression and verified their identity by expression of surface molecules using corresponding CITE-seq data. We present a detailed analysis of the cellular profile in a time-dependent manner of AAA and a comparison of cellular heterogeneity between different disease stages. Macrophages seem to play an important role in early stages (d3-14) of AAA. The later phase of AAA (d28) is dominated by T- and B-lymphocytes. Interestingly, we identify a number previously unrecognized subpopulations and a high heterogeneity of the leukocyte infiltrate in all stages of AAA development.

Conclusions: Future detailed analyses of the molecular profile of AAA-associated leukocytes are essential to understand the underlying pathological mechanisms and to identify potential targets for therapeutic intervention.

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.13 Genomics, GWAS and population genetics; Mendelian randomization*

DYSREGULATION OF IRON METABOLISM-LINKED GENES AT MYOCARDIAL TISSUE AND CELL LEVELS IN DILATED CARDIOMYOPATHY

SAAG SESSION 27: IN THE FOREFRONT OF VASCULAR BIOLOGY

Ilaria Massaiu[1], Jeness Campodonico[2], Massimo Mapelli[3], Elusabetta Salvioni[3], Vincenza Valerio[1], Donato Moschetta[1], Veronika Myasoedova[1], Maria Cappellini[4], Giulio Pompilio[5], Piergiuseppe Agostoni[6], Paolo Poggio[1]
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Background and Aims: In heart failure the biological and clinical connection between abnormal iron homeostasis, myocardial function, and prognosis is known, however, the expression profiles of iron-linked genes are not well defined. Through publicly available datasets, we aim to evaluate the altered iron metabolism in dilated cardiomyopathy (DCM) subjects at whole cardiac tissue and single-cell level.

Methods: Bulk RNA-seq and single-nucleus RNA-seq datasets, respectively of 436 and 38 left ventricle samples from adult non-failed (NF) and DCM subjects, were obtained from public studies. We defined a list of 272 genes directly related to intramyocardial iron metabolism and performed the differential gene expression analysis both at whole cardiac tissue level and in cardiomyocytes, fibroblasts, myeloid, endocardial, and endothelial cells.

Results: From the bulk RNA-seq data, we found 223/272 iron-linked genes expressed at myocardial tissue level and 44 differentially expressed between DCM and NF subjects. At single-cell level, at least 9% of iron-linked expressed genes was significantly regulated in DCM, compared to NF. Specifically, the iron metabolism in DCM cardiomyocytes is altered at several levels (Figure 1), including: 1) imbalance of Fe^{3+} internalization (SCARA5 down-regulation) and reduction of internal conversion from Fe^{3+} to Fe^{2+} (STEAP3 down-regulation), 2) increase of iron consumption to produce hemoglobin (HBA1/2 up-regulation), 3) higher heme synthesis and externalization (ALAS2 and ABCG2 up-regulation), 4) lower cleavage of heme to Fe^{2+}, biliverdin and carbon monoxide (HMOX2 down-regulation), and 5) positive

regulation of hepcidin (BMP6 up-

Iron metabolism-linked genes in cardiomyocytes of DCM patients
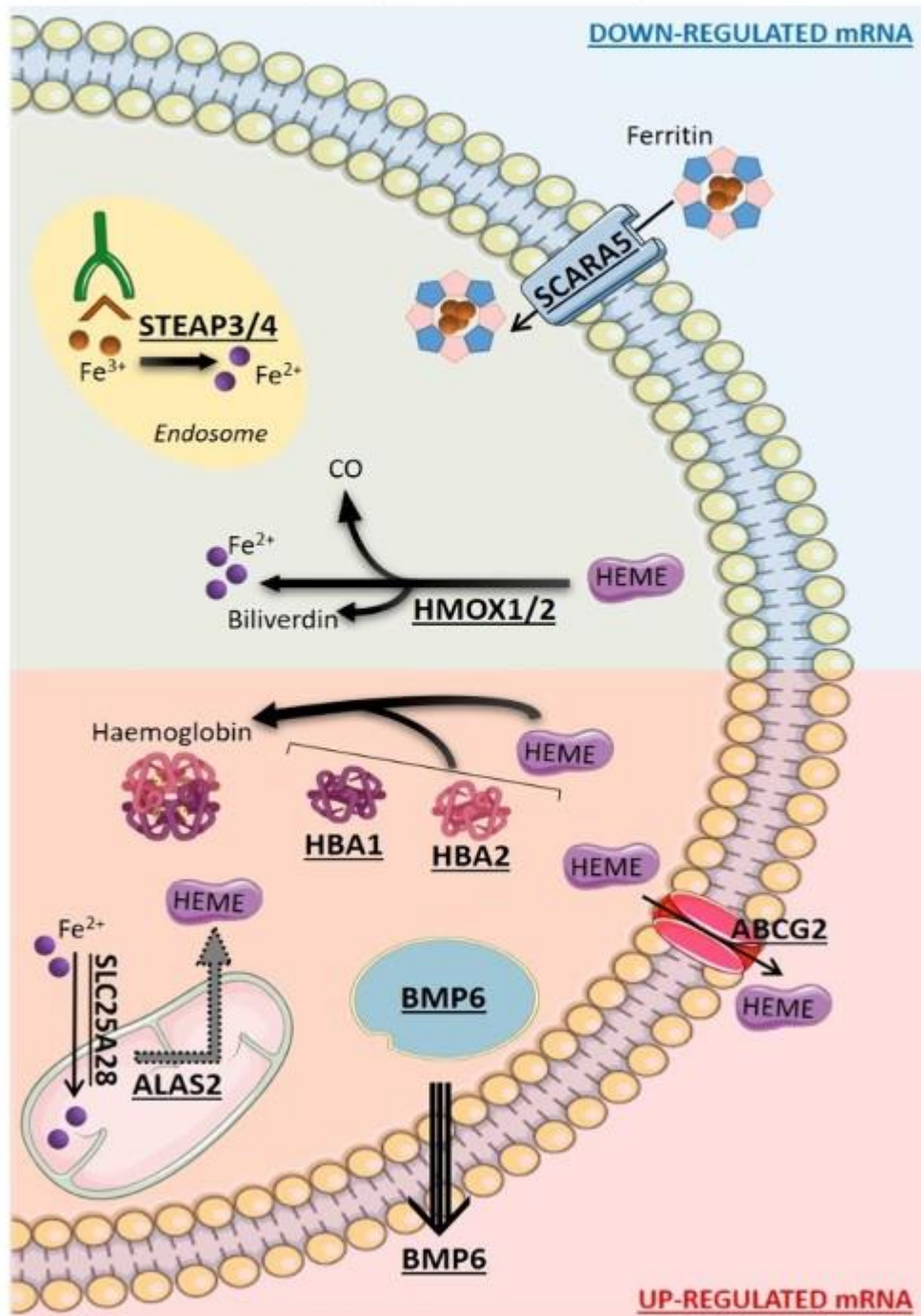
regulation).

Conclusions: In DCM several iron metabolism-related genes are dysregulated possibly contributing to heart failure progression.

SS176 / #1061

Topic: *AS04 Clinical Vascular Disease / AS04.15 Other*

INTRODUCING A NOVEL HEART-ON-CHIP SYSTEM TO INVESTIGATE ENDOTHELIAL-CARDIOMYOCYTE COMMUNICATION IN ISCHEMIA REPERFUSION INJURY.

SAAG SESSION 27: IN THE FOREFRONT OF VASCULAR BIOLOGY

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Background and Aims: Acute myocardial infarction (AMI) is a leading cause of morbidity and mortality. Where reperfusion is the current standard, it often results in myocardial ischemia reperfusion injury (MI-R). MI-R is a multifactorial process with an intricate interplay between multiple cell types, where the primary insult in the coronary vessels results in cardiomyocyte dysfunction and death. As currently no clinically effective cardioprotective agents have been found, it is important to increase our knowledge on how these tissues communicate.

Methods: Our aim is to further investigate the molecular mechanisms of MI-R using a newly developed a 3D heart-on chip model. This model comprises of a vascular channel consisting of human coronary endothelial cells (CAECs) with and a layer of human cardiomyocytes (iPSC-CMs) on top. Both channels are separated by a porous membrane, allowing direct cell-cell communication. It can be mechanistically stretched, individually subjected to flow and secretory output can be collected.

Results: Using live-cell imaging, we were able to show a confluent vascular channel as visualized by F-actin, VE-cadherin and Hoechst, and Troponin-T and Hoechst in our matured CM channel. Our model offers the possibility to study EC-CM interactions under (patho)physiological conditions, as no decrease in cell viability (LDH release) decrease in cellular stress (BNP-1 for CM and IL-8 for EC) was observed (> 7days). Functionally, decreased CM beating frequency was observed in this 3D CM-EC chip-model, indicating increased CM maturation due to the protective endothelial effects under quiescent conditions.

Conclusions: Currently, this model is used to study the molecular underpinnings of MI-R.

Topic: *AS04 Clinical Vascular Disease / AS04.12 Prevention and treatment of cardiovasculardisease; miscellaneous*

EFFECT OF CITRUS BERGAMIA (ENDOBERG®) ON CARDIOVASCULAR RISK PARAMETERS OF RATS FED WITH A HIGH-SUGAR FAT DIET

SAAG SESSION 27: IN THE FOREFRONT OF VASCULAR BIOLOGY

Matheus Belin[1], Taynara Aparecida Vieira[1], Nubia Alves Grandini[1], Juliana Silva Siqueira[1], Thiago Luiz Novaga Palacio[1], Erika Tiemi Nakandakare Maia[1], Fabiane Valentini Francisqueti-Ferron[1], Artur Junio Togneri Ferron[1], Silméia Garcia Zanati Bazan[1], Dijon Henrique Salome Campos[1], Giuseppe Lombardo[2], Giancarlo Aldini[3], Camila Renata Correa[1]
[1]Pathology, Sao Paulo State University, Botucatu, Brazil, [2]Products, AKHYNEX, Milan, Italy, [3]Pharmaceutical Sciences, Università degli Studi di Milano, Milan, Italy

Background and Aims: A diet rich in sugar and fat (HSF) can increase obesity and the risk of cardiovascular disease. *Citrus* plants have anti-inflammatory potential and may act on cardiovascular risk parameters. The objective of this study is to evaluate the effect of EndoBerg® on cardiovascular risk parameters of rats fed with a high-sugar fat diet.

Methods: 48 Wistar rats were distributed into four groups (G). G1: control diet(C); G2: C+EndoBerg®; G3: HSF; and G4: HSF+EndoBerg®; and received gavage (250mg/Kg/day) for 20 weeks. The biochemical parameters (total-cholesterol, VLDL, LDL, HDL, triglycerides, blood-glucose); and the cardiac oxidative stress(COS), by MDA and protein carbonylation, were evaluated by UV-vis-Spectrophotometry. The adiposity index(AI) was measured. Cardiac structure: diastolic-posterior-wall-thickness(PWT), diastolic-thickness-of-interventricular-septum(IST), left-ventricular-mass-index(LVMI); systolic function: PWSV(posterior-wall-shortening-velocity), EF(ejection fraction); and diastolic function: E/E'ratio, and E/A'ratio, were evaluated by Doppler Echocardiography. Systolic blood pressure(SBP) was evaluated by tail-cuff-plethysmography. The data were compared by two-way ANOVA with Tukey's post-hoc($p < 5\%$).

Results: The HSF groups, compared to the C groups, had negative alterations on the biochemical parameters(Table 1); higher AI(Figure 1), COS(Figure 2-A,B), SBP(Figure 3), and had cardiac remodeling and dysfunction(Table 2). EndoBerg® attenuated SBP(Figure 3) and prevented COS(Figure 2-A,B), cardiac remodeling and dysfunction(Table 2).

Table 1. Biochemical parameters in rats fed with a HSF diet after 20 weeks of administering EndoBerg®.

Variable	Groups				Effects		
	C	C + E	HSF	HSF + E	Diet	EndoBerg®	Interactions
Total cholesterol (mg/dL)	53.0 ± 10.6	47.6 ± 8.7	64.9 ± 9.6^a	58.4 ± 8.9^d	<0.001	0.027	0.845
VLDL (mg/dL)	5.0 ± 1.3	6.0 ± 3.3	18.2 ± 6.7^a	17.1 ± 7.0^d	<0.001	0.962	0.489
LDL (mg/dL)	33.1 ± 8.2	27.9 ± 7.8	30.6 ± 8.1	26.3 ± 6.8	0.345	0.032	0.827
HDL (mg/dL)	12.7 ± 3.2	11.1 ± 2.3	16.8 ± 3.7^a	15.3 ± 2.8^d	<0.001	0.075	0.917
Triglycerides (mg/dL)	25.2 ± 6.7	30.1 ± 16.6	90.9 ± 33.5^a	85.3 ± 34.9^d	<0.001	0.962	0.489
Blood Glucose (mg/dL)	80.9 ± 7.1	83.2 ± 9.5	91.4 ± 6.2^a	92.6 ± 8.5^d	<0.001	0.438	0.799

Data presented as mean ± standard deviation. and submitted to Two-way ANOVA with Tukey's post hoc (p<0.05). a = C vs HSF; b = HSF vs HSF + E; c = C vs C + E; d = C + E vs HSF + E. C = Control diet; E = EndoBerg®; HSF = High Sugar Fat diet;VLDL: Very-low-density lipoprotein; LDL: low-density lipoprotein; HDL: high-density lipoprotein.

Table 2. Echocardiographic study rats fed with a HSF diet after 20 weeks of administering EndoBerg®.

Echocardiographic Variable		Groups				Effects		
		C	C + E	HSF	HSF + E	Diet	EndoBerg®	Interactions
Structural	PWT (mm)	1.49 ± 0.06	1.52 ± 0.03	1.92 ± 0.12^a	1.55 ± 0.06^b	<0.001	<0.001	<0.001
	IST (mm)*	1.52 (1.50-1.53)	1.53 (1.53-1.59)	$2.00 (1.88-2.04)^a$	$1.53 (1.53-1.55)^b$	<0.001	<0.001	<0.001
	LVMI (g/g)	1.49 ± 0.21	1.59 ± 0.28	1.80 ± 0.32^a	1.47 ± 0.17^b	0.173	0.112	0.004
Systolic function	PWSV (mm/s)	83.8 ± 4.6	81.4 ± 4.3	67.2 ± 6.3^a	85.5 ± 4.9^{bd}	<0.001	<0.001	<0.001
	EF (%)	0.94 ± 0.02	0.94 ± 0.01	0.91 ± 0.02^a	0.94 ± 0.01^b	<0.001	0.002	0.002
	CD (L/min)	99.5 ± 28.9	100.6 ± 27.1	78.2 ± 2.3^a	105.5 ± 20.2^b	0.235	0.043	0.062
Diastolic function	E/E' ratio (m/s)*	11.8 (10.6-12.4)	11.9 (11.1-13.1)	$18.6 (15.8-19.9)^a$	$11.7 (10.5-12.7)^b$	<0.001	<0.001	<0.001
	E/A ratio (m/s)	1.56 ± 0.07	1.64 ± 0.09^c	0.66 ± 0.07^a	1.57 ± 0.11^{bd}	<0.001	<0.001	<0.001

Data presented as mean ± standard deviation or median with interquartile range (*) and submitted to Two-way ANOVA with Tukey's post hoc (p<0.05). a = C vs HSF; b = HSF vs HSF + E; c = C vs C + E; d = C + E vs HSF + E. C = Control diet; E = EndoBerg®; HSF = High sugar fat diet. PWT: diastolic posterior wall thickness; IST: diastolic thickness of the interventricular septum; LVMI: Left ventricule mass index; PWSV: left ventricle posterior wall shortening velocity; EF: ejection fraction; CD: cardiac debit.
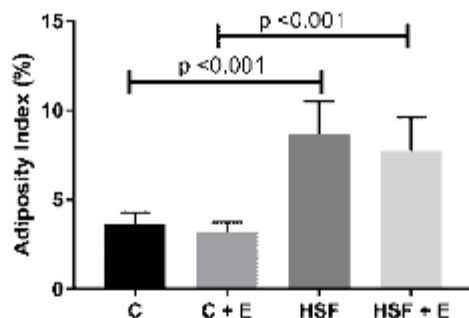
Figure 1. Adiposity Index (%) of rats fed with HSF diet after 20 weeks of administering EndoBerg®.
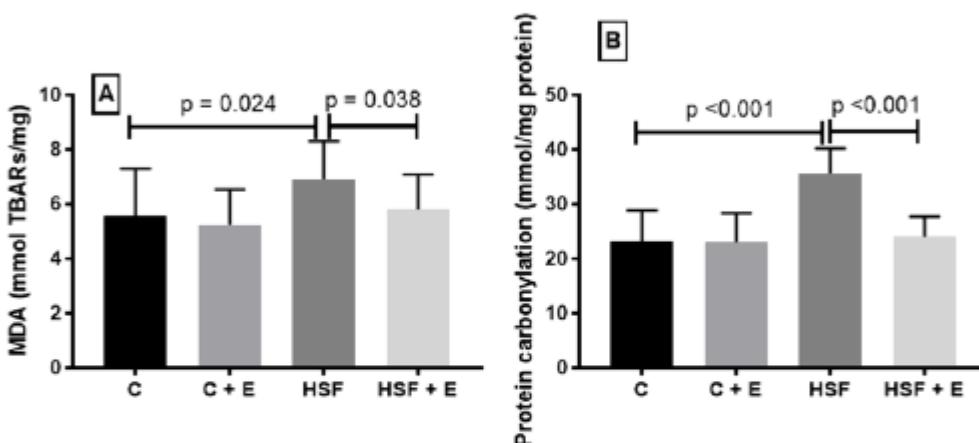
Figure 2. Oxidative Stress in cardiac tissue of rats fed with HSF diet after 20 weeks of administering EndoBerg®. A - Malondyhaldeide (MDA-mmol TBARs/mg); B – protein carbonylation (mmol/mg).
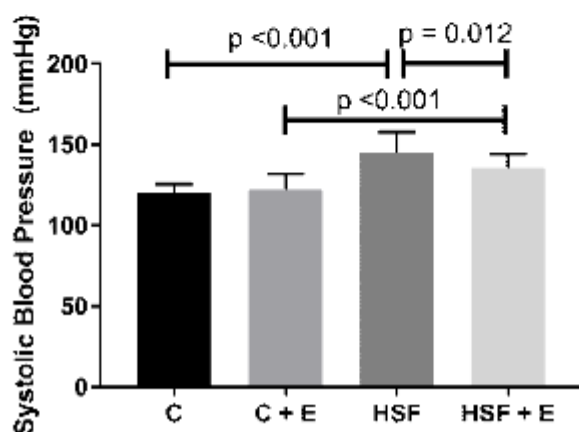
Figure 3. Systolic Blood Pressure (mmHg) of rats fed with HSF diet after 20 weeks of administering EndoBerg®.

Conclusions: Even not controlling the biochemical alterations resulting from obesity and the high-sugar fat diet, EndoBerg® attenuated blood pressure and prevented cardiac oxidative stress and the development of cardiac remodeling and dysfunction. These data suggest that EndoBerg® has a direct action on the cardiac tissue and may help to promote cardiovascular health. More studies are needed to identify the mechanisms of this action.

SS178 / #403

PREDICTIVE VALUE OF HIGH-THROUGHPUT CIRCULATING METABOLITES IN ASSESSING ATHEROSCLEROTIC CARDIOVASCULAR DISEASE RISK: A COHORT STUDY OF 70,000 ADULTS

SAAG SESSION 28: CUTTING-EDGE NEW BIOMARKERS

Danyao Jin[1], Eirini Trichia[2], Nazrul Islam[3], Sarah Lewington[2], Ben Lacey[1]
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Background and Aims: Nuclear magnetic resonance (NMR) spectroscopy has been increasingly used in large-scale studies for metabolic profiling. We aimed to evaluate whether adding high-throughput circulating metabolites to a well-established risk score improves the prediction of 10-year atherosclerotic cardiovascular disease (ASCVD) risk.

Methods: UK Biobank is a prospective study of 0.5 million participants, aged 40-69 at recruitment (2006-2010). Analyses were restricted to 70,409 participants of White ethnicity with metabolic profiling and without ASCVD at baseline. QRISK3 was chosen as the established risk score. The additional predictive value of thirty-nine clinically validated metabolites was evaluated based on Cox proportional-hazards regression in three ways: adding significantly associated metabolites, and adding novel metabolites selected by two machine-learning approaches (elastic-net and extreme gradient boosting algorithms), given the highly correlated metabolites and complex interactions. Predictive values were assessed with measures of discrimination, reclassification and calibration.

Results: Among 70,409 participants with mean age of 44 years at study entry, 5,111 ASCVD cases occurred within 10-year follow-up. Harrell's C-index of QRISK3 was 0.730 (95% CI, 0.718-0.741) for women and 0.699 (95% CI, 0.690-0.707) for men (Table1). Adding selected metabolites (Table2) did not significantly improved any measure of discrimination, reclassification or calibration in women or men

Prediction Performance	Female (95% CI*)	Male (95% CI)
Recalibrated QRISK3		
C-statistics[†]	0.730 (0.718, 0.741)	0.699 (0.690, 0.707)
Adding metabolites associated with ASCVD independently from QRISK3 score[#]		
C-statistics	0.734 (0.723, 0.745)	0.700 (0.692, 0.708)
IDI[‡] (%)	0.19 (0.07, 0.27)	0.13 (0.07, 0.17)
Continuous NRI[§] (%)	10.8 (6.1, 15.3)	6.1 (2.6, 9.6)
events	5.2 (0.5, 9.4)	6.1 (2.7, 9.8)
non-events	5.7 (4.7, 6.6)	-0.1 (-1.5, 1.0)
Categorical NRI (%)	0.3 (-1.0, 1.6)	0.2 (-0.7, 1.0)
events	0.6 (-0.7, 1.9)	-0.2 (-1.2, 0.6)
non-events	-0.2 (-0.4, 0.1)	0.4 (0.2, 0.6)
Adding metabolites with regularization (using Elastic-net[¶])		
C-statistics	0.736 (0.726, 0.746)	0.702 (0.693, 0.711)
IDI (%)	0.23 (0.09, 0.33)	0.08 (-0.02, 0.16)
Continuous NRI (%)	8.1 (3.6, 12.9)	0.9 (-2.7, 4.2)
events	3.3 (-1.1, 7.9)	5.9 (2.4, 9.3)
non-events	4.8 (3.8, 5.9)	-5.0 (-6.2, -3.7)
Categorical NRI (%)	-0.3 (-1.9, 1.0)	1.1 (0.1, 2.0)
events	-0.3 (-1.8, 1.0)	0.7 (-0.3, 1.6)
non-events	0.0 (-0.2, 0.2)	0.4 (0.1, 0.6)
Adding metabolites selected by BorutaSHAP from XGBoost[**]		
C-statistics	0.735 (0.724, 0.745)	0.701 (0.693, 0.711)
IDI (%)	0.23 (0.12, 0.33)	0.11 (0.01, 0.17)
Continuous NRI (%)	10.9 (6.3, 14.9)	5.5 (1.7, 9.5)
events	-0.1 (-4.8, 4.0)	0.9 (-2.6, 4.7)
non-events	11.0 (10.0, 12.0)	4.6 (3.5, 5.6)
Categorical NRI (%)	0.4 (-0.9, 1.9)	0.3 (-0.9, 1.2)
events	0.5 (-0.9, 1.9)	-0.3 (-1.4, 0.5)
non-events	0.0 (-0.2, 0.2)	0.6 (0.3, 0.9)

Comparing prediction performance of 10-year ASCVD risk w/o metabolites. In all models, metabolites are added to recalibrated QRISK3 using Cox proportional-hazards regression. Hyper-parameters of each model are in appendix. *Bootstrap percentile confidence interval, bootstrap for 500 times; [†]Harrell's C-index, measuring the probability that a randomly selected subject with shorter time-to-event will have a higher predicted probability of event than a randomly selected subject with longer time-to-event; [‡]Integrated discrimination improvement, summarising the extent a new model increases risk in events and decreases risk in non-event compared with the old model; [§]Net reclassification improvement, quantifying the appropriateness of the change in predicted probabilities or categorised risk group when changing from old to new model; Categorical NRI is based on a 10% risk threshold. [#]The metabolites associated with ASCVD independently from QRISK3 score were added to QRISK3 in Cox proportional-hazards regression. [¶]Elastic-net is a regression method that perform regularization and variable simultaneously; all metabolites were added to QRISK3 in Cox proportional-hazards regression and penalized by elastic-net. [**]XGBoost (eXtreme Gradient Boosting) is a tree-based algorithm that account for complex nonlinear relationships of variables; BorutaSHAP (SHapley Additive exPlanations) is a wrapper feature selection method to explain how much each factor in a model contributed to the prediction; XGboost was built using all metabolites, and the novel ones were selected by BorutaSHAP, and then added to QRISK3 in Cox proportional-hazards regression.

(Figure).

Clinically validated metabolites	Female				Male			
	Significant associated*	Independent associated†	Elastic-net‡	Boruta SHAP¶	Significant associated	Independent associated	Elastic-net	Boruta SHAP
Cholesterols&Triglycerides								
Total_C					✓		✓	
VLDL_C	✓			✓	✓			
LDL_C	✓			✓	✓			
HDL_C	✓	✓			✓			
Total_TG	✓		✓	✓	✓		✓	
Fatty acids								
Total FA	✓				✓			
Omega-3 FA						✓	✓	
Omega-6 FA					✓			
PUFA								
MUFA	✓			✓	✓			
SFA	✓				✓			
DHA								
LA					✓			
Omega-3 FA to total FA						✓		
Omega-6 FA to total FA	✓			✓				
PUFA to total FA	✓	✓						
MUFA to total FA	✓	✓			✓			
SFA to total FA				✓				✓
DHA to total FA	✓	✓						
LA to total FA	✓	✓	✓					
PUFA to MUFA	✓	✓	✓		✓			
Omega-6 to omega-3 FA								
Apolipoproteins								
ApoB	✓				✓			
ApoA1	✓	✓			✓			
ApoB to ApoA1	✓	✓	✓	✓	✓		✓	✓
Amino acids								
Alanine							✓	
Glycine	✓				✓		✓	✓
Histidine	✓		✓	✓				
Isoleucine			✓					
Leucine								✓
Valine			✓					
BACC								
Phenylalanine			✓				✓	✓
Tyrosine				✓				
Glycolysis related								
Glucose				✓	✓			✓
Lactate			✓				✓	
Fluid balance								
Creatinine								✓
Albumin	✓	✓	✓	✓	✓	✓	✓	✓
Inflammation								
Glycoprotein acetyls	✓	✓	✓	✓	✓		✓	✓

*Association was calculated using Cox proportional-hazards regression with adjustment of established risk factors, including age, education, region, townsend deprivation index, smoking, alcohol intake, body mass index, systolic blood pressure, and baseline diabetes; Significant association was defined as p-value<0.01 after correction of false discovery rate using Benjamini-Hochberg method; †Association was calculated using Cox proportional-hazards regression with adjustment of QRISK3 score; ‡Novel metabolites selected by elastic-net based on Cox proportional-hazards regression, when adding all metabolites into the model; ¶Novel metabolites selected by BorutaSHAP from XGBoost survival model, when adding all metabolites into the model.

Models in women — Models in men
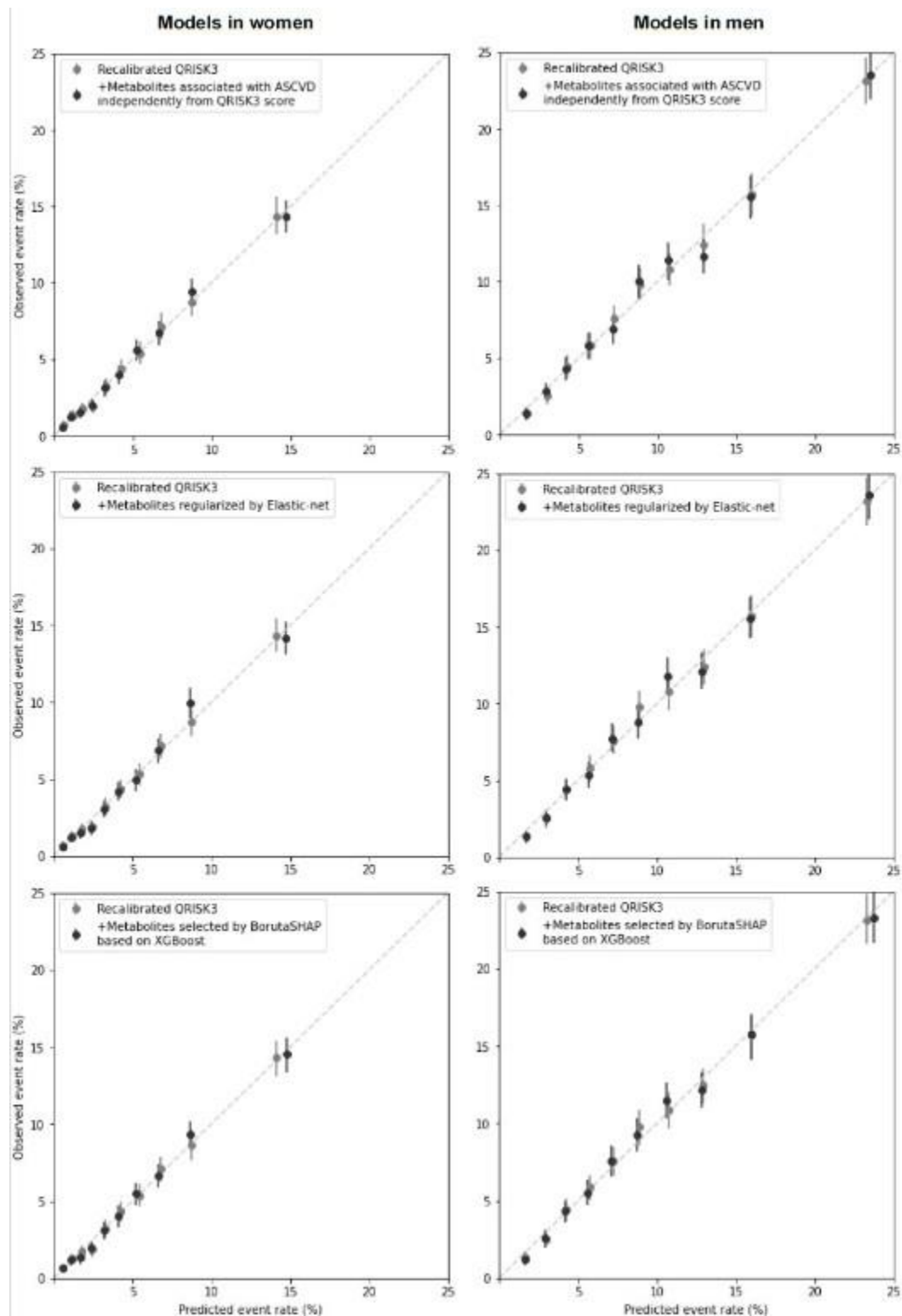
Conclusions: To our knowledge, it is the largest study with blood metabolites measured by NMR spectroscopy, and the first study to use machine-learning algorithms for selecting novel metabolites for prediction of ASCVD risk. However, compared with QRISK3 score, there was no evidence of substantive improvement in prediction of 10-year risk of ASCVD after adding the metabolic biomarkers.

SS179 / #268

Topic: *AS04 Clinical Vascular Disease / AS04.12 Prevention and treatment of cardiovasculardisease; miscellaneous*

PLASMA TSH AND CARDIOVASCULAR RISK IN THE GENERAL POPULATION

SAAG SESSION 28: CUTTING-EDGE NEW BIOMARKERS

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Background and Aims: The association between thyroid stimulating hormone (TSH) and cardiovascular disease has mainly been determined using clinical categories of disease. We tested the hypothesis that TSH on a continuous scale is associated with risk of atrial fibrillation (AF), myocardial infarction (MI), stroke, heart failure (HF), aortic valve stenosis (AVS), and major adverse cardiovascular events (MACE) and whether these associations are likely to be causal.

Methods: We first tested whether plasma TSH on a continuous scale was observationally associated with incident cardiovascular events in a prospective cohort study of 105,224 individuals from the Copenhagen General Population Study followed for a median 7 years. Next, we tested whether a genetic risk score weighted on TSH was associated with cardiovascular endpoints. Finally, using Mendelian randomization, we tested whether the observed associations were likely to be causal.

Results: Using restricted cubic splines, lower concentrations of TSH relative to the population median (=1.53 mIU/L) were associated with higher risk of AF, MI, stroke, HF, AVS, and MACE. Comparing individuals with TSH ≤5th percentile (≤0.54 mIU/L) versus >50th percentile (>1.53 mIU/L), hazard ratios (HRs) ranged from 1.12(1.00-1.26) for stroke to 1.27(1.11-1.46) for HF.

Atrial Fibrillation / Myocardial Infarction

TSH groups %	(mIU/L)	N total	N events (AF)	Hazard Ratio	P	(95% CI)	N events (MI)	Hazard Ratio	P	(95% CI)
					0.01				0.01	
≤ 5%	(≤ 0.54)	5378	427			1.21 (1.09-1.34)	164			1.25 (1.05-1.48)
> 5-10%	(0.54-0.71)	5288	328			1.03 (0.91-1.16)	135			1.09 (0.91-1.32)
>10-25%	(0.71-1.04)	15847	872			0.99 (0.92-1.08)	392			1.14 (1.01-1.29)
>25-50%	(1.04-1.53)	26418	1238			0.97 (0.90-1.04)	557			1.07 (0.96-1.19)
> 50%	(> 1.53)	52293	2240			1.00 (ref)	900			1.00 (ref)

Stroke / Heart failure

TSH groups %	(mIU/L)	N total	N events (Stroke)	Hazard Ratio	P	(95% CI)	N events (HF)	Hazard Ratio	P	(95% CI)
					0.01				0.03	
≤ 5%	(≤ 0.54)	5378	352			1.12 (1.00-1.26)	260			1.27 (1.11-1.46)
> 5-10%	(0.54-0.71)	5288	312			1.11 (0.98-1.25)	185			0.97 (0.83-1.14)
>10-25%	(0.71-1.04)	15847	823			1.08 (0.99-1.17)	487			1.00 (0.89-1.11)
>25-50%	(1.04-1.53)	26418	1190			1.07 (0.99-1.15)	724			0.99 (0.90-1.08)
> 50%	(> 1.53)	52293	1982			1.00 (ref)	1259			1.00 (ref)

Aortic valve stenosis / MACE

TSH groups %	(mIU/L)	N total	N events (AVS)	Hazard Ratio	P	(95% CI)	N events (MACE)	Hazard Ratio	P	(95% CI)
					0.12				< 0.001	
≤ 5%	(≤ 0.54)	5378	111			1.23 (1.00-1.51)	673			1.19 (1.10-1.30)
> 5-10%	(0.54-0.71)	5288	80			1.00 (0.78-1.26)	562			1.09 (1.00-1.19)
>10-25%	(0.71-1.04)	15847	229			1.05 (0.90-1.23)	1492			1.06 (1.00-1.13)
>25-50%	(1.04-1.53)	26418	325			1.00 (0.87-1.15)	2241			1.06 (1.00-1.12)
> 50%	(> 1.53)	52293	567			1.00 (ref)	3707			1.00 (ref)

Axis: 0.8 1.0 1.2 1.4 1.6 (both panels)

Genetic risk estimates per standard deviation decrease in TSH were 1.28(1.08-1.52) for AF, 1.35(1.06-1.71) for MI, 1.06(0.89-1.26) for stroke, 1.19(0.94-1.52) for HF, 1.53(1.03-2.26) for AVS, and 1.09(0.97-1.23) for

MACE.

	Hazard ratio / Risk ratio (95% CI)	P	P for intercept
Atrial fibrillation			
(N=96,484 / Events=7,631)			
Weighted allele score (internally)	1.28 (1.08-1.52)	0.004	
Weighted allele score (externally)	1.28 (1.08-1.53)	0.004	
Simple allele count	1.37 (1.14-1.66)	0.001	
Inverse variance weighted	1.31 (1.08-1.57)	0.01	
Egger	1.35 (0.69-2.65)	0.38	0.92
Median weighted	1.21 (0.98-1.48)	0.07	
Myocardial infarction			
(N=96,484 / Events=4,101)			
Weighted allele score (internally)	1.35 (1.06-1.71)	0.01	
Weighted allele score (externally)	1.35 (1.06-1.71)	0.01	
Simple allele count	1.44 (1.10-1.87)	0.01	
Inverse variance weighted	1.32 (0.99-1.76)	0.06	
Egger	1.62 (0.57-4.60)	0.36	0.69
Median weighted	1.31 (0.97-1.77)	0.08	
Stroke			
(N=96,484 / Events=7,573)			
Weighted allele score (internally)	1.06 (0.89-1.26)	0.52	
Weighted allele score (externally)	1.06 (0.89-1.26)	0.52	
Simple allele count	0.98 (0.80-1.18)	0.81	
Inverse variance weighted	1.02 (0.83-1.24)	0.88	
Egger	1.82 (0.90-3.71)	0.10	0.09
Median weighted	1.02 (0.83-1.25)	0.86	
Heart failure			
(N=96,484 / Events=4,071)			
Weighted allele score (internally)	1.19 (0.94-1.52)	0.15	
Weighted allele score (externally)	1.19 (0.94-1.52)	0.15	
Simple allele count	1.25 (0.96-1.64)	0.10	
Inverse variance weighted	1.20 (0.93-1.53)	0.16	
Egger	0.69 (0.28-1.67)	0.41	0.20
Median weighted	1.20 (0.93-1.53)	0.16	
Aortic valve stenosis			
(N=96,484 / Events=1,611)			
Weighted allele score (internally)	1.53 (1.03-2.26)	0.03	
Weighted allele score (externally)	1.53 (1.03-2.26)	0.03	
Simple allele count	1.47 (0.96-2.27)	0.08	
Inverse variance weighted	1.24 (0.86-1.80)	0.25	
Egger	1.83 (0.48-6.95)	0.38	0.56
Median weighted	1.22 (0.84-1.78)	0.29	
MACE			
(N=96,484 / Events=14,537)			
Weighted allele score (internally)	1.09 (0.97-1.23)	0.14	
Weighted allele score (externally)	1.09 (0.97-1.23)	0.14	
Simple allele count	1.06 (0.93-1.21)	0.39	
Inverse variance weighted	1.07 (0.92-1.23)	0.39	
Egger	1.51 (0.90-2.54)	0.12	0.17
Median weighted	1.08 (0.93-1.25)	0.33	

0.5 1 1.5 4

Risk ratio per 1 standard deviation lower TSH (95% confidence interval)

Conclusions: In 105,224 individuals from the general population low plasma TSH was observationally and genetically associated with increased risk of AF, MI, and AVS suggesting that these observations may be causal.

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.01 Epidemiology of cardiovascular diseases and risk factors*

COMPARISON OF RISK SCORING SYSTEMS IN PATIENTS WITH FIRST DIAGNOSIS OF MYOCARDIAL INFARCTION: A RETROSPECTIVE ANALYSIS

SAAG SESSION 28: CUTTING-EDGE NEW BIOMARKERS

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Background and Aims: Guidelines recommend calculation of risk scores such as SCORE, SCORE2 and pooled ASCVD risk scores. We aimed to compare performance of different scores in patient with myocardial infarction (MI).

Methods: SCORE, SCORE2 and pooled ASCVD risk scores were calculated in a cohort of 1258 patients younger than 65 years old and hospitalized for first diagnosis of MI. SCORE and SCORE2 were calculated using published charts for Turkiye. SCORE risk score was stratified as low to moderate (<4%), high (4-9%) and very high (≥10%) risk categories. SCORE2 risk scores were stratified as low to moderate, high and very high according to age groups. The pooled ASCVD risk scores were calculated from http://www.cvriskcalculator.com/. For comparison purpose, ASCVD risk score was stratified into low to moderate risk (<7.5%), high risk (7.5-20 %) and very high risk (≥20%)
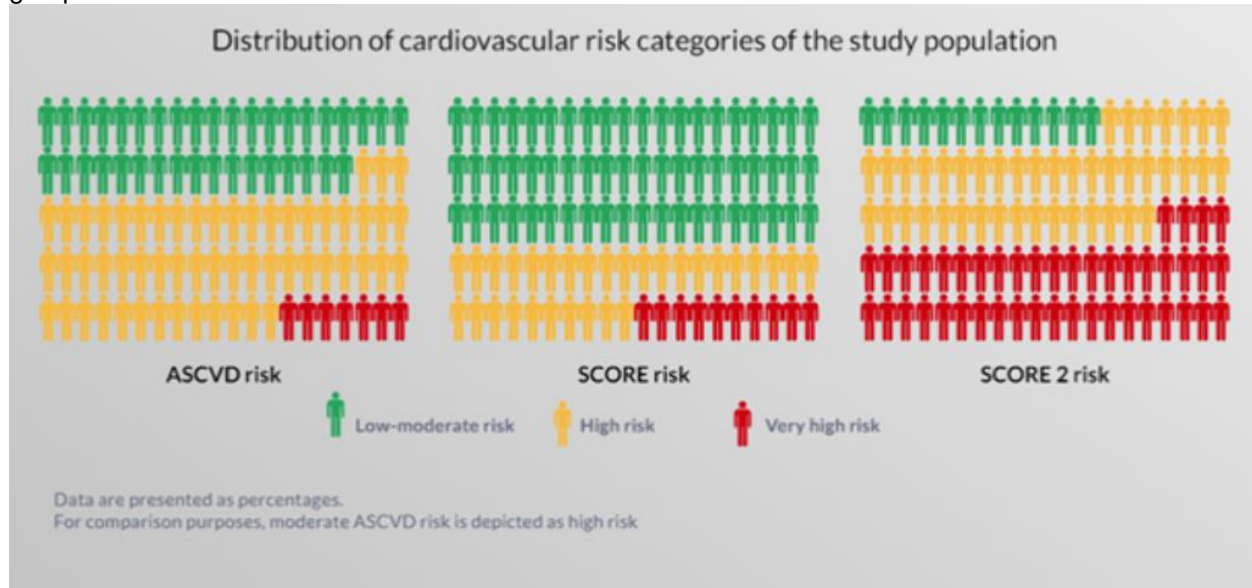
groups.

Results: SCORE 2 stratified most of the patients in very high risk and high risk groups (44% and 43 %), whereas ASCVD stratified only 7% of the patients in the high risk and 56% of the patients in the moderate risk score groups. Only 10% of the study population was stratified as very high risk using SCORE. The proportion of patients stratified as very high risk was significantly higher in SCORE2 compared to SCORE and ASCVD risk (44% vs. 10% vs. 7%, p<

0.01).

Table 1. The demographic, clinical, laboratory characteristics of the study population.

Variables	All (n=1258)	STEMI (n=401)	NSTEMI (n=857)	P value
Age, years	53.6±8.5	52.6±8.1	53.6±8.6	0.04
Male gender, n (%)	997 (79)	357 (89)	640 (74)	<0.01
Hypertension, n (%)	531 (42)	131 (32)	400 (46)	<0.01
Active Smoker, n (%)	703 (56)	311 (77)	392 (45)	<0.01
Laboratory parameters				
Total cholesterol, mg/dL	193±40	185±39	195±42	<0.01
LDL-C, mg/dL	121±33	118±32	121±33	0.08
HDL-C, mg/dL	36±9	36±8	37±9	0.71
NonHDL-C, mg/dL	156±40	149±39	158±41	<0.01
Triglyceride, mg/dL	147[106-205]	133 [98-180]	153 [111-215]	<0.01
Risk scores				
SCORE risk (mean)	4.4±3.3	4.6±3.2	4.2±3.4	0.09
Low risk (<3%), n(%)	472 (37)	132 (30)	340 (39)	<0.01
Moderate risk (3-4%), n(%)	298 (23)	100 (25)	198 (23)	0.47
High risk (5-9%), n(%)	375 (30)	131 (32)	244 (29)	0.13
Very high risk (≥10%) , n(%)	113 (10)	38 (10)	75 (9)	0.68
SCORE2 risk (mean)	8.6±4.5	8.9±4.4	8.4±4.6	0.06
Low-moderate, n(%)	156 (13)	28 (7)	128 (15)	<0.01
High, n(%)	550 (43)	176 (44)	374 (44)	0.94
Very high, n(%)	552 (44)	197 (49)	355 (41)	<0.01
ASCVD risk (mean)	10.5±6.2	10.7±5.6	10.4±6.5	0.47
Low (<5%), n(%)	275 (22)	68 (17)	207 (24)	<0.01
Borderline (5-7,5%), n(%)	188 (15)	60 (15)	128 (15)	0.99
Moderate (7.5-20%), n(%)	704 (56)	245 (61)	459 (54)	<0.01
High (≥20 %), n(%)	91 (7)	28 (7)	63 (7)	0.81

Abbreviations: ASCVD atherosclerotic cardiovascular diseases; HDL high density lipoprotein; LDL low density lipoprotein; NSTEMI non-ST segment elevation myocardial infarction; STEMI ST segment elevation myocardial infarction

* Parametric variables are depicted as mean ± standard deviation and nonparametric variables are depicted as median [25th-75th percentile]

Conclusions: In this retrospective study, SCORE2 stratified a high proportion of MI patients in the very-high and high risk groups. This finding may imply that SCORE2 risk stratification is concordant to real-life findings.

SS181 / #814

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.02 Epidemiology of dyslipidemias*

CHARACTERIZATION OF A CONTEMPORARY COHORT AT HIGH AND VERY HIGH CARDIOVASCULAR RISK: THE PORTRAIT-DYS STUDY

SAAG SESSION 28: CUTTING-EDGE NEW BIOMARKERS

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Background and Aims: This study aims to characterize sociodemographic and clinical characteristics, lipid-lowering therapies (LLT) use, and LDL-C control in a population with increased cardiovascular (CV) risk.

Methods: Cross-sectional observational study using electronic health records of patients followed in 1 hospital and 14 primary care health centers in Portugal between 1/1/2000 and 31/12/2020 (index date). Patients presented at least (i) one year clinical data before inclusion, (ii) one primary care appointment 3 years before index date, and (iii) sufficient data for CV risk classification. Patients were considered as primary prevention (PP) and secondary prevention (SP) groups according to the presence of atherosclerotic cardiovascular disease (ASCVD). CV risk and LDL-C control defined by 2019 ESC/EAS dyslipidemia guidelines. Results were summarized using frequencies and non-parametric statistics.

Results: A total of 51,609 patients were included, with 23,457 classified as high CV risk and 28,152 as very high CV risk. According to prevention level, 43,321 patients were identified in PP and 8288 in SP (Table 1). Patients without any LLT ranged from 30.6% in PP to 15.2% in SP (Figure 1 depicts proportional change in LLT before and after ASCVD diagnosis). Low rates of LDL-C control were observed both according to the type of prevention (5.1% and 7.1% for PP and SP groups, respectively) or risk level (6.6% high and 4.5% very-high risk).

	Primary Prevention		Secondary Prevention	
Eligible Population (n, %)	43,321	31.6	8,288	6.1
Female (n, %)	22,752	52.5	3978	48.0
Age, years (P50, IQR)	70.0	18.0	74.0	20.0
Waist, cm (P50, IQR)	100.0	16.0	100.0	16.0
Body Mass Index, kg/m2 (P50, IQR)	27.4	6.3	27.1	5.9
LDL-C control, ESC 19 (n, %)	2,216	5.1	590	7.1
LDL-C, mg/dL (P50, IQR)	114.0	49.4	97.0	48.0
LDL-C, mmol/dL (P50, IQR)	3.0	1.3	2.5	1.2
Smoking status	**N**	**%**	**N**	**%**
Never	32,957	76.1	6210	74.9
Current	6071	14.0	954	11.5
Former	2749	6.4	696	8.4
General Comorbidities	**N**	**%**	**N**	**%**
Obesity	12,664	29.2	2096	25.3
Hypercholesterolemia	21,029	48.5	2321	28.0
Type 2 Diabetes	26,645	61.5	5295	63.9
Structural Heart Disease	7578	17.5	5257	63.4
Microvascular Disease	2911	6.7	1265	15.3
Familial Hypercholesterolemia	810	1.9	209	2.5
Cardiovascular comorbidities	**N**	**%**	**N**	**%**
Hypertension	32,703	75.5	6717	81.0
Atrial fibrillation	3019	7.0	1805	21.8
Chronic kidney disease	7430	17.2	2352	28.4
Atherosclerotic Cardiovascular Disease	0	0.0	8288	100.0
Unstable angina	0	0.0	422	5.1
Myocardial infarction	0	0.0	2548	30.7
Stroke	222	0.5	5466	66.0
Peripheral artery disease	0	0.0	1096	13.2
Lipid lowering medications	**N**	**%**	**N**	**%**
Any LLT usage	30,078	69.4	7027	84.8
Statin usage	29,289	67.6	6967	84.1
Low intensity	2451	5.7	433	5.2
Moderate intensity	24,622	56.8	5499	66.4
High intensity	2216	5.1	1035	12.5
Statin + ezetimibe usage	1542	3.6	494	6.0
Low intensity + ezetimibe	86	0.2	11	0.1
Moderate intensity + ezetimibe	1101	2.5	494	6.0
High intensity + ezetimibe	355	0.8	205	2.5
Ezetimibe (monotherapy) usage	34	0.1	0	0.00
PCSK9 inhibitors usage	0	0.0	1	0.00
Other LLT usage	122	0.3	38	0.5
Cardiovascular medications	**N**	**%**	**N**	**%**
Renin-angiotensin-system-acting agents	27,472	63.4	6604	79.7
Diuretics	14,726	34.00	4391	53.0
Aldosterone antagonists	1876	4.3	849	10.2
Anticoagulants	3954	9.1	1981	23.9
Diabetes medications	**N**	**%**	**N**	**%**
Glucose lowering drugs	16,304	37.6	3248	39.2
Insulins	2830	6.5	976	11.8

ESC - European Society of Cardiology; IQR - Interquartile range; LLT - Lipid Lowering Therapy; LDL-C - Low-density lipoprotein cholesterol; PCSK9 - Proprotein convertase subtilisin/kexin type 9

Lipid-Lowering Therapy before and after entering secondary prevention
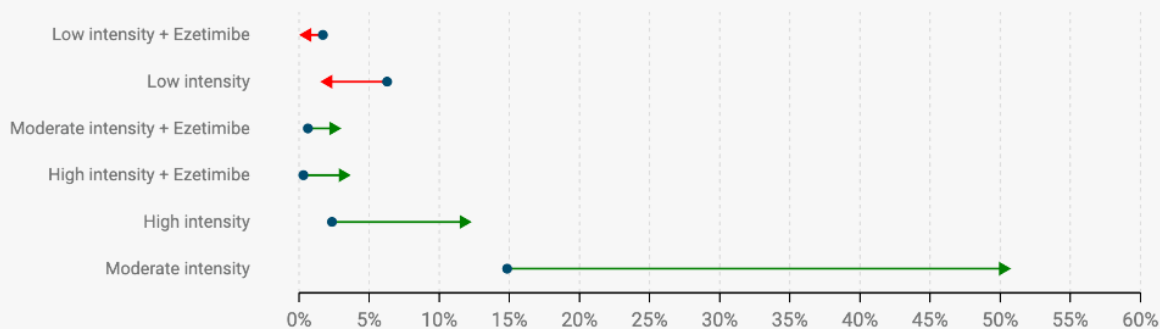
Conclusions: A worrisome gap between guidelines on dyslipidemia management and clinical implementation persists even in those at very high-risk or with established ASCVD.

SS182 / #59

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.08 Novel risk factors and biomarkers*

SYSTEMIC OXIDATIVE STRESS AND CIRCULATING LONG NON-CODING RNAS AS POTENTIAL NOVEL CARDIOVASCULAR RISK FACTORS IN THE GENERAL POPULATION

SAAG SESSION 28: CUTTING-EDGE NEW BIOMARKERS

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Background and Aims: A significant proportion of the general population escape CVR lowering approaches basing on the currently used CVR estimators, which mostly rely on traditional CVR factors. We studied systemic oxidative stress and circulating long non-coding RNAs (lncRNAs) as potential novel CVR factors.

Methods: Systemic oxidative stress was assessed by OxyScore and AntioxyScore in 896 adults (17-65 years old) considering <30 years old as controls. Standardized values of carbonyl groups, 8-hydroxy-2'-deoxyguanosine, and oxidized LDL were included in OxyScore, and total antioxidant capacity, catalase activity, and superoxide dismutase activity in AntioxyScore. lncRNAs CoroMarker, KCNQOT1, UCA1, LeXis, MALAT-1, MIAT and Wisper were measured in plasma of a subset of 142 patients. CVR was determined by QRisk-lifetime.

Results: OxyScore and AntioxyScore were associated with CVR independently of sex and age ($p<0.05$), traditional CVR factors (smoking, SBP, cholesterol, LDL, blood glucose, BMI, eGFR, family history; $p<0.01$), and antihypertensive ($p<0.001$) and statin ($p<0.01$) treatments. Circulating KCNQ1OT1 correlated with age and blood glucose ($p<0.05$ and $p<0.01$), UCA1 with LDL ($p<0.05$), and CoroMarker, UCA1, and LeXis with cholesterol ($p<0.05$). Interestingly, Wisper was associated with OxyScore ($p<0.01$) independently of traditional CVR factors.

Conclusions: The association between systemic oxidative stress and CVR suggests that integrating multimarker scores such as OxyScore and AntioxyScore into CVR estimators might improve CVR assessment. Moreover, we show for the first time the associations between a panel of lncRNAs and traditional CVR factors, pointing to their potential as clinical biomarkers. Finally, we describe Wisper as a novel lncRNA associated with oxidative stress, and which specific role deserves further investigation.

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.14 Epigenetics and microRNA*

DIFFERENTIALLY METHYLATED DNA LOCI BETWEEN EASTERN AND WESTERN FINNS ASSOCIATE WITH CHD RISK FACTORS

SAAG SESSION 28: CUTTING-EDGE NEW BIOMARKERS

Joanna Ciantar[1], Saara Marttila[1], Sonja Rajić[1], Pashupati Mishra[1], Terho Lehtimäki[1], Olli Raitakari[2], Emma Raitoharju[1]
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Background and Aims: The coronary heart disease (CHD) mortality rate is unequally distributed in Finland, with a higher risk in the East than in the West. Although differences in genetics and lifestyle choices partly explain the increased risk of CHD in Eastern Finns, the molecular mechanisms mediating the discrepancy between the sub-populations are still largely unknown. This study aims to investigate whether there is a difference in DNA methylation levels between Eastern and Western Finns and whether this might mediate the discrepancy in CHD risk.

Methods: 'The Cardiovascular Risk in Young Finns Study' (n=1529), a longitudinal population cohort which includes genome-wide DNA methylation and cardiometabolic data, was utilized for this study. An Epigenome-Wide Association Study (EWAS) was performed on individuals originating from East and West Finland, followed by linear regression to determine association with cardiometabolic phenotypes.

Results: The EWAS identified 82 differentially methylated CpG sites (FDR <0.05) between Eastern and Western Finns. Of these, 21 CpG sites had a difference in median methylation levels of ≥2.5% and 10 of these 21 sites are regulated by genetic variation according to the GoDMC database. Linear regression analysis revealed an association between the methylation levels at some non-genetically regulated CpG sites with triglyceride and HDL cholesterol levels as well as blood pressure.

Conclusions: The difference in DNA methylation levels between East and West Finns at certain CpG locations is associated with risk factors for CHD. These results indicate that DNA methylation may play a role in the increased rate of CHD mortality in Eastern Finns.

SS184 / #1454

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.08 Novel risk factors and biomarkers*

GENETIC RISK SCORE FOR HUMAN SERUM LIPIDOME AND ITS ASSOCIATION WITH ANGIOGRAPHIC CORONARY ARTERY DISEASE

SAAG SESSION 28: CUTTING-EDGE NEW BIOMARKERS

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Background and Aims: The modest added predictive value of the existing genetic risk scores (GRSs) over the traditional risk factors for cardiovascular disease (CVD) could be partly due to missing genetic components in the current GRSs. We aimed to test association of GRS for human serum lipidome with coronary artery disease (CAD).

Methods: We calculated GRS for human serum lipidome (GRS$_{Lipidome}$) using genetic data from the Ludwigshafen Risk and Cardiovascular Health (LURIC) study participants. The GRS was constructed using genetic summary data from the most comprehensive genome-wide association study of human serum lipidome to date. We then investigated the association of GRSs with CAD in the LURIC study participants. The association test of the GRS$_{Lipidome}$ with CAD was performed using logistic regression adjusted for the risk factors used in Framingham risk score (FRS), that are age, sex, systolic blood pressure, total cholesterol, HDL cholesterol, smoking habit and medication for hypertension. In addition to the association study of the GRS with CAD, we also assessed added predictive value of the GRS$_{Lipidome}$ on the top of the FRS related risk factors.

Results: The GRS$_{Lipidome}$ was associated with CAD risk in the LURIC participants with p-value of 0.01. However, there was no statistically significant added predictive value of the GRS$_{Lipidome}$ over the used traditional FRS related risk factors

Conclusions: This study showed that GRS$_{Lipidome}$ is a new risk factor for CAD in an European cohort, however with no added predictive value over the traditional risk factors used in FRS.

SS185 / #25

Topic: *AS04 Clinical Vascular Disease / AS04.12 Prevention and treatment of cardiovasculardisease; miscellaneous*

EFFICACY AND SAFETY OF CATHETER ABLATION VERSUS ANTI-ARRHYTHMIC DRUGS FOR THE TREATMENT OF PATIENTS WITH ATRIAL FIBRILLATION; A SYSTEMATIC REVIEW AND META-ANALYSIS OF 194,288 PATIENTS

SAAG SESSION 29: NEW TREATMENTS FOR CVD

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Background and Aims: Background Atrial fibrillation (AF) is the most prevalent cardiac arrhythmia worldwide. In individuals with AF, rhythm control can be achieved via anti-arrhythmic drugs (AAD) or catheter ablation (CA). However, there is a shortage of data comparing the efficacy and safety of AAD and CA. Therefore, we conducted this systematic review and meta-analysis to compare the efficacy and safety of catheter ablation versus anti-arrhythmic drugs for the treatment of patients with atrial fibrillation.

Methods: Methods We conducted a PRISMA-compliant systematic review and meta-analysis. We ran an electronic search of PubMed, Scopus, Web of Science, and Cochrane CENTRAL to identify relevant published studies. Data were extracted and analyzed using the Review Manager software (version 3.5 for Windows).

Results: Results Twenty-one studies (n=194,288 patients) were included in this meta-analysis. CA was superior to AAD in terms of AF recurrence (RR 0.54 [0.44, 0.67], P>0.00001), hospitalization for CVS reasons (RR 0.65 [0.46, 0.91], P=0.01), therapeutic crossover (RR 0.24 [0.10, 0.55], P=0.0007), and the need for further intervention (RR 0.27 [0.14, 0.54], P=0.0002). On the other hand, AAD was associated with less cardiac tamponade risk than CA (RR 6.67 [1.51, 29.40], P=0.01). There were no differences between the two groups regarding complications, cardioversion, pulmonary vein stenosis, stroke/TIA, all-cause death, or death from CVD (all P>0.05).

Identification of studies via databases and registers

Identification

Records identified from: PubMed (n = 3414), Scopus (n = 2406), WOS (n = 553), and CENTRAL (n = 432), All Databases (n = 6805)

Records removed *before screening*:
Duplicate records removed (n = 1284)
Records marked as ineligible by automation tools (n = 0)
Records removed for other reasons (n = 0)

Screening

Records screened (n = 5521)

Records excluded (n = 5290)

Reports sought for retrieval (n = 231)

Reports not retrieved (n = 0)

Reports assessed for eligibility (n = 231)

Reports excluded: 210
Reviews (n = 13)
Protocol (n = 32)
Not meeting our inclusion criteria (n = 165)

Included

Studies included in review (n = 21)
Reports of included studies (n = 21)

Conclusions: Conclusion CA is more effective than AAD for AF patients; however, it is associated with a higher risk of cardiac tamponade. Therefore, more precise patient selection for CA is recommended.

SS186 / #1172

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.17 Other*

COST-EFFECTIVENESS OF ICOSAPENT ETHYL FOR PREVENTING CARDIOVASCULAR EVENTS

SAAG SESSION 29: NEW TREATMENTS FOR CVD

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Background and Aims: Patients with cardiovascular (CV) disease and patients with diabetes mellitus and one or more CV risk factors remain at high risk for major adverse CV events due to atherogenic triglyceride-rich lipoproteins, even after treatment with statins. The REDUCE-IT study was a multi-center, randomized, double-blind, placebo-controlled, event-driven study which showed that among statin-treated patients with cardiovascular disease or diabetes mellitus and elevated triglycerides, the intake of icosapent ethyl reduces the number of first, subsequent, and total ischemic events by about 25 percent. Our aim is to analyze the cost-effectiveness of icosapent ethyl for a Central European population.

Methods: For this purpose, we make use of a Markov cohort state transition model, based on time-to-event data from the Ludwigshafen Risk and Cardiovascular Health Study (LURIC) study which has included patients at intermediate to high risk of CV events. We restricted our analysis to patients a) with stable atherosclerotic vascular disease using statins at baseline and b) patients meeting the essential inclusion criteria of the REDUCE-IT study.

Results: At an assumed relative risk reduction of 25 percent and an annual drug price of 3,250 Euros, QALYs gained of 0.95 and 0.97 in men and women fulfilling inclusion criteria in close analogy to the inclusion criteria of REDUCE-IT, respectively, savings through treatment were 1,650 and 1,590 Euros, and ICERs were 44,550 and 47,950 Euros in men and women, respectively.

Conclusions: Icosapent ethyl is cost-effective for reducing CVE in eligible patients on the foil of the willingness to pay in developed healthcare systems.

Topic: *AS04 Clinical Vascular Disease / AS04.09 Lipid-lowering therapies*

EFFECT OF ALLOPURINOL ON CAROTID INTIMA-MEDIA THICKNESS; A SYSTEMATIC REVIEW AND META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS

SAAG SESSION 29: NEW TREATMENTS FOR CVD

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Background and Aims: Background Increased carotid intima-media thickness (CIMT) is an early indicator of atherosclerosis and a well-established risk factor for CVD. Previous studies showed the possible effect of lowering serum uric acid on reducing atherosclerotic changes and cardiovascular events. Therefore, this systematic review and meta-analysis aimed to investigate the effect of the uric acid-lowering agent (Allopurinol) on the reduction of carotid intima-media thickness.

Methods: Methods We conducted a PRISMA-compliant systematic review and meta-analysis. We ran an electronic search of PubMed, Scopus, Web of Science, and Cochrane CENTRAL to identify relevant published randomized clinical trials. We assessed the quality of included studies using the RoB-Cochrane tool. Data were extracted and analyzed using the Review Manager software (version 3.5 for Windows).

Results: Three studies (n=295) were included in this meta-analysis. Allopurinol (n=151) was superior to the control group (n=144) in terms of the mean change of CIMT (MD -0.04 [-0.06, -0.02], P<0.0001) at the end of the follow-up period which was at least three months

duration.

Identification of studies via databases and registers

Identification

Records identified from: PubMed (n = 329), Scopus (n = 25), WOS (n = 22), and CENTRAL (n = 11), All Databases (n = 387)

Records removed *before screening*:
Duplicate records removed (n = 28)
Records marked as ineligible by automation tools (n = 0)
Records removed for other reasons (n = 0)

Screening

Records screened (n = 359)

Records excluded (n = 335)

Reports sought for retrieval (n = 24)

Reports not retrieved (n = 0)

Reports assessed for eligibility (n = 24)

Reports excluded: 21
Conference abstract (n = 2)
Protocol (n = 6)
Not meeting our inclusion criteria (n = 13)

Included

Studies included in review (n = 3)
Reports of included studies (n = 3)

Summary of studies

Study ID	Country	Duration	Total sample size	Intervention	comparator	population
Andrews2018	USA	started 4/1/2011	n=63	Allopurinol (300mg daily)	placebo	Stage 3 CKD, asymptomatic hyperuricemia
Higgins2014	Scotland	Started 2012	n=80	Allopurinol (300mg daily)	placebo	Recent ischaemic stroke, TIA
Liu2015	China	between October 2009 and 2012	n=152	Allopurinol	conventional	T2DM, asymptomatic HUA

Risk of bias graph:
- Random sequence generation (selection bias)
- Allocation concealment (selection bias)
- Blinding of participants and personnel (performance bias)
- Blinding of outcome assessment (detection bias)
- Incomplete outcome data (attrition bias)
- Selective reporting (reporting bias)
- Other bias

Legend: Low risk of bias | Unclear risk of bias | High risk of bias

Study or Subgroup	Mean Difference	SE	Weight	Mean Difference IV, Random, 95% CI
Andrews 2018	-0.02	0.0204	18.4%	-0.02 [-0.06, 0.02]
Higgins,2014	-0.02	0.0379	6.2%	-0.02 [-0.09, 0.05]
liu2015	-0.05	0.0049	75.5%	-0.05 [-0.06, -0.04]
Total (95% CI)			100.0%	-0.04 [-0.06, -0.02]

Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 2.60$, df = 2 (P = 0.27); $I^2 = 23\%$
Test for overall effect: Z = 4.37 (P < 0.0001)

Favours [Allopurinol]   Favours [control]

Conclusions: We found uric-acid lowering treatment with allopurinol was effective in slowing the progression or even reduction of CIMT which directly indicates less atherosclerotic changes and leads to a reduction of cardiovascular diseases.

SS188 / #740

Topic: *AS04 Clinical Vascular Disease / AS04.10 Anti-thrombotic therapies*

INTERACTION BETWEEN CLOPIDOGREL AND CALCIUM CHANNEL BLOCKER: REALITY OR MYTH?

SAAG SESSION 29: NEW TREATMENTS FOR CVD

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Background and Aims: There have been debates about whether calcium-channel blocker (CCB) reduces the antiplatelet effect of clopidogrel. We aimed to verify the interaction between clopidogrel and CCB in patients who underwent percutaneous coronary intervention (PCI).

Methods: The primary endpoint was the major adverse cardiac and cerebrovascular events (MACCE) including all-cause death, myocardial infarction, stent thrombosis, or stroke. The safety endpoint was the major bleeding, defined as Bleeding Academic Research Consortium type 3-5.

Results: Between 2003 and 2018, a total of 11,714 patients were enrolled and grouped into two groups according to the use of CCB. Baseline characteristics are summarized in Table 1. There were no significant differences in PRU between the CCB group and the non-CCB group (218.4 ± 76.7 vs. 215.8 ± 84.7, p=0.156). The two groups showed no significant differences in the MACCE or major bleeding during five years of follow-up (Figure 1). The administration of CCB did not predict the MACCE in the Cox regression analysis. *(HR 0.909, 95% CI 0.750~1.101,Table*

Table 1. Baseline characteristics

	CCB (N=8897)	Non-CCB (N=2817)	p-value
Age	64.1 ± 11.0	65.1 ± 10.5	<0.001
Male Sex(%)	6110 (68.7%)	1841 (65.4%)	0.001
BMI	24.4 ± 3.1	24.8 ± 3.2	<0.001
Index presentation			<0.001
Stable angina	3611 (40.6%)	1299 (46.1%)	
Unstable angina	2455 (27.6%)	1011 (35.9%)	
Non-ST-segment elevation MI	1543 (17.3%)	317 (11.3%)	
ST-segment elevation MI	1288 (14.5%)	190 (6.7%)	
Predisposing factors			
Hypertension	5028 (56.5%)	2021 (71.7%)	<0.001
Diabetes mellitus	3001 (33.7%)	1056 (37.5%)	<0.001
Dyslipidemia	5684 (63.9%)	1871 (66.4%)	0.015
Chronic kidney disease	1680 (18.9%)	752 (26.7%)	<0.001
Congestive heart disease	685 (7.7%)	195 (6.9%)	0.186
Peripheral artery disease	1105 (12.4%)	348 (12.4%)	0.952
Myocardial infarction	607 (6.8%)	232 (8.2%)	0.013
Previous PCI	1052 (11.8%)	516 (18.3%)	<0.001
Previous CABG	99 (1.1%)	51 (1.8%)	0.006
CVA	569 (6.4%)	244 (8.7%)	<0.001
Laboratory factors			
LV ejection fraction(%)	58.1 ± 10.7	60.8 ± 9.9	<0.001
Hemoglobin(g/dL)	13.6 ± 1.8	13.4 ± 1.9	<0.001
eGFR(mL/min/1.73 m2(MDRD))	80.1 ± 27.0	74.3 ± 26.8	<0.001
Total Cholesterol(mg/dL)	175.9 ± 45.0	168.3 ± 42.5	<0.001
HbA1C(%)	6.6 ± 1.4	6.6 ± 1.4	0.790
P2Y12 reaction units (PRU)	218.4 ± 76.7	215.8 ± 84.7	0.156
High platelet activity(>208PRU)	4979 (56.0%)	1568 (55.7%)	0.796
Angiographic features			
ACC/AHA lesion			<0.001
A/B1 type	3762 (42.3%)	1476 (52.4%)	
B2/C type	5135 (57.7%)	1341 (47.6%)	
Multi-vessel disease	3475 (39.1%)	1056 (37.5%j)	0.141
Concomitant medication			
Aspirin	8614 (96.8%)	2795 (99.2%)	<0.001
Clopidogrel	8897 (100.0%)	2817 (100.0%)	1.000
Cilostazol	931 (10.5%)	288 (10.2%)	0.742
RAS inhibitor	5244 (58.9%)	1683 (59.7%)	0.463
Beta-blocker	5140 (57.8%)	1529 (54.3%)	<0.001
Statin	7874 (88.5%)	2505 (88.9%)	0.561

2.)

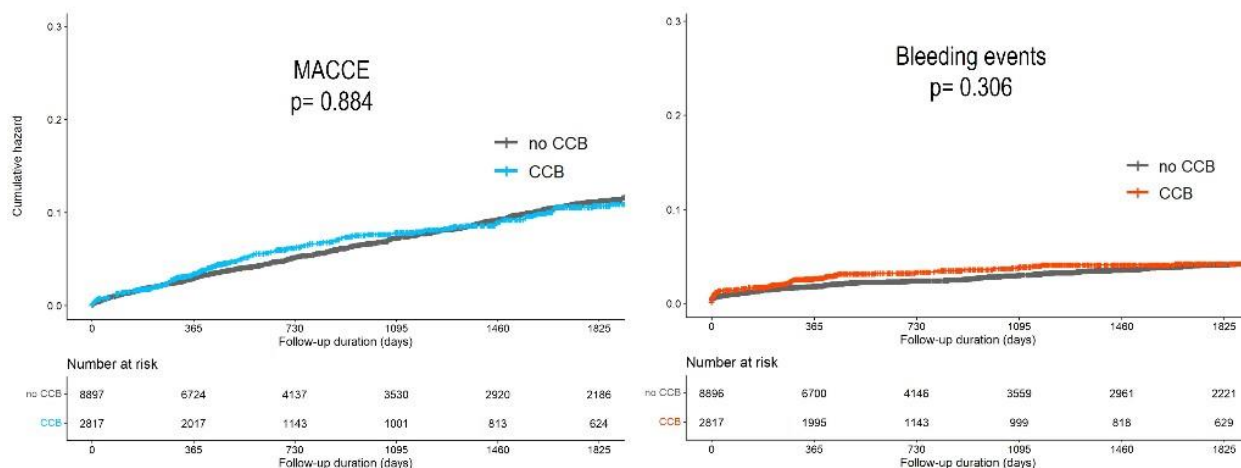*Figure 1. Correlation between the use of CCB and clinical outcomes(MACCE, bleeding events)*

Table2. Multivariable Cox Regression For Clinical Outcome

	Multivariable analysis	
	HR (95% CI)	P-value
Age	1.030 (1.022-1.039)	<0.001
BMI (kg/m²)	0.933 (0.908-0.960)	<0.001
Hypertension	1.296 (1.082-1.552)	0.005
Diabetes mellitus	1.083 (0.919-1.276)	0.342
Chronic kidney disease	0.960 (0.744-1.238)	0.751
Congestive heart disease	0.997 (0.765-1.298)	0.980
Myocardial infarction	1.024 (0.785-1.336)	0.862
LV ejection fraction(%)	0.973 (0.996-0.980)	<0.001
Hemoglobin(g/dL)	0.958 (0.914-1.005)	0.078
eGFR(mL/min/1.73 m2(MDRD))	0.991 (0.986-0.995)	<0.001
Total Cholesterol(mg/dL)	0.999 (0.997-1.001)	0.184
P2Y12 reaction units (PRU)	1.001 (1.000-1.002)	0.018
Aspirin	0.559 (0.366 (0.853)	0.007
Cilostazol	1.243 (0.956-1.616)	0.104
RAS inhibitor	1.316 (1.099-1.575)	0.003
Beta-blocker	0.937 (0.793-1.107)	0.445
Statin	1.542 (1.188-2.001)	0.001
Calcium-channel blocker	0.909 (0.750-1.101)	0.330

Conclusions: Coadministration of CCB did not diminish the antiplatelet effect of clopidogrel in patients undergoing PCI.

SS189 / #1011

Topic: *AS04 Clinical Vascular Disease / AS04.11 Anti-inflammatory therapies*

DEFICIENCY OF OLFACTORY RECEPTOR 2 WORSENS CARDIAC FUNCTION AND INCREASES INFARCT SIZE AFTER MYOCARDIAL INFARCTION

SAAG SESSION 29: NEW TREATMENTS FOR CVD

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Background and Aims: Myocardial infarction (MI) leads to inflammation and scar formation, which is in part modulated by macrophages. Their activation is classically driven by pattern recognition receptors. Recent evidence implied that Olfactory receptor (Olfr) 2 regulates macrophage activation in the context of vascular inflammation and atherosclerosis. This study investigates whether cardiac macrophages express Olfr2 and whether the receptor affects their differentiation and function following MI.

Methods: To test for the functional relevance of Olfr2, we induced MI by ischemia reperfusion (I/R) in wild-type (WT) and Olfr2-deficient (Olfr2$^{-/-}$) mice and performed echocardiography and histological analysis.

Results: Cardiac tissue sections showed Olfr2 colocalization with cardiac macrophages as detected by immunofluorescence. We further performed high-parametric flow cytometry and corroborate Olfr2 expression in cardiac macrophages day7 post MI. To understand the therapeutic potential of Olfr2 modulation post-MI, WT and Olfr2$^{-/-}$ mice underwent I/R surgery followed by histological and echocardiographic analysis. Contrary to our hypothesis, we observed significantly increased scar formation determined by Phalloidin and Wheat Germ Agglutinin staining in Olfr2$^{-/-}$ mice compared to WT on day 7 post MI (WT: 11.5±2.9%; Olfr2$^{-/-}$:18.4±3.2%; p-value: 0.029). Echocardiographic analysis showed significantly reduced ejection fraction in Olfr2$^{-/-}$ mice compared to WT day 7 post MI (WT: 52.16±7,6%; Olfr2$^{-/-}$:37.75±6.9%; p-value: 0.009). The decreased ejection fraction was accompanied by a decrease in cardiac output as a sign of cardiac apex aneurysm formation (WT: 14.92±1,0%; Olfr2$^{-/-}$:12.02±2.0%; p-value: 0.017).

Conclusions: Our data suggest a protective role for Olfr2 in tissue repair after myocardial infarction. Olfr2 might be a novel therapeutic target to control the inflammatory response in MI.

SS190 / #1341

Topic: *AS04 Clinical Vascular Disease / AS04.12 Prevention and treatment of cardiovasculardisease; miscellaneous*

ENZYMATIC RELEASE OF ENDOGENOUS VASCULAR CALCIFICATION INHIBITOR: CALCIFICATION BLOCKING FACTOR

SAAG SESSION 29: NEW TREATMENTS FOR CVD

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Background and Aims: Adrenal glands synthesize and secrete an array of well-known compounds like glucocorticoids, amine peptides and mineralocorticoids which influence cardiovascular and cardiorenal physiology and pathophysiology. In the current study, we investigated the systemic regulation of vascular calcification processes by adrenal glands and the enzymatic release of a recently identified inhibitor of vascular calcification from its parent protein chromogranin A.

Methods: Calcification blocking factor (CBF) was identified in bovine adrenal gland homogenate using sequential chromatographic fractionation. The fractions from each chromatographic step were assessed *in vitro* in aortic smooth muscle cells, *ex vivo* in aortic rings and *in vivo* in vitamin D3 plus nicotine renal failure rat model (VDN) for effects on calcification.

Results: The 19 amino acid peptide 'CBF', with a strong effect on reduction of vascular calcification, was identified using mass spectrometry and pertinent databases. The incubation of elongated CBF peptides with enzymes calpain 1 and kallikrein demonstrated that CBF is cleaved from the parent protein chromogranin A. End stage renal disease patients have significantly low levels of CBF as compared to healthy controls. Disturbed activities of the responsible enzymes in CKD patients might be a mechanism by which CBF plasma levels are altered. These findings offer new options to treat or prevent vascular calcification.

Conclusions: In conclusion, we identified a novel 19 aa peptide that modulates cardiovascular calcification. In addition, we identified the enzymes responsible for release of this peptide from its parent protein. These findings suggest a novel function of adrenal glands in vascular calcification.

SS191 / #39

LONG-TERM RESULTS OF PERCUTANEOUS CORONARY INTERVENTIONS IN LESIONS OF THE LEFT CORONARY ARTERY TRUNK.

SAAG SESSION 29: NEW TREATMENTS FOR CVD

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Background and Aims: To assess the long-term clinical and angiographic results of stenting the trunk of the left coronary artery.

Methods: Materials and methods. From 2010 to 2020 we performed 318 stentings of the trunk of the left coronary artery (LCA) in patients with coronary artery disease. In most cases, the patients were male 75.2% (239), the mean age was 62.5±8.28 years. Dyslipidemia was noted in 81.8%, arterial hypertension in 84.1%, diabetes mellitus in 31%, 67% of patients were smokers. In 3 patients, a history of CABG was performed.

Results: In 92.3% of cases, direct stenting of the LCA trunk was performed. When stenting the LCA trunk in 100% of cases, drug-eluting stents were used. The mean pressure of stent implantation was 13.9±1.6 atm, implantation time was 17.5±0.6 s. The average diameter of the implanted stent was 3.7±0.34 mm with an average length of 24.3±9.1 mm. Long-term results of percutaneous interventions in patients with lesions of the LCA trunk were followed up from 3 months to 4.8 years. After LCA trunk stenting, repeated coronary angiography was performed in 25% (81). At control coronary angiography, the rate of restenosis was 15.2% (48). In 2.5% (8) of cases, repeated interventions were performed for restenosis of the LCA trunk, 16% (51) of patients were recommended CABG. Long-term mortality after LCA stenting was 14% (46).

Conclusions: Stenting is a highly effective and fairly safe method of treating patients with lesions of the LCA trunk. To improve the long-term results of LCA stenting, it is necessary to use intravascular imaging techniques.

SS192 / #419

Topic: *AS02 Lipids and Lipoproteins / AS02.14 Other*

LIPIDS AND RAS BLOCKERS PLUS CCB COMBINATIONS. ALTERNATIONS IN PREDIABETIC HYPERTENSIVE PATIENTS, A RANDOMIZED 12-WEEK OPEN-LABEL COMPARATIVE STUDY

SAAG SESSION 30: THE CHANGING LANDSCAPE OF TYPE 2 DIABETES TREATMENT

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Background and Aims: Hypertensive patients often suffer from other metabolism related comorbidities characterized by insulin resistance and dyslipidemia (e.g., diabetes mellitus, metabolic syndrome). Dual combination treatment is advised with current treatment guidelines for the management of Hypertension. ARB and ACE-i are considered to have a beneficial effect on lipids while CCB exert a neutral effect. We present comparative data of the effect of delapril/manidipine versus telmisartan/amlodipine versus valsartan/amlodipine combinations, on lipid parameters after a 12-week treatment, in hypertensive prediabetic patients.

Methods: Data were collected from 158 patients from the outpatient clinic for patients with lipid disorders, hypertension and diabetes of our hospital, during the period 2014-2018. A total of 53 patients were randomized in the delapril/manidipine (DEL/MANI) 30/10mg/day, 51 patients were randomized in telmisartan/amlodipine (TEL/AMLO) 80/5mg/day and 54 patients in valsartan/amlodipine (VAL/AMLO) 160/5mg/day. Baseline characteristics are presented in Table

1A.

Table 1A. *Patients' characteristics at the start of the study*

Characteristics/Combination of drugs	DEL/MANI	TEL/AMLO	VAL/AMLO
N (Men-Women)	53(30-23)	51(35-16)	54(33-21)
Age (Years)	58.08 ±11.73	58.04 ±13. 6	64.44 ±11.64
Smokers (%)	12(22.6%)	13(25.4%)	14(25.9%)
Alcohol Consumers (%)	7(13.2%)	16(31.4%)	8(14.8%)
Body Weight (Kg)	83.67± 13.21	84.72± 12.87	80.83±11.80
Height (m)	1.68[1.62-1.77]	1.72[1.6-1.77]	1.69[1.62-1.75]
BMI (Kg/m²)	28.73 [27.73-30.3]	29.32 [27.37-31.65]	28.09[26.81-29.89]
SBP (mmHg)	156 [151-161]	163 [158-168]	162 [159.25-165]
DBP (mmHg)	100[88-101]	100[95-106]	100[92-103.75]

Results: Table 2A shows the resulting alternations of lipid parameters before and after treatment. LDL-C and TC levels reduced significantly only in the DEL/MANI group (p-value=0.006, reduction rate of -6.7% and p-value=0.009, reduction rate of -4.01%, respectively). No significant difference was observed for TEL/AMLO and VAL/AMLO treatment group. Non-HDL-C was also reduced in DEL/MANI treatment group (p-value=0.008, reduction rate of -5.4%). Comparison between groups in the change of lipid parameters did not show any significant difference from baseline to the end of

treatment.

Table 2A. *alternations of lipid parameters before and after treatment*

Parameter	baseline	After 12 weeks of treatment	% change	p-value
TC (mg/dL)				
DEL/MANI	205.79± 41.06	197.53± 41.6	-4.01 % (-8.26)	0.009
TEL/AMLO	196.43± 44.88	196.65± 50.11	0.11 % (0.22)	0.963
VAL/AMLO	195.2± 37	192.28± 44.23	-1.5 % (-2.92)	0.456
HDL-C (mg/dL)				
DEL/MANI	52.13± 13.72	52.17± 12.35	0.08 % (0.04)	0.962
TEL/AMLO	49.37± 10.91	48.37± 9.85	-2.03 % (-1)	0.33
VAL/AMLO	51.8± 10.39	52.37± 10.87	1.1 % (0.57)	0.48
LDL-C (mg/dL)				
DEL/MANI	126.88± 36.76	118.38± 38.91	-6.7 % (-8.5)	0.006
TEL/AMLO	121.56± 40.04	121.84± 44.29	0.23 % (0.28)	0.945
VAL/AMLO	119.92± 33.01	115.97± 39.83	-3.29 % (-3.95)	0.194
ApoA-I (mg/dL)				
DEL/MANI	146.49± 29.52	147.65± 28.42	0.79 % (1.16)	0.635
TEL/AMLO	144.83± 21.9	144.51± 21.49	-0.22 % (-0.32)	0.868
VAL/AMLO	148.87± 20.22	151.15± 24.29	1.53 % (2.28)	0.369
ApoB (mg/dL)				
DEL/MANI	84.1[68.8-108]	84.81± 23.86	0.84 % (0.71)	0.042
TEL/AMLO	103[77.3-114]	98.45± 26.8	-4.42 % (-4.55)	0.548
VAL/AMLO	89.25[73.28-103.6]	87.61± 20.19	-1.84 % (-1.64)	0.617
ApoE (mg/dL)				
DEL/MAN	36.3[30.7-44]	35.7[31.7-42.7]	-1.65 % (-0.6)	0.372
TEL/AMLO	35.9[32-50.25]	39.2[31-50]	9.19 % (3.3)	0.834
VAL/AMLO	37.55[33.67-42.75]	36.25[32.52-42]	-3.46 % (-1.3)	0.165
Lp(a) (mg/dL)				
DEL/MAN	6.9[3.4-10.4]	6.8[4.1-10.8]	-1.45 % (-0.1)	0.587
TEL/AMLO	9.3[4.35-27.8]	7.9[3.3-19.2]	-15.05 % (-1.4)	0.076
VAL/AMLO	9.25[4.08-22.9]	9.7[3.4-20.08]	4.86 % (0.45)	0.222

Data are presented as a Mean ± Standard Deviation, Median [25o-75th].
*$p < 0.05$ compared to DEL/MANI treatment, **$p < 0.01$ compared to DEL/MANI treatment,
***$p < 0.001$ compared to DEL/MANI treatment
^$p < 0.05$ compared to TEL/AMLO therapy, ^^$p < 0.01$ compared to TEL/AMLO therapy,
^^^$p < 0.001$ compared to TEL/AMLO treatment
$p < 0.05$ compared to VAL/AMLO treatment, $p < 0.01$ compared to VAL/AMLO treatment
, $p < 0.001$ compared to VAL/AMLO treatment

Conclusions: Manidipine has a PPAR-γ agonist action and exerts a favorable effect on lipids. The reduction on TC, LDL and non-HDL-C levels with DEL/MANI combination can be attributed to this action of manidipine.

SS193 / #341

TYPE 2 DIABETES, CHRONIC KIDNEY DISEASE AND MAJOR CARDIOVASCULAR EVENTS IN PATIENTS WITH ESTABLISHED CARDIOVASCULAR DISEASE

SAAG SESSION 30: THE CHANGING LANDSCAPE OF TYPE 2 DIABETES TREATMENT

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Background and Aims: The aim of this study was to investigate the single and joint effects of T2DM and CKD on major cardiovascular events (MACE) in patients with established cardiovascular disease.

Methods: We prospectively investigated 1738 patients with established cardiovascular disease - angiographically proven coronary artery disease (CAD) or sonographically proven peripheral artery disease (PAD) - over 10.0±4.7 years.

Results: MACE occurred more frequently in T2DM-patients (n=575) than in non-diabetic subjects (42.5% vs 29.8%,p<0.001) and in patients with CKD (eGFR<60ml/min/1.73m^2;n=302) than in those without CKD (52.2% vs 30.1%,p<0.001). 996 subjects had neither T2DM nor CKD, 440 had T2DM but not CKD, 172 did not have diabetes but had CKD, and 130 had T2DM and CKD. Compared to the incidence of MACE among patients with neither T2DM nor CKD (26.5%), MACE occurred more frequently in patients with T2DM without CKD (38.2%;p<0.001) as well as in non-diabetic patients with CKD (48.0%;p<0.001); the incidence of MACE was highest in patients with T2DM and CKD (57.8%;p<0.001), in whom it was higher than in those with T2DM but not CKD (p<0.001) or those without T2DM but with CKD (p=0.007); the incidence of MACE was higher in non-diabetic CKD patients than in T2DM patients who did not have CKD (p=0.040). In Cox regression analysis, T2DM (HR=1.53 [1.29-1.83];p<0.001) and CKD (HR=1.85[1.51-2.26];p<0.001) proved to be mutually independent predictors of MACE.

Conclusions: We conclude that T2DM and CKD in patients with established cardiovascular disease are mutually independent predictors of MACE. Cardiovascular disease patients with both CKD and T2DM are at an extreme risk for MACE.

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.01 Epidemiology of cardiovascular diseases and risk factors*

HYPERGLYCEMIA, DIABETES, AND CARDIOVASCULAR AND ALL-CAUSE MORTALITY: AN OBSERVATIONAL AND MENDELIAN RANDOMIZATION STUDY OF THE GENERAL POPULATION

SAAG SESSION 30: THE CHANGING LANDSCAPE OF TYPE 2 DIABETES TREATMENT

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Background and Aims: Individuals with diabetes are at high risk of cardiovascular and all-cause mortality compared to individuals without. Whether this risk is due to hyperglycemia *per se* and is present at glucose and HbA1c-concentrations below the threshold for a diagnosis of diabetes is not clear.

Methods: We investigated the association between high glucose and HbA1c concentrations and cardiovascular and all-cause mortality in 116,032 individuals from the Copenhagen City Heart Study and the Copenhagen General Population Study, using observational and Mendelian randomization analyses.

Results: Compared to the population median non-fasting glucose and HbA1c concentrations of 5.2 mmol/L and 35.0 mmol/mol, respectively, high glucose and HbA1c concentrations associated with higher risk of cardiovascular and all-cause mortality (p<0.001). Compared to individuals with non-fasting glucose concentrations between 4.0-7.9 mmol/L, individuals with non-fasting glucose between 8.0-11.0 mmol/L had a higher risk of cardiovascular death and all-cause death, with hazard ratios of 2.35 (95% confidence interval: 1.12-1.64), p<0.001 and 1.20 (1.12-1.29), p<0.001, respectively. Mendelian randomization analyses did not support a causal association between high glucose concentrations and cardiovascular death or all-cause death (risk ratio per 1 mmol/L higher glucose level: 1.04 (0.56-0.93), p=0.91, and 1.03 (0.70-1.50), p=0.89.

Conclusions: High plasma glucose and HbA1c concentrations below the threshold for a diagnosis of diabetes associate with high risk of cardiovascular and all-cause mortality in the general population. Mendelian randomization analysis did not suggest that these associations are causal.

SS195 / #922

Topic: *AS03 Dyslipidemia and Risk Factors / AS03.02 Epidemiology of dyslipidemias*

OBESITY AND STATIN USE MAY IMPACT THE PREVALENCE OF DIABETES IN FAMILIAL HYPERCHOLESTEROLAEMIA: A WORLDWIDE CROSS-SECTIONAL STUDY BY THE EAS FAMILIAL HYPERCHOLESTEROLAEMIA STUDIES COLLABORATION (FHSC)

SAAG SESSION 30: THE CHANGING LANDSCAPE OF TYPE 2 DIABETES TREATMENT

Alex Lyons[1], Amany Elshorbagy[2,3], Antonio Vallejo-Vaz[4], Christophe Stevens[5], Kanika Dharmayat[1], Julia Brandts[6], Alberico Catapano[7], Tomas Freiberger[8], G. Kees Hovingh[9], Pedro Mata[10], Frederick Raal[11], Raul Santos[12], Handrean Soran[13], Gerald Watts[14], Kausik Ray[15]
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Background and Aims: Statins are the cornerstone of treatment for patients with heterozygous familial hypercholesterolemia (HeFH) but increase the risk of diabetes mellitus in the general population. Obesity increases diabetes risk, with certain world regions reporting a high prevalence of obesity and diabetes in the general population. We explored the global prevalence of diabetes in adults with HeFH, and how obesity and statins may modify this risk.

Methods: Cross-sectional analysis was conducted on 24,784 patients across 43 countries within the EAS FH Studies Collaboration observational registry. The study population was divided into 12 risk categories based on age (tertiles), obesity (yes/no) and statin use (yes/no), and investigated their odds of diabetes using logistic regression.

Results: Results: Diabetes prevalence was higher in the Eastern-Mediterranean, Americas, and Southeast-Asia and Western-Pacific regions than in Europe, and was higher with overweight and obesity (than normal weight), and with statin use (Figure-1A). Adjusting for sex, relative risk of diabetes was 43-times higher in the highest risk category (aged≥55 years, having obesity and using statins) than the lowest category (aged 18-38 years, without obesity or statin use); being non-obese, even with age≥55 years and statin use, was associated with lower relative risk (RR:17.0 [95%-CI:10.6,27.11]; Figure-1B).

Conclusions: Conclusions: Adults with HeFH in most world regions have a higher prevalence of diabetes than in Europe. Obesity markedly augments the risk of diabetes associated with age and statin treatment. Redcing obesity and weight gain are important considerations to reduce risk of diabetes in regions where FH and diabetes are common.

SS197 / #1031

Topic: *AS04 Clinical Vascular Disease / AS04.14 SGTL2 inhibitor and cardiovascular diseases*

ANTI-ANGINAL EFFECTS OF EMPAGLIFLOZIN IN PATIENTS WITH TYPE 2 DIABETIC AND REFRACTORY ANGINA; A RANDOMIZED, DOUBLE-BLIND PLACEBO-CONTROLLED TRIAL (EMPT-ANGINA TRIAL)

SAAG SESSION 30: THE CHANGING LANDSCAPE OF TYPE 2 DIABETES TREATMENT

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Background and Aims: The EMPT-ANGINA study investigated the potential impact of Empagliflozin on angina burden in patients with type 2 diabetes (T2DM) and refractory angina.

Methods: In this double-blind, randomized, placebo-controlled study, 75 patients with T2DM and refractory angina were randomized to the empagliflozin group (n = 37) and the placebo group (n = 38) for eight weeks. The primary endpoint was an improvement in angina. Symptoms, function, and quality of life are assessed by the Seattle Angina Questionnaire (SAQ) Summary Score.

Results: The Mean age of participants in the Empagliflozin and placebo groups were 67.46±9.4 and 65.47±7.0 years, respectively (P=0.304). Patients who received Empagliflozin showed a significant improvement in the mean of primary SAQ Summary Score (192.73±20.70 vs 224±25.36, P<0.001) and secondary endpoints (physical limitation: 47.68±7.93 vs 50.38±8.98, P<0.001, angina stability: 48.92±7.2 vs 56.27±11.3, P<0.001, angina frequency: 48.86± vs 63.19±11.6, P<0.001, treatment satisfaction: 47.27±7.4 vs 54.16±9.6, P<0.001, quality of life: 48.62±8.1 vs 56.86±9.3, P<0.001). Patients in the Empagliflozin arm showed significant improvement in treadmill exercise duration, time until angina, 1mm ST-segment depression onset, and heart rate recovery. The beneficial effect was achieved without clinically important changes in rest or exercise heart rate or blood pressure. There were no significant side effects in the Empagliflozin group (P=0.125).

Conclusions: Empagliflozin could be added safely as a metabolic modulator drug to other anti-anginal agents in patients with concomitant T2DM and refractory angina for reducing angina symptoms and increasing exercise capacity with minor side effects. It could be considered in these patients.

SS198 / #42

Topic: *AS04 Clinical Vascular Disease / AS04.12 Prevention and treatment of cardiovasculardisease; miscellaneous*

TO ASSESS THE IMMEDIATE CLINICAL AND ANGIOGRAPHIC RESULTS OF STENTING THE TRUNK OF THE LEFT CORONARY ARTERY IN VARIOUS CLINICAL FORMS OF CORONARY ARTERY DISEASE.

SAAG SESSION 31: INSIGHTS INTO RISK PREDICTION

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Background and Aims: To assess the immediate clinical and angiographic results of stenting the trunk of the left coronary artery in various clinical forms of coronary artery disease.

Methods: From 2010 to 2020 we performed 318 stentings of the trunk of the left coronary artery in patients with various forms of coronary artery disease. Most patients were male 75.2% (239), the mean age was 62.5±8.28 years. Dyslipidemia was noted in 81.8%, arterial hypertension in 84.1%, 67% of patients were smokers. The patients were divided into 3 groups: 1) stable angina (SCH) 36.2% (115) patients; 2) unstable angina (UA) 36.4% (116) patients; 3) acute myocardial infarction (AMI) 27.4% (87) patients.

Results: In 92.3% of cases, direct stenting of the trunk was performed. In 100% stenting cases, drug-eluting stents were used. The average diameter of the implanted stent was 3.7±0.34 mm with an average length of 24.3±9.1 mm. The immediate angiographic success of the intervention was 98.4% in group 1, 95.5% in group 2, and 95.1% in group 3. In the unstable angina group, AMI developed in 3 cases (0.94%) and in one case (0.3%) acute stent thrombosis with subsequent lethality (0.3%). In the 3rd group, repeated AMI was noted in 2 cases (0.62%), lethality in 7 cases (2.2%) against the background of cardiogenic shock.

Conclusions: Stenting is a highly effective and fairly safe method of treating patients with lesions of the LCA trunk. PCI of the trunk should be carried out in accordance with accepted recommendations, and alternative methods of treatment should be considered in each case.

Topic: *AS04 Clinical Vascular Disease / AS04.12 Prevention and treatment of cardiovasculardisease; miscellaneous*

PREDICTIVE CAPACITY OF INFLAMMATORY BIOMARKERS FOR LEFT VENTRICULAR REMODELING AMONG ST-SEGMENT ELEVATION MYOCARDIAL INFARCTION PATIENTS

SAAG SESSION 31: INSIGHTS INTO RISK PREDICTION

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Background and Aims: The inflammatory response regulates cardiac remodeling and fibrosis after acute ST-segment elevation myocardial infarction (STEMI). These processes are important factors in long-term patient survival, therefore the purpose of our study was to improve the accuracy of the prediction for left ventricular (LV) remodeling 12 months after STEMI by inflammatory biomarkers. Macrophage migration inhibitory factor (MIF) and soluble suppressor of tumorigenicity-2 (sST2) were the biomarkers studied.

Methods: The study prospectively included 134 patients with a confirmed STEMI and <12 hours of symptoms. Transthoracic echocardiography with strain was performed at admission and one year after STEMI. The levels of biomarkers were measured before and after revascularization. Patients were divided into two groups: group 1 included 48 patients with adverse LV remodeling and group 2 consisted of 86 patients without LV remodeling.

Results: Uni- and multivariate log-regression analysis demonstrated that LV ejection fraction, MIF, sST2, longitudinal strain, number of affected coronary vessels were independent predictors of adverse LV remodeling. ROC analysis showed that the cumulative value of these markers (AUC = 0.718; $p < 0.0001$, 95%, CI 0.634-0.792) allows to identify patients with a high risk of developing adverse cardiac remodeling within one year after STEMI.

Conclusions: The study results demonstrate that LV ejection fraction, MIF, sST2, longitudinal strain and number of affected coronary vessels could identify patients with higher risk of adverse cardiac remodeling after STEMI.

SS200 / #1277

Topic: *AS01 Pathogenesis of Atherosclerosis / AS01.06 Vascular biology of the arterial wall: Miscellaneous*

CARDIOVASCULAR OUTCOMES AND ARTERIAL STIFFNESS: DATA FROM LITHUANIAN HIGH-RISK COHORT (LITHIR)

SAAG SESSION 31: INSIGHTS INTO RISK PREDICTION

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Background and Aims: Metabolic syndrome (MS) is associated with an increased risk of developing type 2 diabetes mellitus and its complications, especially cardiovascular events. To study the role of increased arterial stiffness (AS) as potential patophysiological link for cardiovascular outcome, we assessed the predictive value of AS in MS subjects.

Methods: A follow-up study was conducted from 2012 to 2021 at Vilnius University Hospital Santaros Klinikos. All participants were recruited from Lithuanian high-risk cohort (LitHiR) metabolic syndrome (NCEP/ATPIII criteria) subjects without overt cardiovascular disease. At baseline and at the follow-up visits, all participants underwent extensive cardiovascular assessment, including AS measurements. Cox regression analysis and Kaplan-Meier curves were used to assess the differences between subjects with and without cardiovascular events.

Results: At baseline, the study subjects (n=8085) were 52±6 years old, 41% male participants. During the follow-up period (median 5.2 years), totally 1331 subjects (16%) developed a cardiovascular event. At baseline, we observed that the "gold standard" measure of AS, carotid-femoral pulse wave velocity (cfPWV), correlated with mean arterial pressure, total cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides, glucose, BMI, C-reactive protein, and waist circumference (all $p<0.05$). Cox regression analysis revealed that an increase of cfPWV by one unit was associated with the increase in cardiovascular risk by 10% (HR 1.10 [1.06-1.13], $p<0.001$). In a multivariate model, event-free subjects had lower cfPWV ($p<0.001$), higher eGFR ($p<0.05$), and lower total cholesterol ($p=0.001$).

Conclusions: In middle-aged subjects with metabolic syndrome, cardiovascular outcome is linked to increased aortic stiffness, total cholesterol, and kidney function.

SS201 / #355

Topic: *AS04 Clinical Vascular Disease / AS04.15 Other*

DOES HIGH VARIABILITY IN CHOLESTEROL MEASUREMENTS INCREASE THE RISK OF CATARACTS?: A STUDY USING A COMMON DATA MODEL DATABASE

SAAG SESSION 31: INSIGHTS INTO RISK PREDICTION

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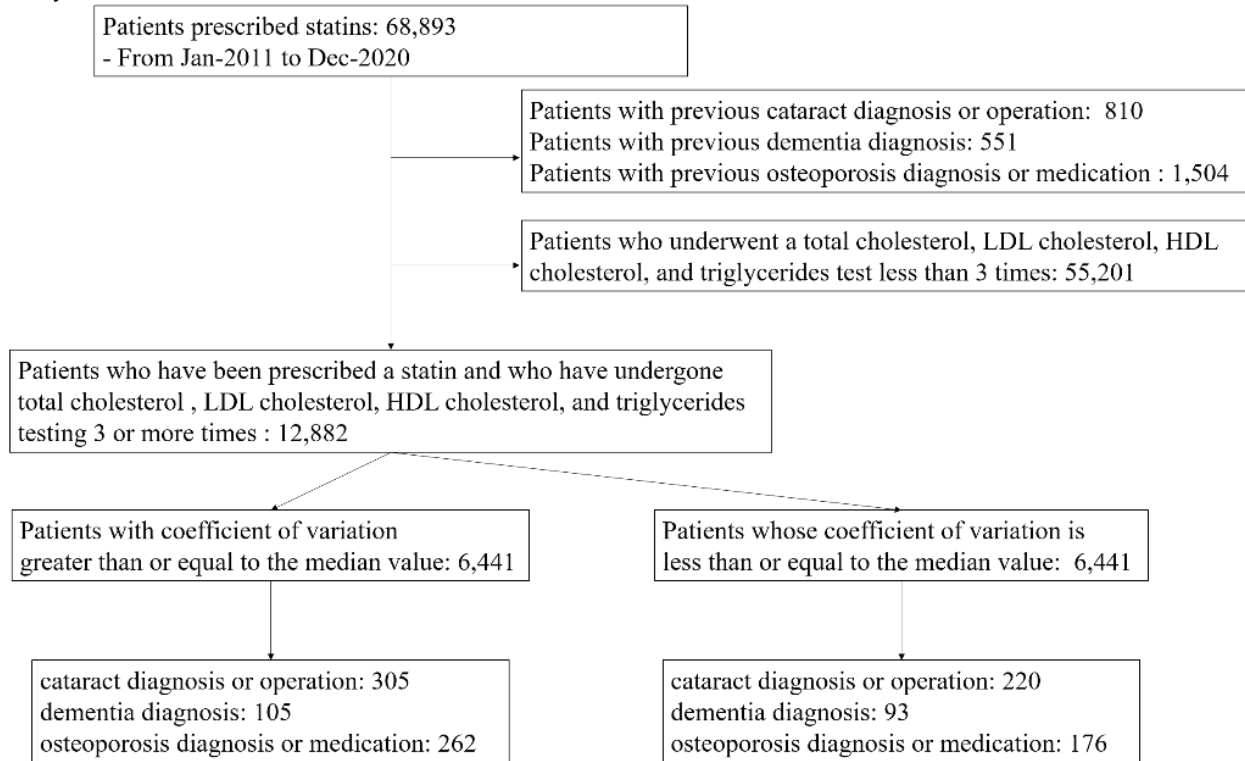
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Background and Aims: The effects of individual variability in various cholesterol measurements on the occurrence of cataracts are controversial. We aimed to determine the effect of variability in cholesterol measurements on cataract, dementia, or osteoporosis development using a common data model (CDM).

Methods: Patients who had been prescribed statin between 2011 and 2020 were included, and patients who had all three or more total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) test results were included. The mean value and standard deviation for each cholesterol test were obtained from each patient, and the coefficient of variation (CV) was obtained through this. Based on demographic variables such as age, sex, and various comorbidities, 1:1 propensity score matching was performed. The odds ratio (OR) was calculated using conditional logistic regression

analysis.

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┌─────────────────────────────────────────────────┐
│ Patients prescribed statins: 68,893               │
│ - From Jan-2011 to Dec-2020                       │
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│ Patients with previous cataract diagnosis or operation:  810            │
│ Patients with previous dementia diagnosis: 551                          │
│ Patients with previous osteoporosis diagnosis or medication : 1,504     │
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│ Patients who underwent a total cholesterol, LDL cholesterol, HDL        │
│ cholesterol, and triglycerides test less than 3 times: 55,201           │
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┌───────────────────────────────────────────────────────────────────────┐
│ Patients who have been prescribed a statin and who have undergone       │
│ total cholesterol , LDL cholesterol, HDL cholesterol, and triglycerides │
│ testing 3 or more times : 12,882                                        │
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┌──────────────────────────────────────┐    ┌──────────────────────────────────────┐
│ Patients with coefficient of variation │    │ Patients whose coefficient of variation is │
│ greater than or equal to the median    │    │ less than or equal to the median value: 6,441 │
│ value: 6,441                           │    │                                        │
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┌──────────────────────────────────────┐    ┌──────────────────────────────────────┐
│ cataract diagnosis or operation: 305  │    │ cataract diagnosis or operation: 220  │
│ dementia diagnosis: 105               │    │ dementia diagnosis: 93                │
│ osteoporosis diagnosis or medication: 262 │  │ osteoporosis diagnosis or medication: 176 │
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Results: Of 12,882 patients, 525 (4.1%) developed cataracts, 198 (1.5%) developed dementia, and 438 (3.4%) developed osteoporosis. There were statistically significant differences in cataract and osteoporosis occurrence. Also, the CV values of LDL-C, HDL-C, and TG and the average of TC, LDL-C, HDL-C, and TG showed statistically significant differences. Conditional logistic regression analysis showed 1.45 times (OR: 1.45, 95% CI 1.20–1.76, $p < 0.001$) higher cataract occurrence and 1.55 times (OR: 1.55, 95% CI 1.26–1.89, $p < 0.001$) higher osteoporosis occurrence in high CV group than in low CV group.
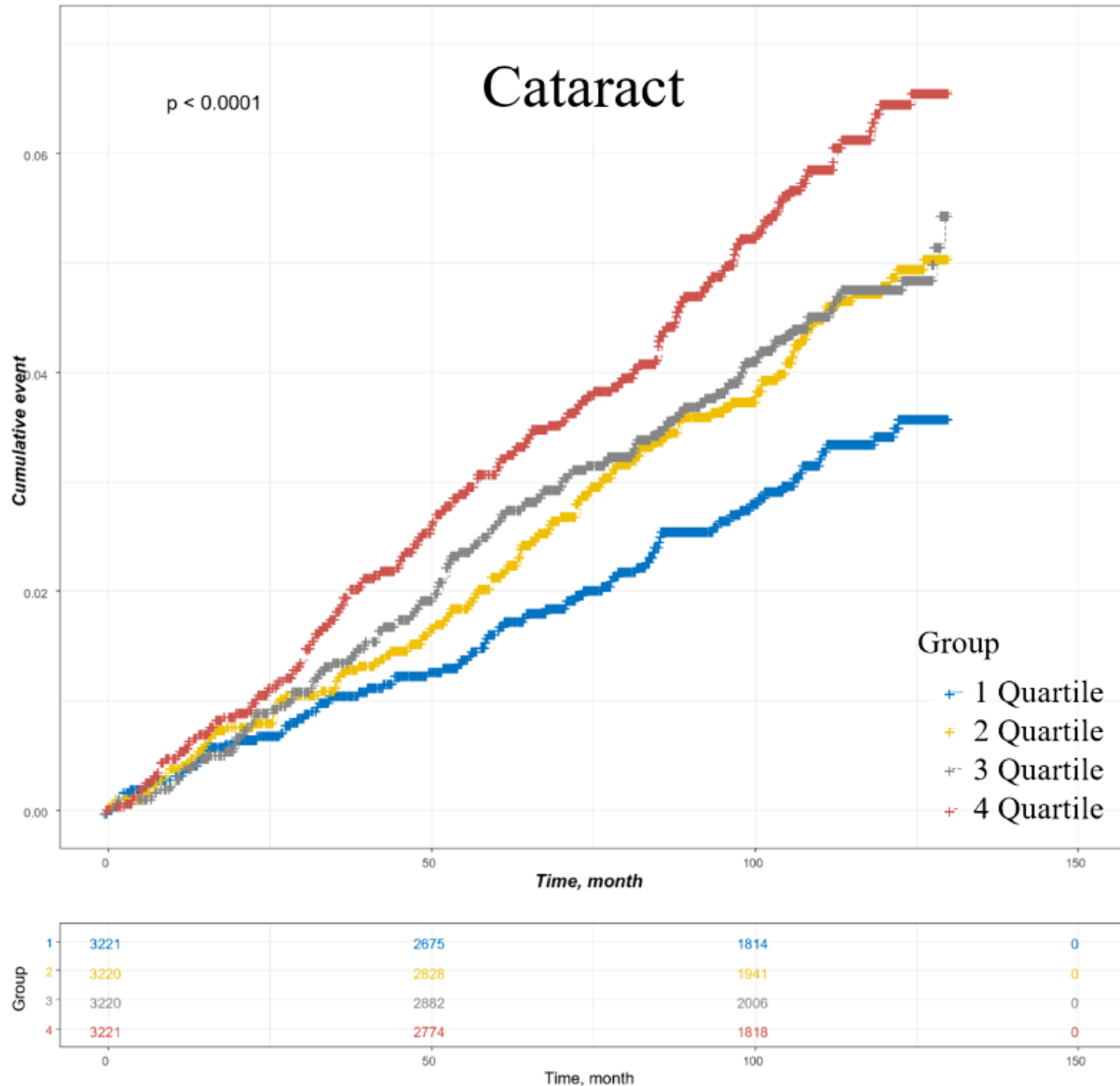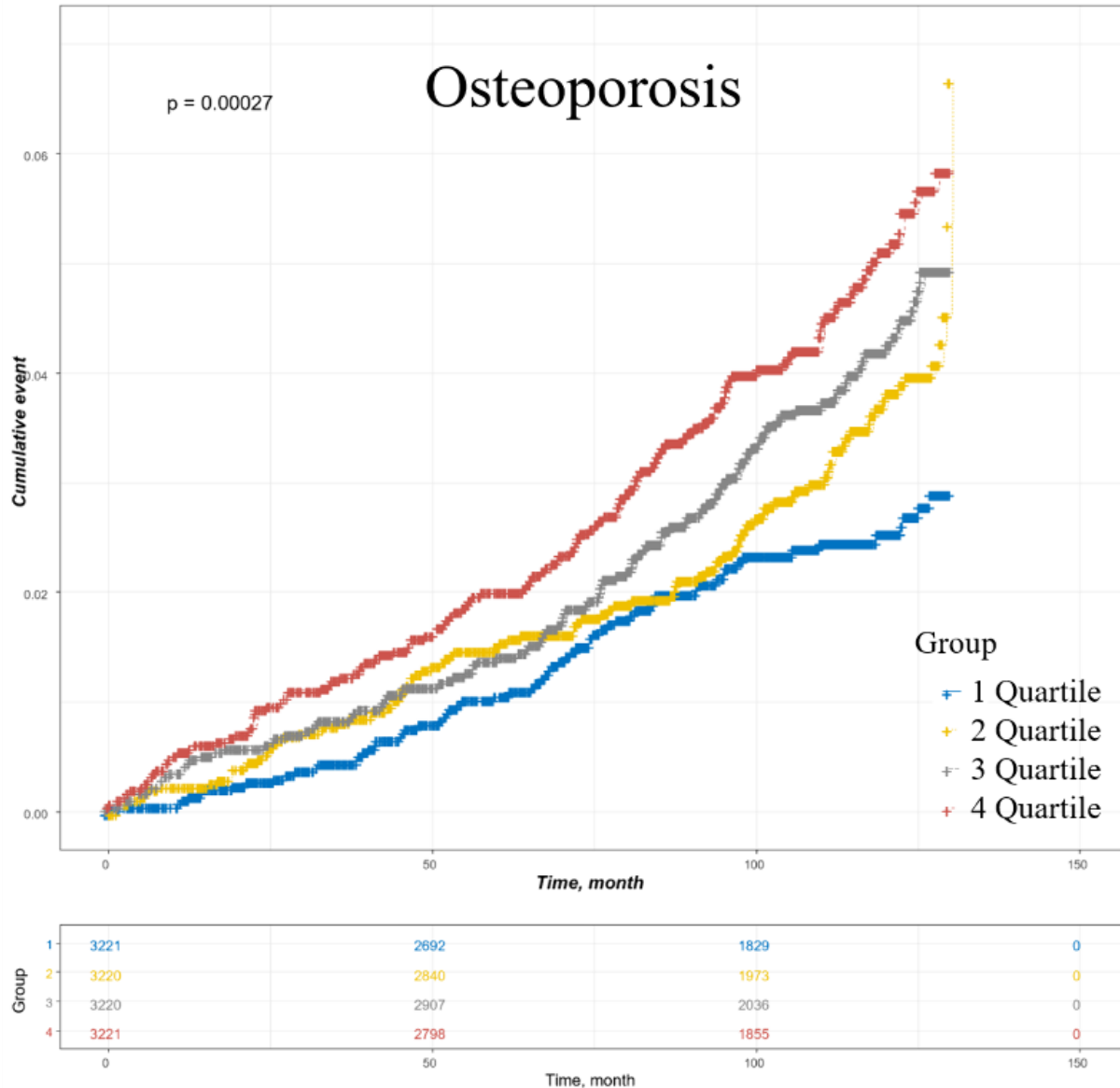
Conclusions: Our study showed that individual variability in TC was associated with the development of cataracts and osteoporosis. It is expected that additional multicenter studies using the CDM will support these claims.

SS202 / #974

THE EFFECT OF CHOLESTERYL ESTER TRANSFER PROTEIN (CETP) ACTIVITY ON CARDIOMETABOLIC AND NON-CARDIOMETABOLIC DISEASES IN EAST-ASIAN AND EUROPEAN POPULATIONS

SAAG SESSION 31: INSIGHTS INTO RISK PREDICTION

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Background and Aims: Inhibition of cholesteryl ester transfer protein (CETP) has been originally evaluated for raising HDL-C for efficacy against coronary heart disease (CHD).Drug target Mendelian randomization (MR) studies have shown that CETP inhibition reduces CHD risk in Europeans, but not in East-Asians, questioning the value of pharmacological CETP inhibition in these subjects.Here, we compared drug target MR effects of CETP inhibition in Europeans and East-Asians, focussing on cardiometabolic efficacy outcomes and relevant safety outcomes.

Methods: We selected variants associated with LDL-C and HDL-C from within and around ±50 kbp of the *CETP* locus from European (n=1,320,016) and East-Asian (n=146,492) GWAS summary statistics from the Global Lipids Genetics Consortium.Coloc was used to evaluate the colocalization between the European well-characterized causal *CETP* variant rs183031, and the HDL-C and LDL-C signals in East-Asians.Subsequently, we conducted a biomarker weighted *cis*-MR to compare the effects of CETP inhibition on 11 plasma biomarkers and 15 cardiometabolic and non-cardiometabolic outcomes between East-Asians and Europeans.

Results: There was strong support for rs183031 affecting HDL-C in both European and East-Asian populations.Given the absence of an LDL-C signal in East-Asians, similar colocalization was not observed for LDL-C.Employing drug target MR weighted by HDL-C, we found that lower CETP had a pronounced effect on LDL-C, Apo-B and LP[a] in Europeans.Conversely, its effect on Apo-A1 was significantly larger in East-Asians.Finally, the effect of lower CETP levels on CHD, angina, stroke, and

heart failure were consistent between
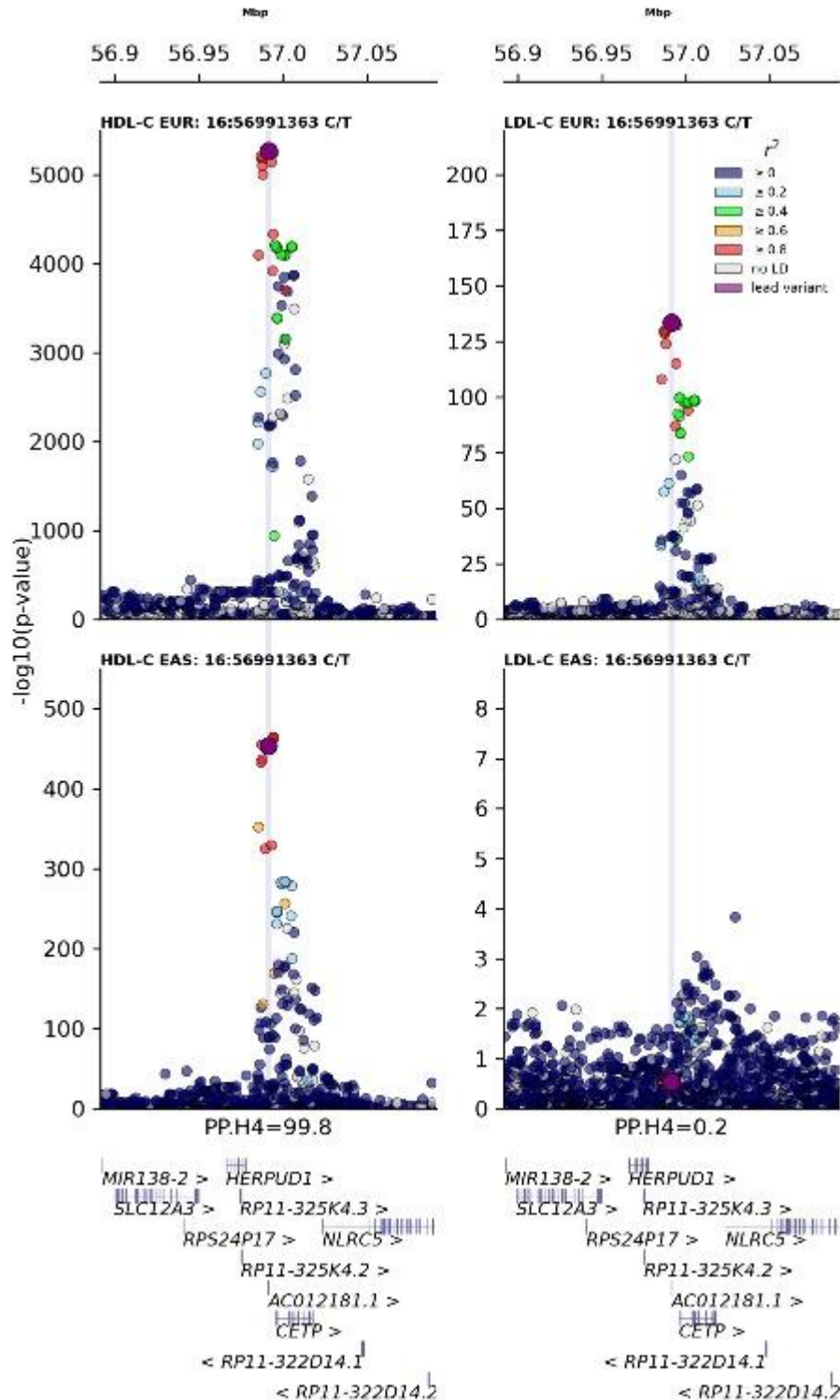
ancestries.

Conclusions: In conclusion, CETP inhibition is anticipated to decrease the risk of cardiometabolic diseases in both European and East-Asian populations.

SS203 / #1506

Topic: *AS04 Clinical Vascular Disease / AS04.12 Prevention and treatment of cardiovasculardisease; miscellaneous*

TRYPTOPHAN METABOLITES AND INCIDENT CARDIOVASCULAR DISEASE IN THE EPIC-NORFOLK PROSPECTIVE POPULATION STUDY

SAAG SESSION 31: INSIGHTS INTO RISK PREDICTION

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Background and Aims: Cardiovascular disease (CVD) remains the largest cause of death globally due to various risk factors. One novel potential contributor to CVD might be the metabolism of the essential amino acid tryptophan (Trp), which through many pathways can produce immunomodulatory metabolites such as kynurenine, indole-3-propionate and serotonin.

Methods: We used the community-based EPIC-Norfolk cohort (46.3% men, age 59.8 ± 9.0) with a median follow-up of 19.5 ± 5.9 years to study associations between the relative levels of Trp metabolites measured with untargeted metabolomics and incident development of CVD. Serum from n=11972 apparently healthy subjects were analysed, of which 6982 individuals had developed CVD at the end of follow-up. Cox proportional hazard models were used to study associations, adjusted for sex, age, conventional cardiovascular risk factors and CRP. All metabolites were Ln-normalised prior to analysis.

Results: Higher levels of Trp were inversely associated with mortality (HR 0.73; CI 0.64-0.83) and fatal CVD (HR 0.76; CI 0.59-0.99). Higher levels of kynurenine (HR 1.33; CI 1.19-1.49) and the [Kynurenine]/[Tryptophan]-ratio (HR 1.24; CI 1.14-1.35) were associated with a higher incident development of CVD. Serotonin was not associated with overall CVD, but we did find associations for myocardial infarction and stroke.

Conclusions: Tryptophan levels were inversely correlated with CVD, while several of its major metabolites (especially kynurenine and serotonin) were positively correlated. These findings indicate that mechanistic studies are required to understand the role of Trp metabolism in CVD with the goal to identify new therapeutic targets.