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.

SE001

Topic: *ASA01 - PATHOGENESIS OF ATHEROSCLEROSIS / ASA01-03 Macrophages in lipid metabolism and atherosclerosis*

A COMPARATIVE GENE EXPRESSION MATRIX IDENTIFIES UNIQUE AND DISEASE STAGE-SPECIFIC GENE REGULATION PATTERNS IN ATHEROMATOUS PLAQUE MACROPHAGES

SAAG SESSION 01: REGULATION OF MACROPHAGES IN ATHEROSCLEROSIS

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Background and Aims : Hypercholesterolemia-driven atherosclerosis is a systemic and chronic inflammatory disease propagated in part by monocytes and macrophages. Yet, our knowledge on gene regulation in these cells over time and in different tissues is limited. With spatio-temporal transcriptomic profiling, we aimed to characterize tissue macrophages during atherosclerosis, which will aid in screening for novel atheromatous plaque macrophage- and disease stage-specific therapeutic targets.

Methods: Apolipoprotein E knock-out mice were studied at baseline (without atherosclerosis), after 1 and 6 months of high-cholesterol diet feeding, representing early and advanced stages of atherosclerosis, respectively. For each disease stage, circulating monocytes, aortic macrophages and peritoneal macrophages were retrieved from the same animals and subjected to bulk-RNA sequencing.

Results: Comparing three cell types at three time points, we identified over 2600 differentially expressed genes (DEG). More than 850 DEGs were selectively regulated in aortic macrophages. A total of 368 DEGs of aortic macrophages were regulated in the atheroma initiation phase and enriched biological processes including macrophage activation, leukocyte migration and proliferation. More than 270 DEGs were relevant in the atheroma progression phase and enriched biological processes including lipid handling and production of IFNγ, IL1β and IL8. Among all DEGs of aortic macrophages, Gpnmb was the only continuously upregulated gene during atheroma initiation and progression, specifically enriched within foamy macrophages according to single-cell RNA sequencing analysis of murine and human datasets.

Conclusions: Using our novel gene expression matrix, we identified uniquely regulated genes in atheromatous aortic macrophages, associated biological processes and their regulation patterns. Our results may support to identify macrophage-directed therapeutic targets at specific disease stages.

SE002

Topic: *ASA01 - PATHOGENESIS OF ATHEROSCLEROSIS / ASA01-03 Macrophages in lipid metabolism and atherosclerosis*

MYELOID PHD2 CONDITIONAL KNOCK-OUT PROMOTES VEIN GRAFT PLAQUE STABILITY BY REDUCING VESSEL WALL M1 MACROPHAGES AND INTRAPLAQUE HAEMORRHAGE

SAAG SESSION 01: REGULATION OF MACROPHAGES IN ATHEROSCLEROSIS

Thijs J. Sluiter[1], Renee Tillie[2], Remco Van De Leijgraaf[1], Jenny De Bruijn[2], Hendrika A.B. Peters[1], Paul H.A. Quax[3], Judith Sluimer[4], Margreet R. De Vries[3]
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Background and Aims : Revascularization interventions using venous bypass grafts are hampered by high failure-rates. Inflammation and intraplaque-angiogenesis are key drivers of vein graft failure and is mediated by macrophages. PHD proteins regulate cellular responses to hypoxia (with PHD2 as the most important regulator), possibly altering macrophage function as well as intraplaque angiogenesis in the hypoxic vein graft wall. We therefore aim to investigate the effect of myeloid PHD2 on plaque stability in hypoxic, atherosclerotic vein grafts.

Methods: Male myeloid PHD2 LysM-Cre conditional knockdown (PHD2cko) $Ldlr^{-/-}$ mice underwent vein graft surgery (n=11/group) by interpositioning donor caval veins into the carotid artery of genotype-matched mice. At POD28, vein grafts were harvested and blood collected for flow cytometry. Immunohistochemistry was used for compositional analysis of the vessel wall calculating intima/media ratios.

Results: PHD2cko reduced the intima/media ratios by 35% compared to control (p=0.0334) and prevented vein graft dissections (0/11 versus 3/11). PHD2cko increased total circulating leukocyte counts (26%, p=0.0303), but reduced total serum monocyte (44%, p=0.0173), $Ly6C^{hi}$ (35%, p=0.2468), $Ly6C^{low}$ (61%, p=0.0173) and $Ly6C^{neg}$ (46%, p=0.0087) counts. Collagenpresence in the vessel was increased by 80% in the PHD2cko-group (p=0.0351) as was intraplaque haemorrhage (27%, p=0.022). anti-inflammatory macrophages, as well as total macrophage number were unaltered compared to control, however pro-inflammatory macrophage presence was reduced by 44% (p=0.0140) in the PHD2cko-group.

Conclusions: Myeloid PHD2cko ameliorates vein graft lesion stability by reducing serum monocyte count, favourably altering macrophage polarization in the vessel wall, reducing intraplaque-haemorrhage and increasing collagen-content, consequently preventing dissections.

SE003

Topic: *ASA01 - PATHOGENESIS OF ATHEROSCLEROSIS / ASA01-03 Macrophages in lipid metabolism and atherosclerosis*

MYELOID CANNABINOID RECEPTOR CB1 DEFICIENCY LIMITS MACROPHAGE PROLIFERATION AND ATHEROSCLEROSIS IN A SEX-SPECIFIC MANNER

SAAG SESSION 01: REGULATION OF MACROPHAGES IN ATHEROSCLEROSIS

Yong Wang[1], Raquel Guillamat Prats[1], Guo Li[1], Michael Lacy[1], Yvonne Jansen[1], Beat Lutz[2], Stephan Herzig[3], Sabine Steffens[1]
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Background and Aims : Cardiovascular diseases are the leading cause of death globally. Research into the mechanisms and signaling of endocannabinoids in atherosclerosis might help finding novel treatments. We aimed to clarify the myeloid-cell-specific effects of CB1 in atherosclerotic plaque formation and macrophage functions.

Methods: Myeloid-CB1 knockout mice were generated on ApoE-/- background. Age- and sex- matched groups were analyzed at baseline, 4 and 16-weeks Western-diet. In vitro, bone-marrow-derived-macrophages(BMDM) were generated from global CB1-/-ApoE-/- and ApoE-/- mice.

Results: Male mice with myeloid-specific-CB1 deficiency had smaller lesions after 4weeks Western-diet and less macrophage accumulation in the aortic roots compared to controls. In addition, male with myeloid-CB1 deficiency showed reduced plaque progression after 16 weeks-diet. No impact of myeloid-CB1 on plaque formation was observed in female. In vitro, male CB1-/- BMDM had lower proliferation rates compared to CB1+/+ macrophages. Likewise, plaques of male myeloid-CB1 deficient contained less proliferating macrophages. This proliferation phenotype was again not observed in female, supporting a sex-specific impact of CB1 in regulating myeloid-cell proliferation in atherogenesis. Furthermore, in vitro stimulation of male BMDM with LPS resulted in reduced TNFα secretion in CB1-deficient cells. Finally, Seahorse assays revealed a reduced oxygen consumption rate in male BMDM stimulated with CB1 agonist-ACEA, which supports a proinflammatory role of CB1 signaling in macrophages.

Conclusions: Our data suggest that the biological function of CB1 signaling in macrophages seems to be sex-dependent, playing a pro-atherosclerotic role of myeloid-CB1 in male, but not female mice. It further highlights the need to consider the biological sex as an important variable in preclinical studies.

SE004

Topic: *ASA01 - PATHOGENESIS OF ATHEROSCLEROSIS / ASA01-03 Macrophages in lipid metabolism and atherosclerosis*

EFFEROCYTOSIS INDUCES A TRANSIENT BURST OF GLYCOLYSIS IN MACROPHAGES TO PROMOTE LACTATE-DRIVEN BINDING AND CONTINUING REMOVAL OF APOPTOTIC CELLS

SAAG SESSION 01: REGULATION OF MACROPHAGES IN ATHEROSCLEROSIS

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Background and Aims : Resolving “M2-like” macrophages stabilize atherosclerotic plaques by clearing apoptotic cells (ACs) through efferocytosis. These macrophages are thought to rely mainly on oxidative phosphorylation, but emerging evidence suggests a possible link between efferocytosis and glycolysis. To help reconcile this issue, we investigated the role of glycolysis in macrophage-mediated efferocytosis.

Methods: Murine bone marrow-derived macrophages and human monocyte-derived macrophages were incubated with Jurkat T-cells, rendered apoptotic by UV-irradiation, to study efferocytosis. The Seahorse analyzer was used to interrogate cellular energy metabolism.

Results: We found that efferocytosis promotes a time-dependent increase in macrophage glycolysis (+50% after 1h, unchanged after 24hrs), which distinguishes this process from more-prolonged glycolysis in pro-inflammatory “M1-like” macrophages. Immunoblotting and gene silencing approaches showed that efferocytosis-induced macrophage glycolysis (EIMG) but not inflammation-induced glycolysis is mediated by post-translational regulation of 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 2 (PFKFB2). In terms of consequences, blocking EIMG with 2-deoxyglucose blocked efferocytosis-induced lactate production, and, most importantly, decreased the uptake of subsequent ACs. This defect in so-called "continual efferocytosis" was rescued by lactate but not by increasing ATP availability with dichloroacetate. Experiments using the actin inhibitor cytochalasin D showed that inhibition of EIMG blocks subsequent AC binding rather than internalization, suggesting that lactate promotes the sensing and/or binding of ACs by macrophages.

Conclusions: These results indicate a role for EIMG-associated lactate production in continual efferocytosis, which has been shown to have a critically important role in promoting atherosclerotic plaque stabilization, particularly during atherosclerosis regression. Accordingly, we now aim to test this hypothesis by targeting macrophage PFKFB2 in a mouse model of atherosclerosis regression.

SE005

Topic: *ASA01 - PATHOGENESIS OF ATHEROSCLEROSIS / ASA01-03 Macrophages in lipid metabolism and atherosclerosis*

POLARIZED PRIMARY HUMAN MACROPHAGES REVEAL SPECIFIC BIOENERGETIC SIGNATURES

SAAG SESSION 01: REGULATION OF MACROPHAGES IN ATHEROSCLEROSIS

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Background and Aims : Classically M1 and alternatively activated M2 macrophages (MF) show different transcriptomic profiles. M1, abundantly present in atherosclerotic plaque, show upregulation of anaerobic glycolysis and lipid synthesis genes. Conversely, M2 show a higher expression of tricarboxylic acid cycle (TCA) and oxidative phosphorylation genes. Fluxomic data on primary human MF metabolism are lacking. This study is aimed at assessing bioenergetic fluxomics of polarized human MF through a novel technique of indirect microcalorimetry.

Methods: MF were obtained from healthy donors (n=13) and polarized in M1, M2 or unstimulated (M0) for 16h. Inflammatory mediators were quantified by qPCR and ELISA and surface markers by flow-cytometry. Steady-state bioenergetics (fluxes: pmol/min for $5x10^4$ cells) was measured by indirect microcalorimetry, based on the integration of four primary flows [oxygen consumption and proton production (Seahorse Analyzer); lactate and NH_4 release (enzymatic methods)] with the stoichiometric equations of metabolic pathways. The following fluxomic parameters were calculated: lipid, glucose and amino-acid consumption, anaerobic glycolysis, TCA, energy expenditure (µJ/min) and efficiency, maximum and anaerobic ATP.

Results: M1 *vs* M2 show higher glucose consumption (281±47 *vs* 154±28;p<0.01), lactate production (558±95 *vs* 315±56;p<0.01), Anaerobic Glycolysis (275±47 *vs* 149±27;p<0.01) and ATP (552±94 *vs* 299±55;p<0.01); and lower amino-acid consumption (10±3 *vs* 28±7;p<0.05), TCA (all parameters p<0.05) and energy efficiency (0.05±0.01 *vs* 0.08±0.01;p<0.01). M0 have an intermediate phenotype characterized by a lower (anaerobic) glycolysis *vs* M1 and oxidative TCA *vs* M2.

Conclusions: Using a novel technique of indirect microcalorimetry, we demonstrated - for the first time in human MF - that M1 and M2 have distinct bioenergetic signatures.

SE006

Topic: *ASA01 - PATHOGENESIS OF ATHEROSCLEROSIS / ASA01-10 Clonal Haematopoiesis*

CLONAL HEMATOPOIESIS OF INDETERMINATE POTENTIAL IN PERIPHERAL ARTERY DISEASE

SAAG SESSION 02: FRESH PERSPECTIVES OF CLINICAL VASCULAR DISEASE

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Background and Aims : Clonal hematopoiesis of indeterminate potential (CHIP) mutations increase the risk of coronary artery disease and CHIP mutant macrophages are thought to be mediators of atherosclerotic pathomechanisms. We analyzed blood and atherosclerotic arterial tissue of patients with peripheral artery disease (PAD) for CHIP mutations.

Methods: Patients (n=32) with PAD who underwent open vascular surgery to treat femoral artery stenosis and occlusion participated in this study. Peripheral blood, monocytes, progenitor cells, plaque tissue from the atherosclerotic femoral lesion, subcutaneous and perivascular tissue and collateral vessel samples were collected. DNA was isolated and CHIP was assessed using a next-generation sequencing approach targeting the most commonly mutated loci.

Results: CHIP mutations were detected in 18 (56%) patients, seven of whom carried more than one mutation. The most commonly affected target genes were TET2 (12 mutations) and DNMT3A (8 mutations). In 14 of the 18 CHIP carriers, CHIP mutations were detected in femoral lesion samples. Unexpectedly, seven patients carried CHIP mutations in DNA prepared from fat deposits. In blood samples, mutations were detected in monocytes (7 cases), progenitor cells (7 cases) or in the remaining population (7 cases). In three patients, mutations were detectable in enriched blood populations or vessel material, but not in DNA from whole blood.

Conclusions: CHIP mutations are common in PAD patients, with TET2 mutations in this small PAD cohort apparently being more common than in CAD or hematological cohorts. Since these mutations can be detected in atherosclerotic lesions, an involvement of inflammatory cells affected by CHIP mutations in disease progression seems plausible.

SE007

Topic: *ASA01 - PATHOGENESIS OF ATHEROSCLEROSIS / ASA01-11 Plaque remodelling*

CO-STIMULATORY TREATMENT ENHANCES THE PROTECTIVE ROLE OF CD8 AND CD4 T CELLS IN MURINE VEIN GRAFTS.

SAAG SESSION 02: FRESH PERSPECTIVES OF CLINICAL VASCULAR DISEASE

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Background and Aims : Background Vein grafts are frequently used to bypass coronary artery occlusions. Unfortunately, vein graft disease (VGD) causes impaired patency that rapidly declines due to extensive intimal hyperplasia (IH). T-cells demonstrate both pro-and anti-atherogenic effects. Therefore, we investigated T-cell involvement in a mouse-model for VGD using pharmacological targeting of the co-stimulatory factor CD137.

Methods: Methods Hypercholesterolemic male ApoE3*Leiden mice underwent vein graft surgery and were treated once with agonistic CD137-antibodies (200ug;S.C.) (n=9/group) or treated twice a week with antagonistic CD137L (200ug;S.C.) (n=9/group). Flow cytometry was performed on blood and non-invasive ultrasound was performed weekly until sacrifice (t28). Vein grafts were processed for morphometric and histological analysis or processed for flow cytometry.

Results: Results Flow cytometry showed increased CD43 glycosylation on both CD4 (5.2-fold, p:0.005) and CD8 (3.5-fold, p:0.01) T-cells systemically after CD137 treatment. Ultrasound analysis showed a 1.7-fold increase (p:0.006) in lumen area after CD137 treatment at t21 and increased by 2.0-fold (p:0.004) at t28. Notably, CD137L treated vein grafts showed a decrease in lumen area, both confirmed histologically. Moreover, agonistic CD137 treatment increased smooth muscle cell accumulation by 18% at t28. Although no differences were observed in the macrophage lesion area after agonistic CD137 treatment. T-cells in the vein grafts from CD137 treated mice showed an increase in IFN-y+ cells, indicating an increase in Th1 and Tc1 cells.

Conclusions: Conclusions Activation of T cells via CD137 enhanced T-cell activation, local IFN-y upregulation and an increased vein graft lumen. Activation of T-cells via CD137 is an attractive target to treat VGD.

SE008

Topic: *ASA01 - PATHOGENESIS OF ATHEROSCLEROSIS / ASA01-12 Other*

THE DIAGNOSTIC POTENTIAL OF PLAQUE-SPECIFIC METHYLATION PATTERNS IN CELL-FREE DNA

SAAG SESSION 02: FRESH PERSPECTIVES OF CLINICAL VASCULAR DISEASE

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Background and Aims : There is an urgent and unmet need for early, non-invasive diagnosis of atherosclerosis that can identify high-risk patients with potentially symptomatic plaques. Recent breakthroughs in the use of circulating fragments of cell-free DNA (cfDNA), have provided major advances in early cancer detection. Furthermore, it has been shown that, using tissue-specific methylation patterns, the contribution of different tissue origins to cfDNA can be quantified. Here we aim to identify plaque-specific methylation patterns and unravel the biological processes behind this epigenetic signature. We hypothesize that an increased contribution of plaque-derived cfDNA, as a proxy for the accumulation of atherosclerotic plaque, can be measured in cardiovascular disease patients.

Methods: DNA methylation patterns were obtained for plaque samples from 50 female and 50 male patients that underwent carotid endarterectomy (CEA) from the Athero-Express Biobank using the Infinium HumanMethylation450 Beadchip Array. cfDNA methylation from 18 patients, spanning three cardiovascular clinical cohorts, was measured using the NEBNext Enzymatic Methyl-seq kit.

Results: We were able to identify a plaque-specific methylation signature encompassing 200 CpG sides, of which a portion was found to be involved in atherosclerotic processes. Furthermore, we built a comprehensive human methylation atlas using methylation data on 18 different tissue and cell types, including plaque, which is used for the deconvolution of cfDNA.

Conclusions: Our plaque-centric methylation atlas together with our well-established biobanks, offer the unique opportunity to investigate cfDNA composition in patients spanning various types of cardiovascular disease. We believe this comprehensive methylation framework would help unravel the potential of cfDNA as an innovative non-invasive biomarker for cardiovascular disease.

SE009

Topic: *ASA01 - PATHOGENESIS OF ATHEROSCLEROSIS / ASA01-12 Other*

RESTING HEART RATE AND ARTERIAL STIFFNESS: A LONGITUDINAL PROSPECTIVE STUDY IN A POPULATION OF HYPERTENSIVE PATIENTS

SAAG SESSION 02: FRESH PERSPECTIVES OF CLINICAL VASCULAR DISEASE

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Background and Aims : The role of resting Heart Rate on the progression of arterial stiffness has not been extensively evaluated. The aim of this study is to investigate the relationship between resting HR and arterial stiffness (evaluated by cfPWV) and its progression, in a population of hypertensive patients, over a 3.7 years follow-up period.

Methods: We enrolled 572 hypertensive outpatients 18–80 aged, followed by the Hypertension Unit of St. Gerardo Hospital (Monza, Italy). Anamnestic, clinical and laboratory data, BP and cfPWV (complior) were assessed at baseline and after a median follow-up time of 3.7±0.5 years.

Results: At baseline the mean age was 53.9±12.7 years, SBP and DBP were 141.2±17.8 and 86.5±10.5 mmHg, HR was 65.6±10.9 bpm and PWV was 8.6±2.0 m/s. Despite an improvement in BP values (from 141.2/86.5 to 132.6/79.2 mmHg,$p<0.001$), during follow-up, PWV increased (ΔPWV 0.5±2.2 m/s). In patients with a ΔHR above as compared to those under the median value (9 bpm), ΔPWV was significantly higher (0.82±2.22 vs 0.27± 2.25 m/s,$p=0.003$). At multivariate analysis, HR was among the significant determinants of both baseline PWV and its progression ($\beta=0.031$,$p<0.001$). Furthermore, ΔHR was a significant determinant of ΔPWV ($\beta=0.019$;$p=0.017$).

Conclusions: In hypertensive patients there is a significant relationship between basal resting HR and basal PWV as well as between the increase of HR and the increase of PWV during the follow-up period. Beyond age and BP, resting HR must be considered as an independent determinant of arterial stiffness. This represents a possible mechanism through which HR contributes to the increase in CV risk.

SE010

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-01 Lp(a)*

CAROTID ENDARTERECTOMY PLAQUES OF PATIENTS WITH ELEVATED LEVELS OF LIPOPROTEIN(A) DEMONSTRATE INCREASED INTRAPLAQUE ANGIOGENESIS

SAAG SESSION 03: THE MANY FACES OF LP(A)

Kim E. Dzobo
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Background and Aims : Elevated lipoprotein(a) [Lp(a)] levels have been demonstrated to be a causal risk factor for the development of cardiovascular disease. Previously, we described that patients with elevated Lp(a) levels have an increased risk of major adverse cardiovascular events (MACE), mostly stroke, 30 days after undergoing carotid endarterectomy (CEA). **Aim:** Unravelling the mechanism underlying this increase in MACE.

Methods: We performed targeted plasma proteomics analysis using the proximity extension assay for 276 proteins on complete plasma from patients undergoing CEA with high levels (>195 mg/dl; N=39) and low levels (<7 mg/dl; N=73) of Lp(a).

Results: Surprisingly, no differences were observed in the expression of systemic plasma proteins. Hence, we started to assess the local effects of Lp(a) on plaque morphology. Plaque phenotyping demonstrated that patients with high levels (>195 mg/dl; N=57) of Lp(a) had a 33% increase in mean vessel density compared to patients with low levels (<7 mg/dl; N=106), as attested by semi-quantitatively scoring of the plaques. This increase in neovascularization correlates with decreased plaque stability, as was indicated by increased intraplaque haemorrhage. *In-vitro* stimulation of Human Arterial Endothelial Cells with physiologically relevant levels of Lp(a) (100 mg/dl), results in enhanced collagen degradation capacity, hyper-sprouting in a 3D spheroid assay and increased blood vessel invasion using a 3D collagen-invasiveness assay. Currently, we are elucidating the signalling pathways underlying this Lp(a)-induced angiogenesis, which we strive to present at the EAS 2022.

Conclusions: Lp(a)-induced intraplaque angiogenesis can contribute to the increased risk of major adverse cardiovascular events in patients with elevated levels of Lp(a).

SE011

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-01 Lp(a)*

ELUCIDATING THE ATHEROGENICITY OF LP(A): THE PRO-INFLAMMATORY ROLE OF DIACYLGLYCEROLS AND LYSOPHOSPHATIDIC ACID IN MONOCYTES

SAAG SESSION 03: THE MANY FACES OF LP(A)

Kim E. Dzobo
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Background and Aims : The atherogenicity of Lp(a) has been attributed to the pro-inflammatory oxidized phospholipids (OxPLs) bound to its apolipoprotein(a) [apo(a)] moiety. While blocking these OxPL epitopes profoundly reduces the monocytic inflammatory response, a residual IL-8 inflammatory response remains. We hypothesized that besides OxPLs, additional features contribute to the atherogenicity of Lp(a).

Methods: We performed lipidomics on complete plasma from healthy individuals with either elevated [median 87 mg/dL (218 nmol/L); N=12] or low [median 7 mg/dL (18 nmol/L); N=13] levels of Lp(a).

Results: Using this unbiased lipidomics approach, we discovered a distinct “lipidome” in individuals with elevated Lp(a) levels as displayed by an upregulation of several lipid species, in particular diacylglycerols (DAGs) and lysophosphatidic acid (LPA). Further fractionation and purification of lipoprotein fractions showed that DAGs and the LPA precursor Lysophosphatidylcholine (LPC) are carried by the Lp(a) particle. Functional assessment of DAG40:6 and DAG38:4-stimulated primary monocytes demonstrated a dose-dependent increase in the pro-inflammatory cytokines IL-8, IL-6 and IL-1β secretion, whereas no effect was observed upon LPA stimulation. Interestingly, DAGs and LPA did not trigger an inflammatory response in human arterial endothelial cells (HAECs), indicating a cell-specific inflammatory response. Activation of monocytes by DAGs and LPA increased their transendothelial migratory capacity. Mechanistically, DAGs are able to activate the inflammasome by upregulating NLRP3 and cleavage of pro-caspase-1. Currently, we are investigating whether apo(a) antisense treatment results in a decrease in plasma DAGs and LPA as well as inflammasome markers.

Conclusions: Residual inflammation after OxPL blocking can partially be attributed to DAGs and LPA carried by the Lp(a) particle.

SE012

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-01 Lp(a)*

LP(A) ASSOCIATES CROSS-SECTIONALLY WITH PLASMA CLOT PROPERTIES IN AFRICANS – EPIDEMIOLOGICAL EVIDENCE

SAAG SESSION 03: THE MANY FACES OF LP(A)

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Background and Aims : Lp(a) consists of apolipoprotein (a), which has structural homology with plasminogen, covalently bound to an LDL-like particle. There is contradictory evidence regarding Lp(a) and clot lysis, with few studies investigating other clot properties. We aim to determine the association between Lp(a) concentration and plasma clot properties in Africans, who have known differences in Lp(a)-CVD associations.

Methods: We cross-sectionally associated serum Lp(a), using a relatively size-independent turbidimetric assay (Roche, Gen2), with turbidimetrically determined plasma clot properties, fibrinogen (Clauss) and plasminogen activator inhibitor-1 activity (PAI-1_{act}) (adjusted for known CVD risk markers) in 1640 apparently healthy Africans (>30y) following ethical approval.

Results: The median Lp(a) concentration of the study population was 67.9 (IQR: 31.0 - 129) nM. Women had significantly higher values than men (70.0 vs 60.1 nM; p=0.02). In univariate regression, Lp(a) was significantly associated with fibrinogen, rate of clot formation (slope), clot density (maximum absorbance) and clot lysis time (CLT) (Table 1). After full adjustment significance remained for fibrinogen, slope and max absorbance, and Lp(a) associated negatively with PAI-1_{act}.

Table 1: Association of Lp(a) with plasma clot properties, fibrinogen and PAI-1_{act}

Variable	Univariate regression		Multivariate regression*	
	β (95% CI)	p	β	p
Fibrinogen (g/L)	0.16 (0.11; 0.21)	<0.0001	0.16 (0.10; 0.21)	<0.0001
Lagtime (min)	-0.02 (-0.07; 0.03)	0.46	-0.04 (-0.10; 0.01)	0.14
Slope (au/s)	0.12 (0.07; 0.17)	<0.0001	0.09 (0.04; 0.14)	0.001
Maximum absorbance (Δau)	0.19 (0.14; 0.24)	<0.0001	0.09 (0.05; 0.14)	<0.0001
CLT (min)	0.08 (0.03; 0.13)	0.003	0.03 (-0.009; 0.08)	0.13
PAI-1_{act} (IU/mL)	-0.04 (-0.09; 0.01)	0.13	-0.05 (-0.10; 0.005)	0.03

* Adjusted for: gender, age, BMI, HbA1c, LDL-C, HDL-C, IL-6, alcohol consumption, fibrinogen (clot properties only) and PAI-1_{act} (CLT only)

Conclusions: This African population had a high median Lp(a) concentration compared to published data for Europeans. Higher Lp(a) was associated with a prothrombotic clot phenotype also after adjusting for LDL-C and other CVD risk factors. Lp(a) associated with CLT in the univariate model but not the fully adjusted model, partly as a result of its negative association with PAI-1_{act}. Serum Lp(a) concentrations,

measured with a relatively size-independent assay, was associated with plasma clot phenotype but not CLT in an epidemiological setting in apparently healthy Africans.

SE013

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-02 TG rich lipoproteins metabolism and lipases*

ANALYSIS OF HYPERTRIGLYCERIDEMIC GENE SCORE IN PATIENTS WITH FAMILIAL DYSBETALIPOPROTEINEMIA

SAAG SESSION 04: ALL ABOUT TRIGLYCERIDES

Martin Satny[1], Veronika Todorovova[1], Michal Vrablík[1], Jaroslav Hubacek[1], Tereza Altschmiedova[1], Vladimir Soska[2], Ondrej Kyselak[2]
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Background and Aims : Familial dysbetalipoproteinemia (FD, type III hyperlipoproteinemia) is an AR inherited disease associated with APOE polymorphism. The typical genotype of FD is APOE2/2 and the phenotype mixed DLP arising in the context of other unknown metabolic or genetic factors. The aim of this work was to determine the hypertriglyceridemic (HTG) score in patients with FD, as a possible determinant of FD development.

Methods: A total of 15 single-nucleotide polymorphisms (SNPs), which condition the HTG development in the Czech population, were analyzed in 101 patients with FD and 90 controls with the APOE2/2 genotype (biobank of post-MONICA and HAPIEE studies). Genetic analyzes of SNPs were performed using PCR-RFLP or real-time PCR. The data were processed by descriptive statistics methods.

Results: A total of 13 SNPs out of 15 examined were associated with the development of DLP, resp. HTG in patients with FD compared to controls (strongest SNPs in APOE and APOA5 genes; $P < 0.005$). The unweighted gene score (sum of risk alleles) was different between the two groups ($P < 0.0002$).

Conclusions: Cumulation of risk genetic variants, assessed by an unweighted score, can distinguish between individuals with the APOE2/2 genotype and those at risk of developing FD.

SE014

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-02 TG rich lipoproteins metabolism and lipases*

POST PRANDIAL METABOLISM OF LIPOPROTEINS IN FAMILIAL CHYLOMICRONEMIA PATIENTS TREATED WITH LOMITAPIDE AND TIPARVOVEC

SAAG SESSION 04: ALL ABOUT TRIGLYCERIDES

Mariagrazia Gueli Alletti[1], Federica Brucato[2], Chiara Scrimali[2], Rossella Spina[2], Valeria Ingrassia[2], Grazia Ida Altieri[2], Francesca Fayer[2], Davide Noto[2], Gabriella Misiano[2], Antonina Giammanco[2], Carlo M. Barbagallo[2], Antonina Ganci[2], Angelo Baldassare Cefalù[2], Alberto Zambon[3], Maurizio Averna[4]
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Background and Aims : Familial chylomicronemia syndrome (FCS) is a rare recessive monogenic disease characterized by triglycerides (TG) levels >10 mmol/L. FCS is causally associated with mutations in candidate genes but most patients have mutations in lipoprotein lipase (LPL). Defects in LPL result in reduced clearance of chylomicrons (CM) and development of acute pancreatitis. Treatment of FSC patients is based on combined action of a lipid- and carbohydrate-reduced diet in addition to available hypolipidemic therapies that often fails to achieve a desired TG levels. Recently several innovative drugs have been developed: tiparvovec, lomitapide, volanesorsen and monoclonal antibodies.

Methods: Five patients carrying FCS causative mutations in candidate genes of FCS were collected. Each patient was given a modified oral fat load to avoid a risk of pancreatitis induced by postprandial hyperchylomicronemia but sufficient to assess the change in postprandial chylomicron levels. The meal was supplemented with retinol palmitate (RP) as CM biomarker. We compared TG and RP levels after administration of an oral fat load before and after lomitapide or tiparvovec. The trend in postprandial TG levels was evaluated by taking hourly samples for nine hours and a single sample at 24 hours later.

Results: Here we present preliminary data of four patients treated with lomitapide for twenty-six weeks and the only patient that received tiparvovec in Italy.Area Under Curve of patients on lomitapide therapy were reduced roughly by 87% for TG, 27% for non-HDL-C, while no improvement was observerd for tiparvovec

Conclusions: Lomitapide was effective in improving post prandial metabolism of lipoproteins in subjects with FCS. No benefits were observed for tiparvovec.

SE015

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-03 HDL*

ELEVATED FREE CHOLESTEROL TRANSFER TO HDL UPON TRIGLYCERIDE-RICH LIPOPROTEIN LIPOLYSIS PROTECTS FROM INCIDENT MYOCARDIAL INFARCTION IN A PROSPECTIVE STUDY OF PATIENTS WITH TYPE 2 DIABETES

SAAG SESSION 04: ALL ABOUT TRIGLYCERIDES

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Background and Aims : Both low and extremely high concentrations of HDL-cholesterol (HDL-C) are associated with elevated cardiovascular risk, resulting in the U-shape relationship with cardiovascular disease (CVD). To explain this relationship, we proposed the reverse remnant cholesterol transport (RRT) hypothesis featuring, as a first step, transfer to HDL of free cholesterol from triglyceride-rich lipoproteins (TGRLs) upon lipolysis (Ma Feng et al. Europ J Prevent Cardiol 2019). To further assess our hypothesis, we evaluated whether the rate of this process was capable of predicting CVD in a prospective DIABHYCAR cohort involving patients with Type 2 diabetes followed for up to 6 years.

Methods: CVD cases (n=178) were matched according to age, sex and smoking habits to CVD-free controls (n=178). Statin treatment was excluded. Free cholesterol transfer to HDL upon TGRL lipolysis by lipoprotein lipase was measured using fluorescent TopFluor® cholesterol.

Results: The above-median free cholesterol transfer rate at baseline was associated with protection from incident acute myocardial infarction (AMI; odds ratio, 0.45 [0.23 to 0.91; p=0.026] vs. below-median). Adjustment for HDL-C and CV risk factors did not weaken this relationship. In contrast, the association between baseline HDL-C and incident AMI did not reach significance (odds ratio for above- vs. below-median, 0.69 [0.36 to 1.32], p=0.26). Elevated cholesterol transfer to HDL was also associated with reduced total mortality at a short follow-up of <4 years (p=0.02).

Conclusions: Free cholesterol transfer to HDL upon TGRL lipolysis in a pathway of RRT predicts incident CVD better than HDL-C, further strengthening links between HDL, triglyceride metabolism and atherosclerosis.

SE016

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-09 Lipid and lipoprotein metabolism*

SEARCHING FOR HYPERTRIGLYCERIDEMIA THROUGH ROUTINE LABORATORY TEST EXPLOITATION

SAAG SESSION 04: ALL ABOUT TRIGLYCERIDES

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Background and Aims : Familial Chylomicronemia Syndrome (FCS) is a rare genetic disorder of lipid metabolism characterized by severe hypertriglyceridemia and consequent risk of recurrent pancreatitis. Classical observational data suggest a 1:1,000,000 prevalence of FCS, however this estimate has not been validated.

Methods: Electronic medical records of the Niguarda Hospital, from January 2016 to December 2018, were retrospectively queried based on single fasting plasma TG levels of ≥880 mg/dL. After the exclusion of secondary causes of hypertriglyceridemia (diabetes, alcohol misuse, etc.) and responses to lipid-lowering treatment, probable patients with FCS were identified and underwent a clinical, biochemical and genetic evaluation.

Results: Out of 143615 charts queried with valid TG value, 116 patients with TG levels of ≥880 mg/dL were identified. Thirty-one subjects did not have any documented secondary causes of chylomicronemia and 15 were prospectively enrolled (age 46.9±14.7, 8 male). Median TG levels were 926 mg/dL (317-1141) and 9 subjects (60%) had history of at least one pancreatitis. FCS was clinically defined through FCS Score calculation in 8 subjects and prevalence of the disease was estimated in 0.008%. Molecular analysis of candidate genes confirmed FCS diagnosis in 5 patients (4 for mutations in *LPL* and 1 for *GPIHBP1*). Carriers of FCS causative mutations had higher TG levels and higher frequency of pancreatitis compared to non-genetic hypertriglyceridemia. Prevalence of FCS was recalculated in 0.006%.

Conclusions: FCS may be more common than classically estimated in a large hospital system and is likely underdiagnosed. Increasing efforts should be made toward early identification of FCS and specialist referral for this challenging disease.

SE017

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-02 Epidemiology of dyslipidemias*

HIGH TRIGLYCERIDE METABOLISM AND INCREASED MORTALITY: A POPULATION-BASED STUDY OF 30,000 INDIVIDUALS

SAAG SESSION 04: ALL ABOUT TRIGLYCERIDES

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Background and Aims : We tested the hypothesis that high triglyceride metabolism, marked by high plasma glycerol and β-hydroxybutyrate, is associated with increased all-cause mortality independently of elevated triglyceride-rich lipoproteins and body mass index (BMI).

Methods: We included 30,000 individuals nested within 109,751 individuals from the Copenhagen General Population Study. During a median follow-up of 11 years, 9,897 individuals died, while none were lost to follow-up. Two markers of triglyceride metabolism, plasma glycerol and β-hydroxybutyrate, were measured using nuclear magnetic resonance spectroscopy.

Results: In a multivariable adjusted model including triglycerides and BMI, higher plasma glycerol and β-hydroxybutyrate were each associated with higher risk of all-cause mortality. For glycerol, the multivariable adjusted hazard ratio for all-cause mortality was 1.34 (95%CI: 1.25–1.43) in individuals with glycerol>79.7 µmol/L (highest quartile) versus individuals with glycerol<52.0 µmol/L (lowest quartile). For β-hydroxybutyrate, the multivariable adjusted hazard ratio for all-cause mortality was 1.19 (1.12–1.27) in individuals with β-hydroxybutyrate >154.4 µmol/L (highest quartile) versus individuals with β-hydroxybutyrate <91.4 µmol/L (lowest quartile). For glycerol and β-hydroxybutyrate combined, individuals with both plasma glycerol and β-hydroxybutyrate above the median had the highest risk of all-cause mortality with a hazard ratio of 1.24 (1.18–1.31), when compared to individuals with both plasma glycerol and β-hydroxybutyrate below the median level.

Conclusions: We observed higher risk of all-cause mortality with higher triglyceride metabolism marked by higher levels of plasma glycerol and β-hydroxybutyrate. These novel findings implicate triglyceride metabolic rate as a risk factor for all-cause mortality independent of plasma triglyceride levels and BMI per se.

SE018

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-05 Inherited dyslipidemias*

NEXT GENERATION SEQUENCING IN SEVERE HYPERTRIGLICERIDEMIA: IDENTIFICATION OF A NOVEL NONSENSE MUTATION OF CREB3L3 GENE

SAAG SESSION 04: ALL ABOUT TRIGLYCERIDES

Federica Brucato[1], Roberto Scicali[2], Chiara Scrimali[1], Mariagrazia Gueli Alletti[1], Rossella Spina[1], Valeria Ingrassia[1], Grazia Ida Altieri[1], Davide Noto[1], Gabriella Misiano[1], Antonina Giammanco[1], Carlo M. Barbagallo[1], Antonina Ganci[1], Angelo Baldassare Cefalù[1], Francesco Purrello[2], Maurizio Averna[1]
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Background and Aims : Hypertriglyceridemia (HTG) is a common form of dyslipidemia associated with an increased risk of cardiovascular disease and pancreatitis. The severe forms are characterized by very high plasma levels of triglycerides (TG). Monogenic autosomal recessive forms are characterized by homozygous or compound heterozygous loss-of-function mutations of genes involved in the intravascular lipolysis of the triglyceride-rich lipoproteins, namely lipoprotein lipase, apolipoprotein C2, apolipoprotein A5, glycophosphatidylinositol (GPI)-anchored high-density lipoprotein-binding protein 1, lipase maturation factor 1, and glycerol-3-phosphate dehydrogenase 1. Mutations in CRE-binding protein 3–like 3 (CREB3L3) and glucokinase regulator have been associated to dominant familial hypertriglyceridemia.

Methods: We performed a NGS analysis to study the coding exons and intron/exon boundaries of genes affecting the main pathways of triglyceride synthesis and metabolism in outpatients with severe hypertriglyceridemia.

Results: In the majority of subjects no functionally mutations in the LPL, APOC2, APOA5, GPIHBP1, and LMF1 genes were detected. Two patients were found to be carriers of mutations in CREB3L3 gene. A 54 years old woman with very high TG levels (up to 1900 mg/dL) was found to be carrier of a novel nonsense heterozygous mutation (c.610C>T p.Arg204Ter) while a 51 years old woman with TG levels up to 1000 mg/dL was heterozygous for an already known pathogenic mutation (c.718G>A p.Glu240Lys). The p.Arg204Ter variant is predicted to result in the formation of a premature stop codon and synthesis of a truncated protein devoid of function.

Conclusions: NGS is a powerful tool for the genetic diagnosis of HTG and mutations of CREB3L3 gene may be associated with severe forms of hypertriglyceridemia.

SE019

Topic: *ASA01 - PATHOGENESIS OF ATHEROSCLEROSIS / ASA01-12 Other*

TAHINI CONSUMPTION EXHIBITS BENEFICIAL EFFECTS IN CARDIOVASCULAR INDICES AND ANTIOXIDANT BIOMARKERS IN HEALTHY MALES POSTPRANDIALLY.

SAAG SESSION 05: WE ARE WHAT WE EAT: MODULATING CVD RISK WITH DIET

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Background and Aims : Sesame (*Sesamum indicum* L.), rich in polyunsaturated fatty acids, proteins, vitamin E and lignans is well known for its antioxidant, antihypertensive, hypolipidemic and appetite control properties. The aim of the study was to investigate the postprandial effect of tahini (sesame paste) consumption on cardiovascular indices and oxidative stress biomarkers.

Methods: Research protocol was approved by the Medical Ethics Committee of 'Laiko' University Hospital and registered with ClinicalTrials.gov, where the full trial protocol can be accessed (Identifier: NCT04608747). After a 12-h fast, twenty healthy men (mean age: 28y, mean BMI: 25.81 kg/m^2) consumed 50g of tahini and blood and urine samples were obtained before and 1, 2, 3 and 4h postprandially. Assessment of hemodynamic parameters was performed at 0 and 4h. Blood glucose, triglycerides, vascular adhesion molecules (ICAM-1, VCAM-1, E-selectin), ferric-reducing ability of plasma (FRAP) and total phenolic content and urinary 8-iso-prostaglandin F2α were also measured.

Results: Plasma glucose was found to be significantly lower 1h ($p<0.001$), 3h ($p=0.032$) and 4h ($p<0.001$) after tahini consumption compared to baseline. Significant decrease in DBP ($p=0.010$) and pulse rate ($p=0.002$) was observed 4 h postprandially. Significant increases in serum triglycerides ($p<0.001$), flow-mediated dilatation ($p=0.022$) and urinary 8-iso-prostaglandin F2α levels ($p=0.016$) were observed 4h postprandially. Moreover, a trend of increase in total phenolic content ($p=0.092$) was observed 1 h postprandially. No changes were observed in other indices measured, compared to baseline.

Conclusions: This is the first study to report that tahini consumption can lower blood pressure, pulse rate, improve endothelial function and may exert antioxidant properties.

SE020

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-13 Other*

THE USE OF SEAWEED-DERIVED PHYTOSTEROLS TO DEFEAT ALZHEIMER'S DISEASE

SAAG SESSION 05: WE ARE WHAT WE EAT: MODULATING CVD RISK WITH DIET

Nikita Martens[1,2], Melissa Schepers[1,3], Na Zhan[2,4], Frank Leijten[2], Gardi Voortman-Minderman[2], Assia Tiane[1,3], Ben Rombaut[1,3], Janne Poisquet[1], Nienke Van De Sande[2], Anja Kerksiek[5], Folkert Kuipers[6], Johan W. Jonker[6], Hongbing Liu[4], Dieter Luetjohann[5], Tim Vanmierlo[1,3], Monique T. Mulder[2]
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Background and Aims : Accumulating evidence indicates a key role for a disturbed cerebral cholesterol transport in the development and progression of AD. We showed that memory of AD mice improves upon activation of brain cholesterol turnover by synthetic activators of liver X receptors (LXRα/β). However, serious side effects including hepatic steatosis render these LXRα/β activators unsuitable for patients. We found that the seaweed *Sargassum fusiforme*, containing preferential LXRβ-agonist 24(S)-saringosterol, prevented memory decline and reduced Aβ deposition in an AD mouse model without inducing hepatic steatosis. We examined the effects of 24(S)-saringosterol on cognition and neuropathology in AD mice.

Methods: Six-month-old male APPswePS1ΔE9 mice and wildtype C57BL/6J littermates received 24(S)-saringosterol (0.5 mg/25 g body weight/day) (APPswePS1ΔE9 n=20; C57BL/6J n=19) or vehicle (APPswePS1ΔE9 n=17; C57BL/6J n=19) via oral gavage for 10 weeks. Cognition was assessed using object recognition and object location tasks. Sterols were analyzed by gas chromatography/mass spectrometry, Aβ and inflammatory markers by immunohistochemistry, and gene expression by qPCR. Hepatic lipids were quantified after Oil-Red-O staining.

Results: Administration of 24(S)-saringosterol prevented cognitive decline in APPswePS1ΔE9 mice without affecting the Aβ plaque load. 24(S)-Saringosterol prevented the increase in inflammatory marker Iba1 in the hippocampus and cortex of APPswePS1ΔE9 mice. 24(S)-Saringosterol did not affect the expression of lipid metabolism-related LXR-response genes in the hippocampus nor the hepatic neutral lipid content.

Conclusions: Thus, administration of 24(S)-saringosterol prevented cognitive decline in APPswePS1ΔE9 mice independent of effects on Aβ load and without adverse effects on liver fat content. The anti-inflammatory effects of 24(S)-saringosterol may contribute to the prevention of cognitive decline.

SE021

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-11 Gut microbiome*

GUT LEAKAGE AND CARDIAC BIOMARKERS AFTER PROLONGED STRENUOUS EXERCISE IN HIGHLY TRAINED ENDURANCE ATHLETES

SAAG SESSION 05: WE ARE WHAT WE EAT: MODULATING CVD RISK WITH DIET

Susanne Kristine Aune[1,2], Martin Bonnevie-Svendsen[1,3], Christoffer Nyborg[1,3], Marius Trøseid[1,4], Ingebjørg Seljeflot[1,2,5], Jonny Hisdal[1,3], Ragnhild Helseth[2,5]
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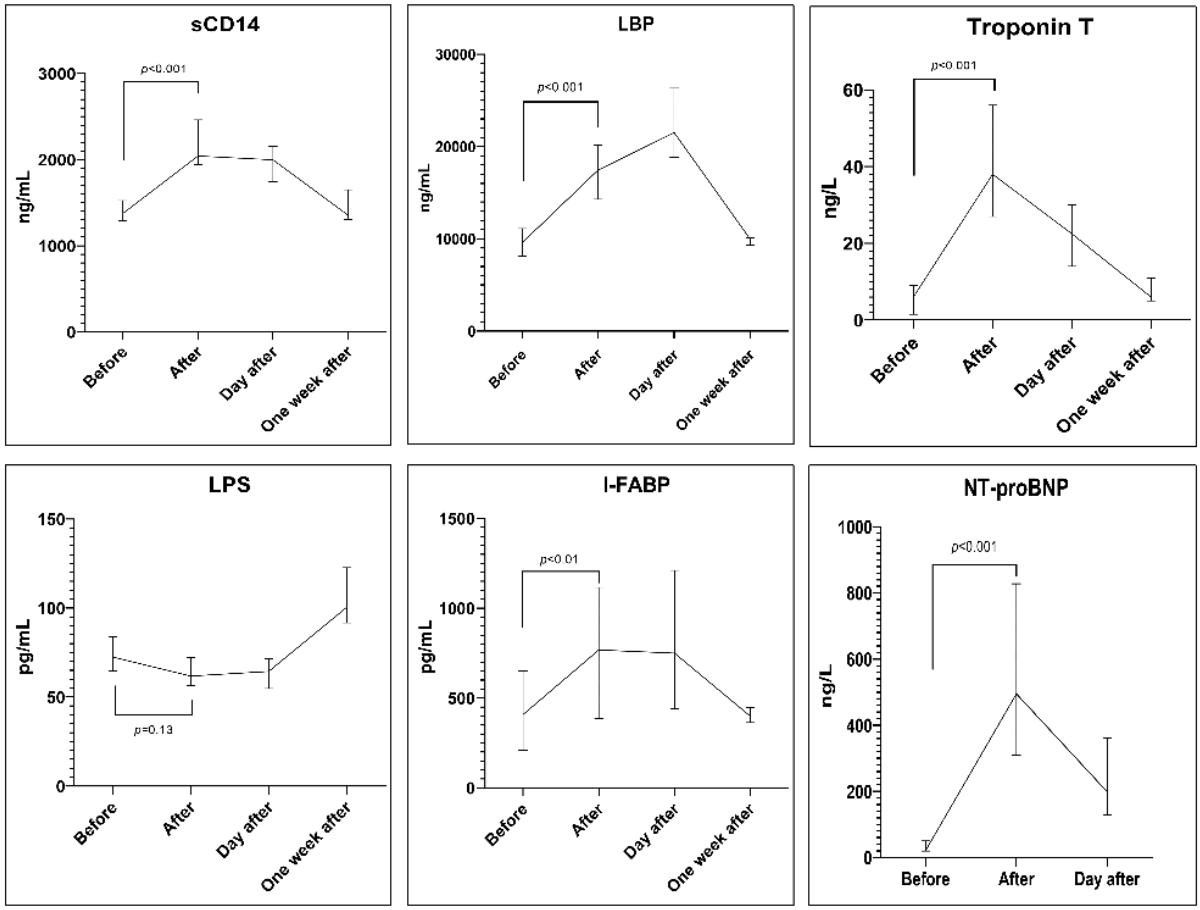
Background and Aims : Transient increase in the cardiac biomarkers Troponin T (TnT) and NT-proBNP are observed during strenuous exercise, even in healthy athletes. Gut leakage, the translocation of bacterial lipopolysaccharide (LPS) into the circulation, is associated with atherosclerosis and cardiovascular disease, but has also been reported after prolonged endurance exercise. We aimed to explore the link between exercise-induced gut leakage and cardiac biomarker release.

Methods: Participants in Norseman Xtreme Triathlon (Norseman) were included (*n*=42, age 43±9 years, 9 (21%) women). Blood samples were taken before, immediately after, and the day after the race for determination of gut leakage markers LPS, LPS-binding protein (LBP) and soluble cluster of differentiation 14 (sCD14), intestinal injury marker intestinal fatty-acid binding protein (I-FABP), TnT and NT-proBNP. Friedman tests, following Wilcoxon tests were applied for different time point comparisons, and Spearmans rho for correlation analyses.

Results: Median finish time was 14h 33min (13h 42min, 15h 29min). TnT and NT-proBNP increased significantly to 38 ng/L (27, 56) and 495 ng/L (310, 828) after the race ($p<0.001$, both). LBP and sCD14 also increased significantly ($p<0.001$, both), as did I-FABP ($p<0.01$). LPS remained unchanged ($p=0.13$). No significant correlations between changes in gut leakage markers and changes in cardiac markers were observed after adjusting for multiple testing ($p>0.05$,

all).



Conclusions: In this cohort of Norseman Xtreme Triathlon participants, both cardiac and gut leakage markers increased after strenuous exercise, but were not intercorrelated. Thus, in healthy athletes, the exercise-induced increase in cardiac biomarkers and gut leakage seem to occur independently.

SE022

Topic: *ASA04 - CLINICAL VASCULAR DISEASE / ASA04-07 Nutrition, nutraceuticals*

REGISTRY DATA INDICATE HIGHER RISK OF EATING DISORDERS IN INDIVIDUALS WITH FAMILIAL HYPERCHOLESTEROLEMIA COMPARED WITH AGE AND SEX MATCHED CONTROLS

SAAG SESSION 05: WE ARE WHAT WE EAT: MODULATING CVD RISK WITH DIET

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Background and Aims : Events of illness, loss of family members and maintaining a restrictive diet are some of many risk factors for developing an eating disorder. Familial hypercholesterolemia (FH) is a hereditary condition that predisposes for premature atherosclerotic cardiovascular disease and death. Consequently, medical and dietary treatment are initiated from childhood. The aim of this study was to examine whether having FH is associated with a risk of developing an eating disorder.

Methods: A prospective matched cohort comparing individuals with genetically verified FH (n=5602), with age and sex matched controls from the general population (n=110526). An incident of eating disorder was defined as ICD10 code F50 (primary or secondary diagnosis) from the Norwegian Patient Registry (hospital or special health service data) or the Norwegian Cause of Death Registry. Risk of eating disorder was analyzed using Cox regression (matched analysis) and expressed as hazard ratios (HR).

Results: Registry data (2008-18) revealed 35 cases of eating disorder in the FH population (frequency 0.62%) and 424 in the control group (frequency 0.38%). Individuals with FH showed higher risk of developing an eating disorder than controls (age and sex adjusted HR =1.65 (95% CI: 1.16-2.35)). Stratification on sex gave an excess risk in women with FH (HR=1.78 (95% CI: 1.24-2.54)), whereas men with FH did not possess same risk (HR=0.83 (95% CI: 0.20-3.41)).

Conclusions: Results from Norwegian registries suggests that individuals with FH have higher risk of developing eating disorders compared with age and sex matched controls. The results must be interpreted with caution due to the limited data.

SE023

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-03 Diabetes, insulin sensitivity and resistance*

RISK OF FRAGILITY FRACTURES IN INDIVIDUALS WITH DIABETES. AN OBSERVATIONAL AND MENDELIAN RANDOMIZATION STUDY.

SAAG SESSION 06: NOVEL PERSPECTIVES OF DIABETES

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Background and Aims : Smaller observational studies indicate that individuals with diabetes have a high risk of fragility fractures, possibly because of microvascular disease. We aimed 1) to investigate the observational risk of fragility fractures in individuals with diabetes, and 2) to use Mendelian randomization to assess whether glucose levels have a causal effect on fragility fracture risk.

Methods: We included 116,076 individuals from the Copenhagen City Heart Study and the Copenhagen General Population Study, whereof 548 individuals had a diagnosis of type 1 diabetes and 5,985 individuals a diagnosis of type 2 diabetes. First, we assessed the prospective risk of fragility fractures of the hip, spine, and arm as a function of diabetes status. Second, we used one-sample Mendelian randomization to assess the potential causal effect of high glucose concentrations on fracture risk using genetic variants known to be associated with elevated glucose levels as instrumental variables.

Results: Compared with individuals without diabetes, individuals with type 1 diabetes and type 2 diabetes had a higher risk of fragility fracture, with a hazard ratios (HR) of 1.50 (95% confidence interval (CI): 1.19-1.88) and 1.22 (1.13-1.32), respectively. In Mendelian randomization analysis, the risk ratio of fragility fracture was 1.46 (95%CI: 1.03-2.08) per 1mmol/L genetically instrumented higher glucose level.

Conclusions: In a general population setting, a diagnosis of type 1 or type 2 diabetes was prospectively associated with a high risk of fragility fracture. Mendelian randomization analysis supported a causal effect of high glucose levels on fragility fracture risk, suggesting that glycemia might have negative impact on bone or bone vasculature.

SE024

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-03 Diabetes, insulin sensitivity and resistance*

ACCELERATED VASCULAR AGEING AND RETENTION OF LDL IN TYPE 2 DIABETES

SAAG SESSION 06: NOVEL PERSPECTIVES OF DIABETES

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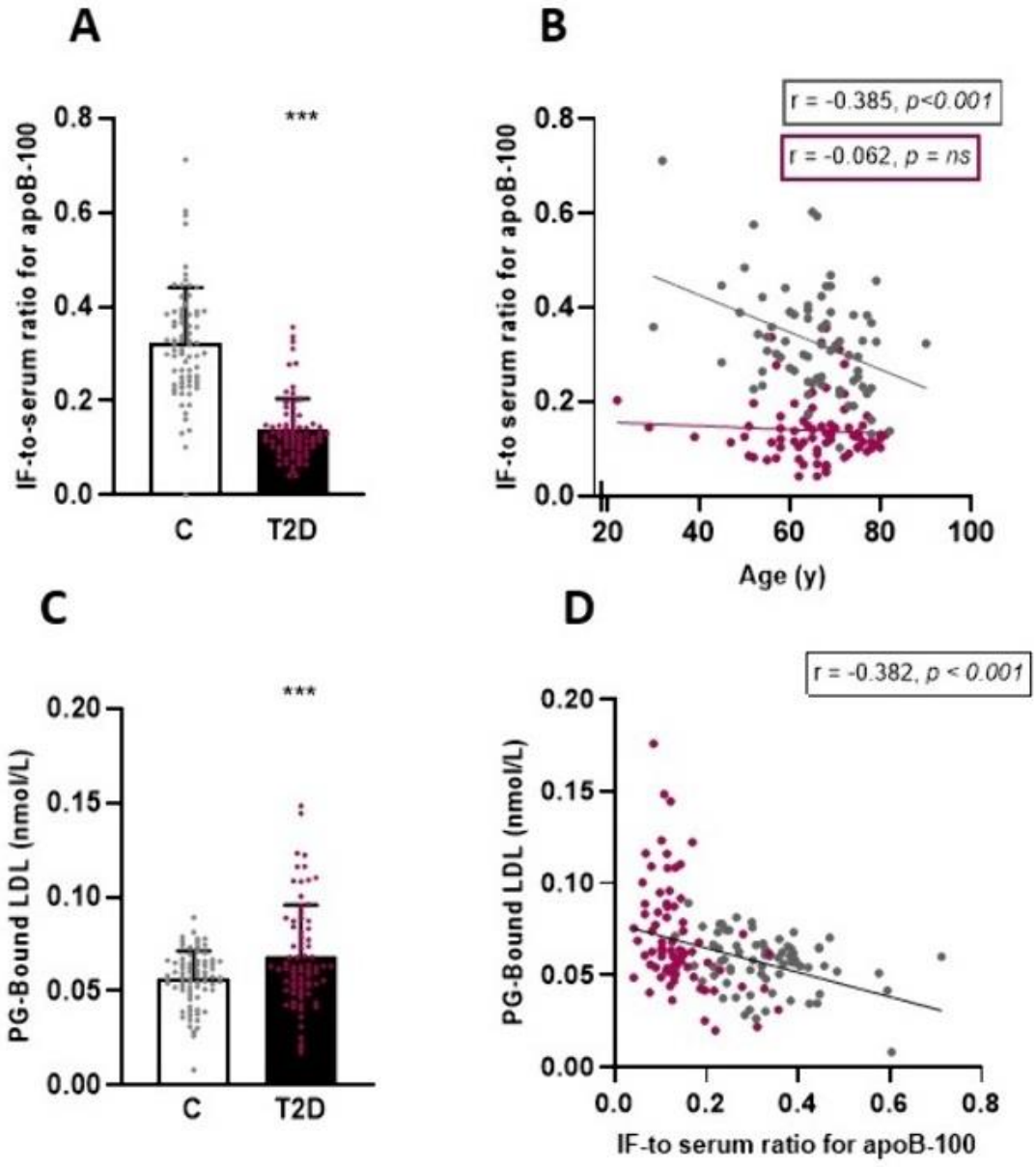
Background and Aims : Patients with Type 2 Diabetes (T2D) are disproportionately affected by atherosclerosis compared with non-diabetics. We previously reported that the interstitial fluid-to serum ratio (IF:S) for atherogenic lipoproteins is reduced in T2D. Further measures related to pathophysiological functions of atherogenic lipoproteins in serum and IF may unveil novel insights in transvascular transport of cholesterol in atherosclerosis.

Methods: We recruited 75 T2D patients and 75 age-and sex-matched controls and obtained serum and IF after overnight fast. IF collected from abdominal skin blisters was compared with serum obtained from peripheral blood. Cholesterol, TG, proteins, and lipoprotein lipids were determined using FPLC and ELISA The binding of serum, isolated VLDL, and LDL to proteoglycans (PGs) was evaluated through ex vivo binding to human aortic PGs.

Results: As anticipated, T2D displayed a significantly reduced IF:S of apoB-100 ($p<0.001$). A reduced IF:S for apoB-100 was linked to increasing age in controls ($r=-0.385$, $p<0.001$) but not in T2D. Serum and LDL from T2D patients had increased affinity for PGs ($p<0.001$), and enhanced binding was associated with a reduced IF:S for apo-B100 ($r=-0.336$ serum, $r=0.382$ LDL, $p<0.001$). VLDL binding to PGs did not differ between the

groups.



Conclusions: Serum LDL from T2D patients have an increased binding to PGs in association with a reduced transvascular ratio of LDL. In accordance with the “response to retention” hypothesis of atherosclerosis, this indicates an increased peripheral accumulation of LDL cholesterol in T2D, which can be seen as a process of “premature ageing”.

SE025

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-08 Novel risk factors and biomarkers*

PLASMA APOLIPOPROTEIN CONCENTRATIONS AND NEW-ONSET DIABETES IN SUBJECTS WITH PREDIABETES

SAAG SESSION 06: NOVEL PERSPECTIVES OF DIABETES

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Background and Aims : To investigate the association of plasma apolipoprotein concentrations with the incidence of new-onset diabetes (NOD) in subjects with prediabetes.

Methods: NOD was assessed in 307 participants with impaired fasting glucose levels (fasting plasma glucose [FPG]: 110–125 mg/dL). NOD was defined as a first FPG value ≥ 126 mg/dL during follow-up. Apolipoprotein plasma concentrations were determined by mass spectrometry. Kaplan–Meier curves were drawn using a ternary approach based on terciles and incident NOD. The association between plasma apolipoproteins and the incidence of NOD was determined using Cox proportional-hazard models.

Results: During a median follow-up of 5-year, 115 participants (37.5%) developed NOD. After adjustment for age, sex, BMI, FPG, HbA_{1c}, and statin use, the plasma levels of apoC-I, apoC-II, apoC-III, apoE, apoF, apoH, apoJ, and apoL1 were positively associated with the risk of NOD. After further adjustment for plasma triglycerides, apoE (1 SD natural-log-transformed hazard ratio: 1.28 [95% CI: 1.06; 1.54]; p = 0.010), apoF (1.22 [1.01; 1.48]; p = 0.037), apoJ (1.24 [1.03; 1.49]; p = 0.024), and apoL1 (1.26 [1.05; 1.52]; p = 0.014) remained significant. Besides, Kaplan–Meier survival curves showed that patients with lowest plasma apoE levels (<5.97 mg/dL) had significantly less risk of NOD (log-rank test, p = 0.002).

Conclusions: The plasma apoE concentrations were positively associated with the risk of NOD in individuals with prediabetes, independently of traditional risk factors. The positive but non-causal association of apoF, apoJ, and apoL1 levels with the risk of NOD also pave the way for further investigations.

SE026

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-04 Lipoprotein receptors*

THE LOW-DENSITY LIPOPROTEIN RECEPTOR FUELS THE CELLULAR CHOLESTEROL-MTORC1 AXIS REQUIRED FOR CD8+ T CELL ACTIVATION

SAAG SESSION 07: CELL TALK TIME: RECEPTORS AND INTRACELLULAR TRAFFICKING

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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 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 cells. T cells from FH (familial hypercholesterolemia) patients, carrying mutations in the LDLR gene, were tested.

Results: LDLR mRNA and protein expression increased after in vitro activation of CD8, but not CD4, 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.

SE027

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-08 Cellular lipid metabolism and lipid droplets*

GENOME-WIDE CRISPR-CAS9 SCREEN TO IDENTIFY NEW PROTEINS INVOLVED IN INTRACELLULAR CHOLESTEROL TRANSPORT

SAAG SESSION 07: CELL TALK TIME: RECEPTORS AND INTRACELLULAR TRAFFICKING

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Background and Aims : Cholesterol is a fundamental component for cellular survival. LDL cholesterol is transported into the cell via endocytosis by the LDL receptor. Cholesterol is released in lysosomes and transported to other cellular structures. Not all proteins involved in this transport to the ER are identified. Therefore, we aimed to set up a genome-wide CRISPR-Cas9 screen to identify unknown proteins involved in intracellular cholesterol transport.

Methods: To study this, Hec-1B cells were used. These cells lack the gene encoding SQLE, a rate-limiting enzyme for cholesterol production. Therefore, they are fully dependent on exogenous cholesterol. The cells were cultured under several circumstances and the effect on their growth was measured.

Results: We showed that these cells will not survive for seven days without cholesterol being released from the lysosomes. Moreover, introducing the SQLE gene into the cells made the cells survive in sterol-depleted medium. Therefore, Hec-1B cells ± SQLE will be infected with a genome-wide sgRNA CRISPR library and cultured in lipid-derived medium supplemented with LDL. The cells will be cultured for 12 days and cells with important genes affected will starve within this time frame. DNA of the surviving cells will be cross-referenced to the genes in the library. Missing genes are either important housekeeping genes, which will be a hit in both cell lines, or are involved in cholesterol transport, which will only be absent in the parental cells.

Conclusions: Using the Hec-1B cell line, we have set up a sensitive genome-wide live-dead CRISPR-Cas9 screen to identify proteins involved in intracellular cholesterol transport.

SE028

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-08 Cellular lipid metabolism and lipid droplets*

LINKING CELLULAR LIPID METABOLISM PROFILES TO THE OUTCOMES OF CHOLESTEROL-LOWERING THERAPY IN A GENERAL POPULATION COHORT STUDY

SAAG SESSION 07: CELL TALK TIME: RECEPTORS AND INTRACELLULAR TRAFFICKING

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Background and Aims : Alterations in cellular lipid metabolism are linked to differential treatment outcomes in familial hypercholesterolemia patients. However, whether similar effects exist in the general population is not known.

Methods: Methods: Previously, we established a multiparametric analysis platform to quantify lipid uptake and storage in leukocytes from human subjects (BioRxiv, doi:10.1101/2021.04.19.440471). Here we use this pipeline to analyse 400 subject samples from the FINRISK 2012 cohort study, including 200 recipients of cholesterol-lowering medication. For each subject we have access to drug reimbursement, NMR-metabolomics and clinical follow-up data.

Results: We observe large inter-individual variation of LDL uptake and lipid mobilization, the velocity with which cells deplete their lipid reservoirs, varying up to 8-fold. In subjects on a high potency statin, LDL uptake shows a negative correlation with LDL-cholesterol as well as cholesterol and cholesterol esters in VLDL and LDL particles. In the same subject group LDL uptake displays a positive correlation with triglycerides in VLDL particles. These correlation profiles are reduced in subjects on less potent statins and absent in control individuals. Likewise, lipid mobilization shows a differential correlation profile with certain lipid types and lipoprotein subclasses, which is dependent on whether the subjects are on cholesterol-lowering therapy.

Conclusions: Our study highlights that each individual has a defined cellular lipid uptake and storage potential, providing deeper insight into differential treatment outcomes of cholesterol-lowering therapy in the general population.

SE029

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-09 Lipid and lipoprotein metabolism*

CONSEQUENCES OF (INTESTINAL) LAL DEFICIENCY ON WHOLE BODY LIPID METABOLISM

SAAG SESSION 07: CELL TALK TIME: RECEPTORS AND INTRACELLULAR TRAFFICKING

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Background and Aims : Lysosomal acid lipase (LAL) is the only enzyme responsible for the degradation of cholesteryl esters and triglycerides in the lysosome at an acidic pH. Mutations in its gene cause two rare autosomal recessive diseases, depending on the residual activity of the enzyme. One of the most common symptoms of LAL deficiency is lipid malabsorption throughout the small intestine accompanied by macrophage infiltration. We aim to investigate the consequences of whole-body and intestinal LAL deficiency on lipid metabolism and absorption.

Methods: We collected three parts of the small intestine (duodenum, jejunum, ileum) and livers from mice with a global (LAL KO) or intestine-specific deletion of LAL (iLAL KO) and wild-type littermates. We isolated RNA and protein and quantified lipids. Lipoprotein secretion and cholesterol absorption were also assessed.

Results: We found massive lipid accumulation in the small intestine of LAL KO mice, particularly in macrophages in the lamina propria, associated with elevated cholesterol absorption. Despite drastically reduced LAL activity in iLAL KO enterocytes, villus morphology, lipid concentrations, and expression of lipid transporters and inflammatory genes were unaltered in the small intestine of iLAL KO mice.

Conclusions: Although small intestinal LAL expression and lipid accumulation are substantial in LAL KO mice, iLAL KO animals do not recapitulate this phenotype. Therefore, loss of LAL in enterocytes alone is not sufficient to cause lipid deposition in the small intestine, implying that macrophages play an important role in this process. Further studies are needed to determine the involvement of LAL in enterocytes and macrophages in lipid absorption.

SE030

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-09 Lipid and lipoprotein metabolism*

ANGIOPOIETIN-LIKE 3 (ANGPTL3) RESIDES ON HDL AND LDL WITH THE LATTER FORM HAVING THE HIGHEST LIPASE INHIBITORY ACTIVITY

SAAG SESSION 07: CELL TALK TIME: RECEPTORS AND INTRACELLULAR TRAFFICKING

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Background and Aims : Angiopoietin-like 3 (ANGPTL3) is an inhibitor of lipoprotein lipase (LPL) and endothelial lipase (EL). The impact of ANGPTL3 lowering has been shown to depend on the type of dyslipidemia: reduced LDL cholesterol in patients with familial hypercholesterolemia and reductions in plasma triglycerides in patients with chylomicronemia. This differential effect may be related to a difference in the association of ANGPTL3 with lipoproteins. We therefore hypothesized that ANGPTL3 resides on lipoproteins and that this affects its ability to suppress lipase activity.

Methods: To investigate whether ANGPTL3 resides on LDL and/or HDL *ex vivo*, recombinant ANGPTL3 was incubated with ultracentrifugation-isolated LDL and HDL fractions derived from healthy volunteers. In addition, plasma from healthy volunteers and HDL deficient patients (due to rare genetic variants in *ABCA1* or *LCAT*) was fractionated by fast protein liquid chromatography and distribution of ANGPTL3 among lipoprotein fractions was determined by ELISA. ANGPTL3 activity was studied by measuring lipolysis and uptake of ^{3}H-trioleate by brown adipocyte T37i cells.

Results: *Ex vivo* binding experiments revealed that ANGPTL3 associates to both HDL and LDL. In healthy volunteers, approximately 75% of lipoprotein-associated ANGPTL3 resides in HDL fractions whereas patients without HDL carried 50% of their ANGPTL3 in LDL fractions. Unbound ANGPTL3 did not suppress T37i lipase activity but when given with HDL or LDL, ANGPTL3 suppressed lipase activity by 21.4±16.4% (p=0.03) and 25.4±8.2% (p=0.006) , respectively.

Conclusions: ANGPTL3 preferentially resides on HDL but can also be found on LDL where it has its highest lipase inhibitory activity.

SE031

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-13 Other*

CHARACTERIZATION OF THE LPS AND 3OHFA CONTENTS IN THE LIPOPROTEIN FRACTIONS AND LIPOPROTEIN PARTICLES OF HEALTHY SUBJECTS

SAAG SESSION 07: CELL TALK TIME: RECEPTORS AND INTRACELLULAR TRAFFICKING

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Background and Aims : Atherosclerosis is a chronic inflammatory disease that is caused by the accumulation of LDL particles in the intima, causing the activation of immune cells and triggering an inflammatory response. LPS is a potent activator of the innate immune response and it can be transported by lipoproteins. Since humans are much more sensitive to LPS than other mammals, and very low amounts of LPS can cause inflammation and elicit an immune response, the aim of this study is to characterize the distribution of LPS and its immunogenic portion, the 3-hydroxy fatty acids (3OHFAs), among all lipoprotein types of healthy subjects.

Methods: A group of 25 healthy subjects was studied. VLDL, IDL, LDL and HDL fractions were separated by ultracentrifugation and the amount of each 3OHFA was measured by MS in each lipoprotein fraction to calculate LPS concentration. Lipoprotein particle concentrations were measured by NMR.

Results: LDL and HDL fractions transported the highest concentration of LPS (35.7% and 31.5% respectively), followed by IDL (18.9%) and VLDL (13.9%); but VLDL particles carried much more LPS molecules per particle (0.55 molecules/particle) than LDL or HDL ($p<0.01$). The distribution of LPS and all 3OHFAs among lipoprotein fractions showed high interindividual variability.

Conclusions: These findings suggest that LPS may be studied as a potential biomarker and may help understand the role of LPS in atherosclerosis in those cases where the disease cannot be explained by traditional risk factors.

SE032

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-09 Lipid and lipoprotein metabolism*

REAL-WORLD USE OF PCSK9 INHIBITORS IN FAMILIAL HYPERCHOLESTEROLEMIA: RESULTS FROM THE ITALIAN PCSK9I AIFA REGISTRIES.

SAAG SESSION 08: OPTIMIZE TREATMENT IN FAMILIAL HYPERCHOLESTEROLEMIA

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Background and Aims : Background. Data on the real-world use of PCSK9 inhibitors (PCSK9i) in FH are limited. **Aims.** Evaluate the pattern of prescription and benefit of PCKS9i in clinical practice in Italy.

Methods: Methods. Clinical information was extracted from the Italian PCSK9i AIFA Registries, which included patients who were prescribed with alirocumab or evolocumab from February 2017 through December 2019. 3033 patients with heterozygous (HeFH) (23.7% genotyped) and 81 with homozygous FH (HoFH) (46.9% genotyped) were considered.

Results: Results. At baseline, the majority were receiving maximal lipid-lowering therapies (LLTs) and 42.4% of HeFH and 28.4% of HoFH reported statin intolerance. Mean LDL-C levels were 199.1±52.7 mg/dl in HeFH and 245.9±103.8 mg/dl in HoFH. Evolocumab and alirocumab were prescribed in 47% and 53% of HeFH, respectively; HoFH received only evolocumab. At the data cutoff, 2562, 1973 and 1025 HeFH and 69, 56 and 30 HoFH patients had a potential follow-up of 6, 12 and 24 months, respectively. At 6-month follow-up, LDL-C fell by 56% to 88 mg/dl in HeFH and by 45% to 147 mg/dl in HoFH. This reduction was similar at the different time-points. Overall, 39% of HeFH and 17% of HoFH achieved recommended EAS/ESC LDL-C goals.

Conclusions: Conclusion. The use of PCSK9i resulted in about 50% reduction in LDL-C. Approximately 2 over 5 HeFH and 1 over 6 HoFH achieved the recommended LDL-C goals. These results show that the addition of PCSK9i is very useful in managing FH, even though the full achievement of EAS/ESC LDL-C goals in severe FH may require multiple LLTs.

SE033

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-05 Inherited dyslipidemias*

PREDICTIVE FACTORS OF STATIN INITIATION DURING CHILDHOOD IN A COHORT OF 245 CHILD-PARENT PAIRS WITH FAMILIAL HYPERCHOLESTEROLEMIA: IMPORTANCE OF GENETIC DIAGNOSIS

SAAG SESSION 08: OPTIMIZE TREATMENT IN FAMILIAL HYPERCHOLESTEROLEMIA

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Background and Aims : Children with heterozygous familial hypercholesterolemia (HeFH) are undertreated despite international guidelines advocating for early statin initiation during childhood; dramatically increasing their risk of premature atherosclerotic cardiovascular disease (ASCVD).The objective of the study was to identify childhood and parental factors associated with statin early initiation in HeFH children to promote early treatment.

Methods: Design, Setting and Participants: National register-based (REFERCHOL) multicenter, retrospective and prospective cohort study. We selected HeFH children aged 8-18 years and their FH parents, followed between 2014 and 2020. Demographic and clinical characteristics at last visit to the lipid clinic were collected. Vascular damage in parents was defined as a history of ASCVD, and/or a coronary artery calcium score above 100, and/or at least one carotid stenosis (>50%). **Main outcome:** Statin initiation in HeFH children.

Results: We included 245 child-parent pairs. The children age was 14±3 years, and only 135 (58%) were under statin treatment. In multivariate analysis, the predictive childhood factors associated with being

treated by a statin were: genetic diagnosis (OR=2.5, 95%CI [1.3; 4.9], p=0.01), older age (OR=4.4, 95%CI [1.8; 10.6], p=0.01), and longer follow-up duration (OR=1.3, 95%CI [1.1; 1.6], p<0.001); whereas the predictive parental factor associated with child treatment was the presence of vascular damage (OR=2.4, 95%CI [1.0; 5.7], p=0.04).

Conclusions: A positive genetic diagnosis during childhood and vascular damage in parents were independently associated with statin treatment in HeFH children. Genetic diagnosis seems an important tool for cardiovascular prevention in these future adults.

SE034

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-05 Inherited dyslipidemias*

NUTRITIONAL APPROACH IN PAEDIATRIC PATIENTS WITH FAMILIAL HYPERCHOLESTEROLAEMIA

SAAG SESSION 08: OPTIMIZE TREATMENT IN FAMILIAL HYPERCHOLESTEROLEMIA

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Background and Aims : Cardio-protective diet and promotion of healthy lifestyle habits are worldwide recognised as first-step treatments of Familial Hypercholesterolemia (FH), yet their impact on lipid profile is still poorly studied in paediatric patients. The aim of this retrospective, observational study on good clinical practice is to investigate the effect of nutritional and lifestyle intervention on plasma lipid profile and dietary habits in FH children at the first step of treatment.

Methods: 63 FH children (mean age 7.9 years, 36 female) were included (on-going study). Tailored nutritional advice was given to each patient, according to STEP one indications. Dietary habits were evaluated through the Food Frequency Questionnaire (FFQ), physical and sport activity were evaluated asking each patient how many hours did they spent every week practicing physical and/or sport activities, blood samples for lipid profile were collected at first access (T0) and after six months (T1). T test for paired samples and Wilkoxon signed rank test were used for the analysis.

Results: the lipid profile at T0 and T1, mean±standard deviation (mg/dl) was: total cholesterol: 283.6±50.3 and 266.2±44.1 (p<0.01), LDL-C: 214.7±47.4 and 197.9±45.7 (p<0.01), non-HDL-C: 230.3±48.8 and 212.2±45.8 (p<0.01), HDL-C: 53.3±13.6 and 53.9±12.1 (p=0.09), triglycerides 90.1±46.9 and 84.6±34.3 (p=0.5). In the dietary habits (weekly portions) we observed an improvement (p≤001) for fruit, vegetables, fish, pulses, whole foods, and a reduction (p<0.01) for meat, sausages, cheese, junk foods consumption.

Conclusions: in our cohort of FH paediatric patients plasma lipid profile and dietary habits are improved after targeted nutritional and lifestyle treatment.

SE035

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-05 Inherited dyslipidemias*

TREATMENT GAPS IN PAEDIATRIC PATIENTS WITH HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLAEMIA

SAAG SESSION 08: OPTIMIZE TREATMENT IN FAMILIAL HYPERCHOLESTEROLEMIA

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Background and Aims : Patients with Homozygous Familial Hypercholesterolaemia (HoFH) require intensive combination lipid lowering therapy (LLT) from diagnosis to avoid premature atherosclerotic cardiovascular disease (ASCVD). However, not all LLT are licensed for use in children and adolescents. We aimed to compare use of LLT in children, adolescents and adults with HoFH.

Methods: We extracted descriptive data from two international databases for patients who were <18 years old at the time of HoFH diagnosis. We analysed the relationship between number of LLT prescribed (including apheresis) and achieved LDL-C, stratified by age at last database record.

Results: Baseline characteristics of the 412 patients included are shown in Table 1. Median age at diagnosis and at last database record was 6.0 (IQR 3.0-9.5) and 17.0 (IQR 10.0-27.8) years old, respectively. Despite similar untreated LDL-C levels, children (aged 0-10 years) had higher overall on-treatment LDL-C levels than adolescents and adults (11.1 [9.4-14.6], 9.3 [6.0-12.1] and 8.6 [5.9-12.3] mmol/L respectively, p<0.001). Among patients aged 0-10 years, those who received lipoprotein apheresis had lower treated LDL-C levels compared to those who did not (Figure 1). Number of LLT prescribed increased with age (Figure 1). Among those who were <18 years old at last database record, ASCVD was already present in 79/216 patients (37%) (Table

Table 1 - Baseline characteristics of patients diagnosed with Homozygous Familial Hypercholesterolemia before the age of 18 years old

	Overall (n=412)
Male – n (%)	200 (49)
Age of diagnosis – mean (SD)	6.87 (4.66)
Xanthomata (any type) at diagnosis – n (%)	329/374 (88)
Corneal arcus at diagnosis – n (%)	73/371 (20)
BMI (kg/m²) – median (IQR)	21.73 [18.66, 24.84]
Hypertension – n (%)	37/392 (9)
Untreated lipids (mmol/L) – median (IQR)	
Total cholesterol	19.79 [17.02, 23.14]
LDL-C	17.80 [14.70, 20.73]
HDL-C	0.88 [0.70, 1.10]
Triglycerides	1.25 [0.90, 1.73]
Genetic diagnosis – n (%)	286/412 (69)
Two *LDLR* variants	265/286 (93)
Other*	21/286 (7)
***LDLR* functionality– n (%)**	240/265 (91)
Defective/Defective	131 (55)
Defective/Negative	30 (13)
Negative/Negative	79 (33)
ASCVD <18 years old – n (%)**	79/216 (37)

BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; HDL-C high-density lipoprotein cholesterol; *LDLR*, low-density lipoprotein receptor; ASCVD, atherosclerotic cardiovascular disease

*Other diagnoses include: homozygous *LDLRAP1*, double heterozygous (*LDLR* + *PCSK9* / *LDLR* + *APOB*), clinically HoFH but no mutation found.

**Composite of myocardial infarction, percutaneous intervention, carotid artery bypass grafting, angina pectoris, peripheral artery disease, ischemic cerebrovascular disease, aortic valve replacement, aortic stenosis

1).

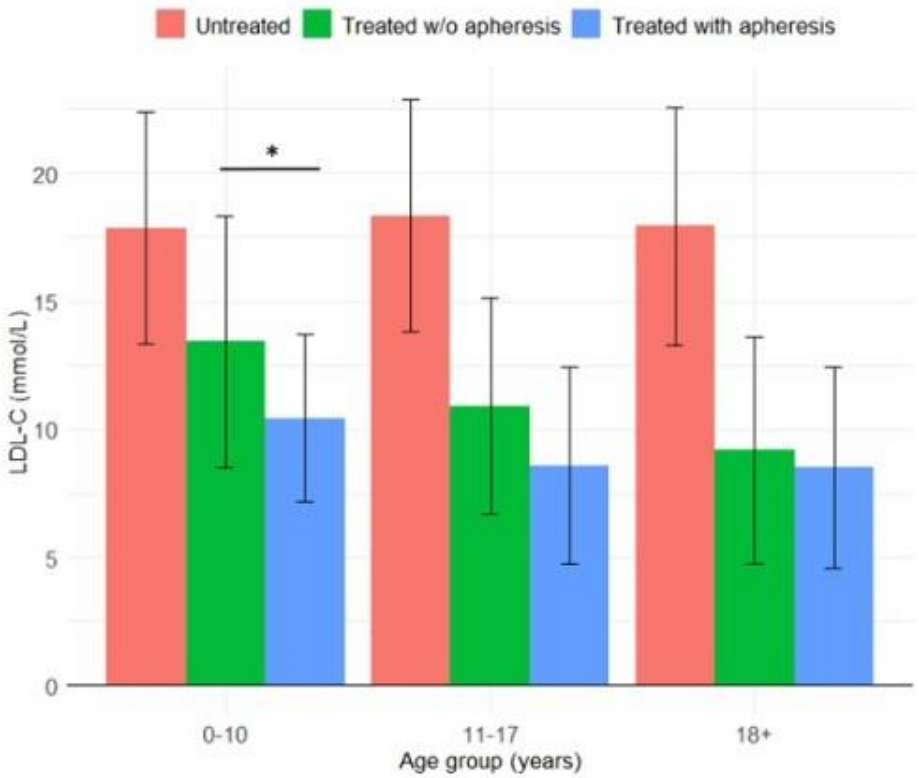

Figure 1 – Untreated and on-treatment LDL-C levels in HoFH patients per age group at last database record.

Age-group (years)	0-10	11-17	≥18
Number	105	111	194
Number of LLT † (mean, SD)	1.5 (1.0)	2.1 (1.2)	2.4 (1.1)
On lipoprotein apheresis – n (%)	57 (54%)	68 (61%)	97 (50%)

LDL-C, low-density lipoprotein cholesterol; HoFH, homozygous familial hypercholesterolemia; LLT, lipid-lowering therapy. Bars represent mean±SD values.
* p<0.05. Comparisons between untreated and treated groups all p<0.01, bars not shown.
† Including lipoprotein apheresis

Conclusions: Although LDL-C generally remains uncontrolled in paediatric patients with HoFH, use of multiple LLT therapies including apheresis is associated with lower LDL-C levels. This emphasizes the importance of making combination therapies accessible to all patients with HoFH regardless of age to prevent premature ASCVD.

SE036

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-05 Inherited dyslipidemias*

CORONARY ARTERY CALCIUM IS STRONGLY ASSOCIATED WITH PULSE WAVE VELOCITY AND LDL-CHOLESTEROL BURDEN IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA

SAAG SESSION 08: OPTIMIZE TREATMENT IN FAMILIAL HYPERCHOLESTEROLEMIA

Antonina Giammanco[1], Alessandro Mattina[2], Angelo Baldassare Cefalù[1], Giulio Geraci[1], Davide Noto[1], Emilio Nardi[1], Carlo M. Barbagallo[1], Francesca Fayer[1], Rossella Spina[1], Francesco D'Ignoto[1], Antonella Cardella[1], Ludovico La Grutta[3], Tommaso Smeraldi[4], Massimo Midiri[4], Maurizio Averna[5]
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Background and Aims : Familial hypercholesterolemia (FH) is a genetic disorder characterized by high plasma levels of low-density lipoprotein cholesterol (LDL-C) and severe cardiovascular (CV) diseases. Coronary artery calcification (CAC) assessment and arterial stiffness measured as pulse wave velocity (PWV) are accurate in CV risk assessment, but data on HeFH are lacking. Furthermore, aortic stenosis evaluated by Doppler echocardiography with markers of severity, aortic valve area (AVA) and mean gradient (MG) are still unresolved. In this study we evaluated CAC, PWV and the relationship aortic valve calcium/stenosis severity in a population of HeFH patients in order to improve risk stratification and therapy timing.

Methods: One-hundred genetically characterized HeFH patients were recruited at our outpatient clinic. In all patients,CAC, PWV measurement and LDL-C burden calculation were assessed. Physiologic/structural determinants of aortic valve area (AVA)/mean gradient (MG) relationship associated with aortic stenosis were also evaluated.

Results: Mean age was 45±16 years. 25% of patients had hypertension;15% were in secondary prevention. On univariate analysis, we found strong positive correlations between CAC and both PWV ($r=0.52$ $p>0.0001$) and total LDL-C burden ($r=0.52$ $p<0.0001$). No other associations with lipid parameters were found. Multivariate analysis showed that CAC was independently associated with PWV adjusted for sex, total LDL-C burden, systolic blood pressure, smoking, LDL-C, HDL-C and statin treatment.

Conclusions: Arterial stiffness is independently correlated with CAC in HeFH patients with similar total LDL-C burden and CV risk profile. The assessment of PWV in HeFH patients could represent a valuable tool to refine the CV risk and therapeutic choices.

SE037

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-08 Novel risk factors and biomarkers*

VON WILLEBRAND FACTOR AS A PREDICTOR OF RECURRENT CARDIOVASCULAR EVENTS IN PATIENTS WITH PREMATURE CORONARY ATHEROSCLEROSIS

SAAG SESSION 09: THROMBOSIS AND BLEEDING IN ATHEROSCLEROSIS

Soreiia A. Urazgildeeva[1,2,3], Irina V. Alekseeva[4], Lubov V. Vasina[5], Stepan G. Grigoriev[6], Mariya V. Muzalevskaya[2], Elena V. Baranova[7], Valeria D. Shurygina[3]
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Background and Aims : Von Willebrand factor (vWF) is known as a key player in thrombogenesis, but also considered to be a marker of endothelial dysfunction. However, there is still no consensus on the importance of vWF level as an additional risk factor of cardiovascular events (CVE). **The Objective** of our study was to estimate the link between the recurrence of CVE and the level of vWF in patients with premature coronary atherosclerosis.

Methods: In two-year prospective study 80 middle-aged patients with premature coronary atherosclerosis, manifested as acute coronary syndrome under the age of 55 for males and 60 for females were investigated. The vWF levels were determined by EIA kit (Uscn Life Science Inc., Cloud-Clone Corp., USA). Incidence of new CVE was evaluated.

Results: The vWF levels were found to be within the normal range (7.35-20.0 ng/ml) in 12 patients. In the rest 68 patients the vWF levels were elevated: 57.43±18.66 ng/ml. During the follow-up 13 new CVE were registered only in patients with increased levels of vWF. The most important factors of the new CVE (age, body mass index, vWF level, hyperglycemia, carotid atherosclerosis) were identified by discriminant analysis and included in the prognostic model. Statistical significance of the model was highly reliable ($p<0.001$) and represented 92.5% coincidence of the forecast results with the observation results. The level of vWF demonstrated the highest significance in predicting the risk of recurrent CVE.

Conclusions: Elevated levels of vWF may be considered as a predictor of new CVE in patients with premature coronary atherosclerosis.

SE038

Topic: *ASA04 - CLINICAL VASCULAR DISEASE / ASA04-06 Aneurysms and other non-atherosclerotic arteriopathies*

METHOTREXATE ASSOCIATED WITH A LIPID CORE NANOPARTICLE PREVENTED THE DILATION AND DISSECTION OF THE AORTIC ARCH IN MICE WITH MARFAN SYNDROME.

SAAG SESSION 09: THROMBOSIS AND BLEEDING IN ATHEROSCLEROSIS

Maria Carolina Guido[1], Natalia M. Lopes[1], Camila I. Albuquerque[1], Elaine R. Tavares[1], Leonardo Jensen[2], Priscila O. Carvalho[1], Lygia V. Pereira[3], Ricardo R. Dias[4], Francisco R.M. Laurindo[5], Raul C. Maranhão[1]
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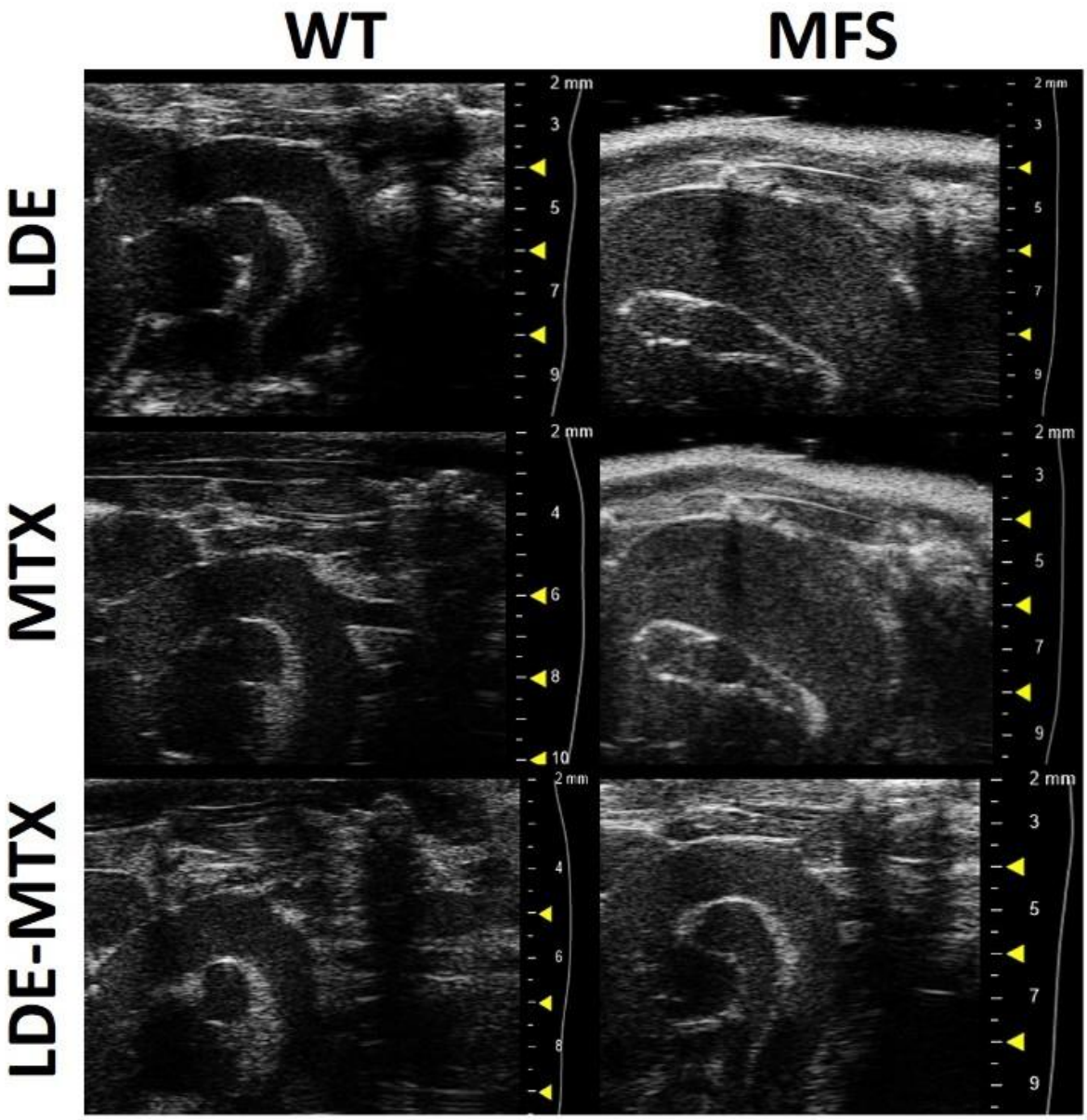
Background and Aims : Fibrillin-1 mutations result in rupture of the aorta, the main cause of mortality in patients with Marfan syndrome (MFS). When MTX is associated to LDE, the cell uptake is increased, which endows MTX with enhanced action mechanisms and the drug toxicity is diminished. The aims of this study was to investigate whether treatment with LDE-MTX can prevent the development of aortic arch lesions in a murine model of MFS.

Methods: MFS and wild-type mice were allocated in 3 groups of treatment: LDE only; commercial MTX; LDE-MTX. The treatment occurred weekly at a dose of 1mg/kg, between the 3rd and 6th month of life. The animals were submitted to echocardiography, morphometry and protein expression of the aortic arch.

Results: LDE-MTX showed smaller lumen area of ascending and descending aorta and aortic arch in MFS mice. LDE-MTX also decreased aortic dissections and the protein expression of the CD68, CD3, TNF-α, caspase 3 and type 1 collagen. Moreover, LDE-MTX reduced TGF-β, ERK1/2 and the SMAD3 expression. CD68 and CD3 expression was positively correlated with the lumen area of the aortic arch, indicating the importance of inflammation for the aortic dilation. The increase in bioavailability of intracellular adenosine in LDE-MTX was suggested by the higher expression of A2a adenosine receptor and the lower expression of adenosine

deaminase.



Conclusions: By increasing the bioavailability of intracellular adenosine, LDE-MTX reduce the processes of inflammation, apoptosis and fibrosis that are consequent to fibrillin-1 mutation. By these means, LDE-MTX may prevent the development of the dilation and dissection in the aortic arch.

SE039

Topic: *ASA04 - CLINICAL VASCULAR DISEASE / ASA04-10 Anti-thrombotic therapies*

RIVAROXABAN ATTENUATES VALVULAR CALCIFICATION IN PATIENTS WITH SEVERE AORTIC STENOSIS

SAAG SESSION 09: THROMBOSIS AND BLEEDING IN ATHEROSCLEROSIS

Michal Zabczyk[1,2], Magdalena Kopytek[1], Jacek Tarasiuk[3], Sebastian Wronski[3], Anetta Undas[1,2], Joanna Natorska[1,2]
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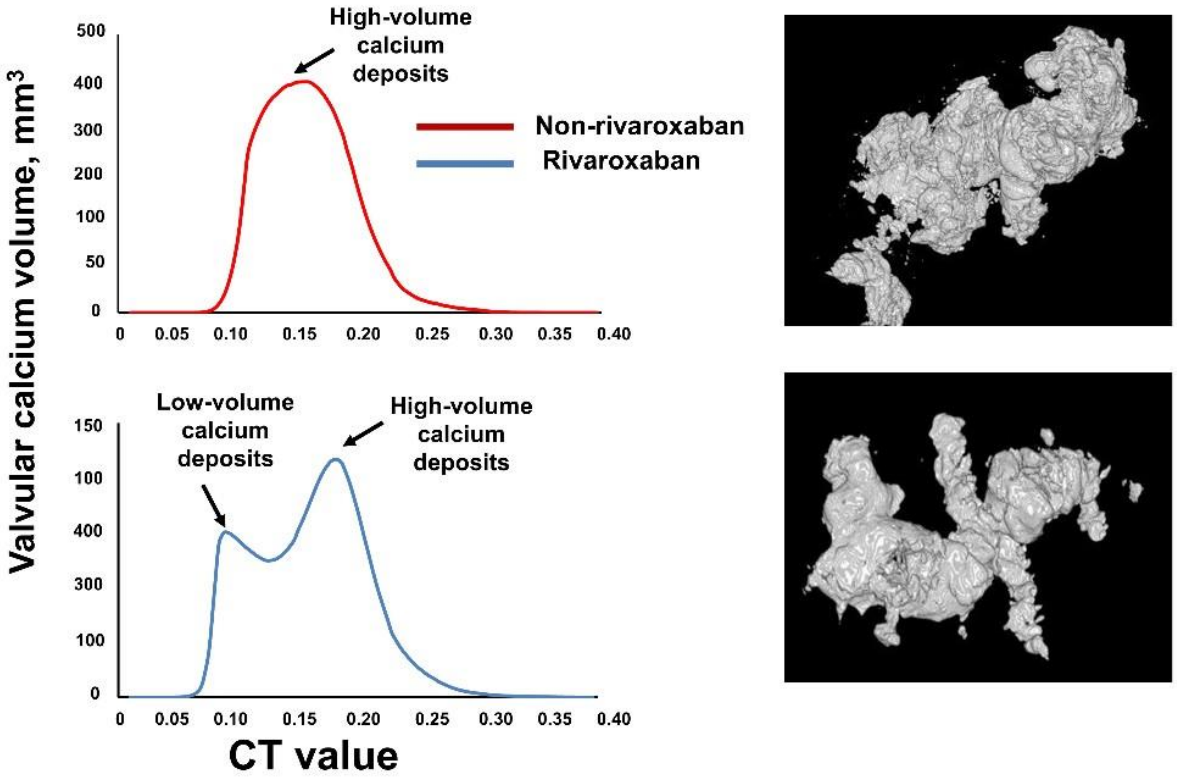
Background and Aims : It has been shown that rivaroxaban attenuates calcification of atherosclerotic plaque in mice. It is unclear whether rivaroxaban at therapeutic concentrations can inhibit aortic valves calcification in patients with aortic stenosis (AS).

Methods: We enrolled 30 patients with severe AS aged 66±7.3 years (meangradient 53mmHg, max gradient 88mmHg), including 19 individuals with AS and concomitant atrial fibrillation taking rivaroxaban (20mg/daily for at least 2 years, AS-RIVA). Stenotic aortic valves were obtained during valve replacement surgery. Valvular calcification was estimated ex vivo using micro-computed tomography (micro-CT). Total calcification volume, high-density calcification and soft, less mineralized calcification were measured. Valvular expression of NFκB and osteocalcin was evaluated by immunostaining.

Results:

FIGURE 1.



Micro-CT analysis showed 30% lower calcium volume in AS-RIVA valves compared to patients not taking rivaroxaban (110.75 [98.5-126.1]mm^3 vs. 365.2 [276.5-474.5]mm^3, P<0.001) (Fig 1). Moreover, the soft calcification (calculated as a ratio of soft calcification volume/total volume) was 24.7% higher in AS-RIVA valves. Valvular expression of NFκB and osteocalcin was 44% and 33% decreased in AS-RIVA patients, compared to those without such treatment. Solely in AS-RIVA patients the expression of both proteins was associated with the soft calcium volume (r=0.67, p=0.003), but not with high-density calcification or total calcium volume.

Conclusions: Rivaroxaban can inhibit the increase in valvular calcium volume. Our study might suggest that rivaroxaban, even at a low dose, could slow down the rate of AS progression, at least in AS patients with mild-to-moderate AS and indications for anticoagulant therapy. Supported by the Polish National Science Centre (UMO-2018/29/B/NZ5/02629).

SE040

Topic: *ASA04 - CLINICAL VASCULAR DISEASE / ASA04-09 Lipid-lowering therapies*

FINAL RESULTS FROM THE PAN-EUROPEAN OBSERVATIONAL HEYMANS STUDY SUGGEST A MISMATCH BETWEEN GUIDELINES AND PCSK9I REIMBURSEMENT CRITERIA

SAAG SESSION 10: DO WE SUCCEED IN DYSLIPIDEMIA TREATMENT IN REAL WORLD?

Kausik K. Ray[1], Eric Bruckert[2], Pasquale Perrone-Filardi[3], Christoph Ebenbichler[4], Anja Vogt[5], Ian Bridges[6], Mahendra Sibartie[7], Nafeesa Dhalwani[8]
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Background and Aims : We report the final analysis from a cohort study that 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 lowering therapy (LLT) and lipid values were collected from medical records.

Results: Overall, 1,951 patients were enrolled (baseline characteristics: Table 1). The mean study follow-up was 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). For attainment of recommended LDL-C goals see Figure 1. At evolocumab initiation and during follow-up, background oral LLT did not materially change: 41-44% did not receive statin or ezetimibe, 40-42% received statin ± ezetimibe, 11-14% received statin without ezetimibe.

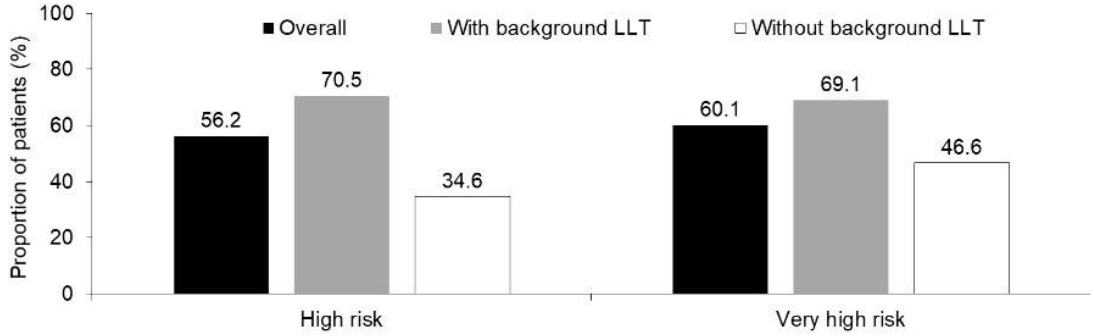
Conclusions: In Europe, evolocumab-treated patients had baseline LDL-C levels almost 3x higher than the present threshold for PCSK9i use recommended in guidelines, likely reflecting disparities between local reimbursement criteria and guidelines. Although evolocumab led to a >50% median reduction in LDL-C, only ~60% of patients achieved an LDL-C <1.4mmol/L. LDL-C goal attainment was higher among patients receiving evolocumab with background LLT. However, despite this, ~40% received evolocumab as monotherapy. Expanded use of PCSK9i combined with moderate/high-intensity statin and/or ezetimibe may increase the likelihood of achieving current LDL-C

goals.

Table 1 – Patient characteristics and background LLT at baseline

Characteristic	All patients (N=1951)
Male sex, n (%)	1219 (62)
Mean (SD) age, years	60 (11)
Primary prevention, n (%)	300 (15)
Secondary prevention, n (%)	1651 (85)
History of intolerance to any statin, n (%)	1175 (60)
Background LLT at baseline	
No background LLT, n (%)	780 (40)
Statin (± ezetimibe), n (%)	823 (42)
Statin without ezetimibe, n (%)	251 (13)
Ezetimibe without statin, n (%)	348 (18)

Figure 1 – 2019 ESC/EAS LDL-C goal[1] attainment by subgroup



1. Mach F *et al. Eur Heart J* 2020;41:111–88; LLT, lipid-lowering therapy, i.e. statin ± ezetimibe

SE041

Topic: *ASA04 - CLINICAL VASCULAR DISEASE / ASA04-09 Lipid-lowering therapies*

EFFICACY & SAFETY OF PCSK9-INHIBITORS: A SYSTEMATIC REVIEW AND META-ANALYSIS OF REAL-WORLD DATA

SAAG SESSION 10: DO WE SUCCEED IN DYSLIPIDEMIA TREATMENT IN REAL WORLD?

Janneke W.C.M. Mulder[1], Kim Steward[1], Annette M.H. Galema-Boers[1], Eric Boersma[2], Jeanine E. Roeters Van Lennep[1]
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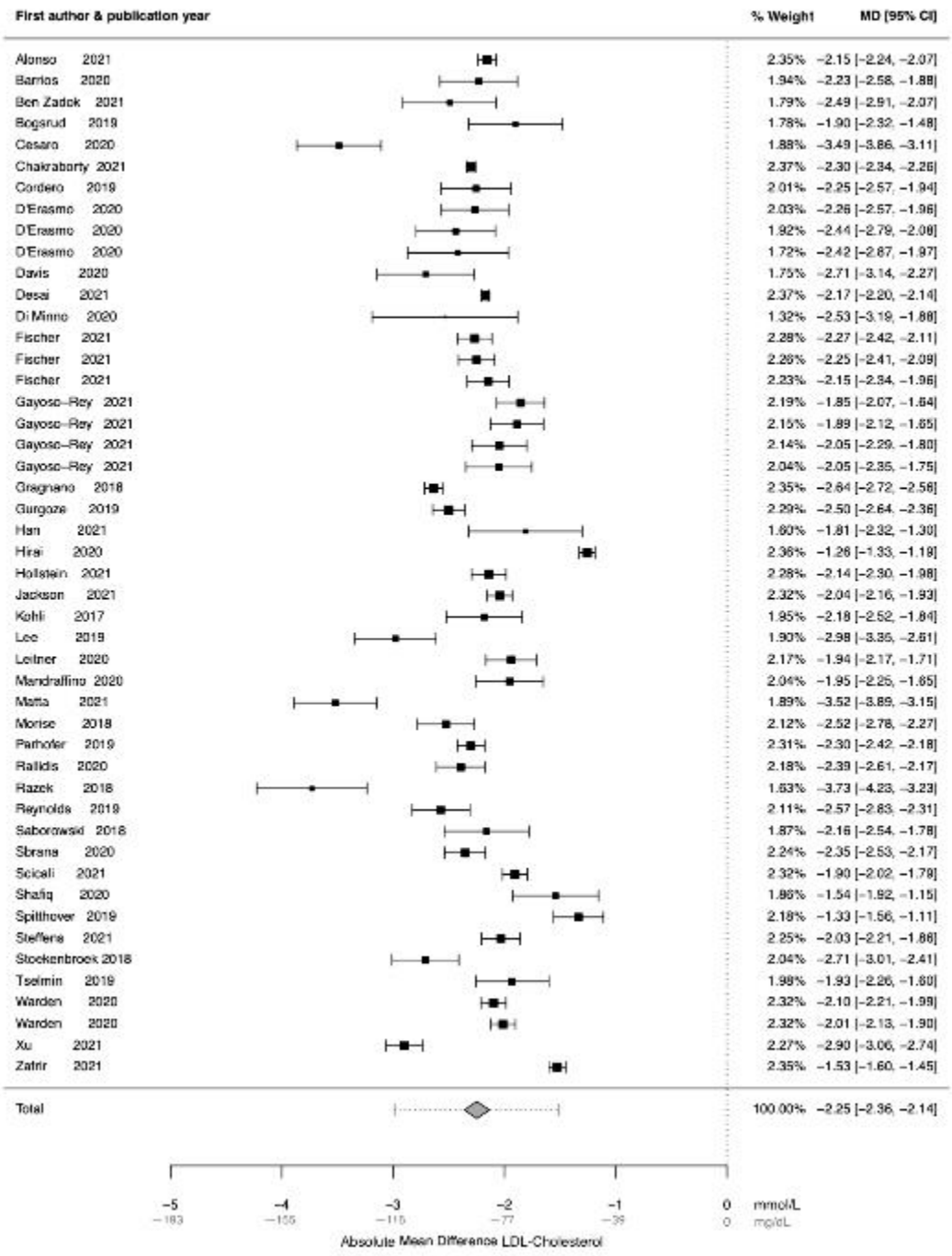
Background and Aims : To investigate the real-world efficacy and safety of PCSK9-inhibitors.

Methods: A systematic search of observational studies with PCSK9-inhibitor use of >=3 months was performed through Embase, Medline, Web of Science Core Collection, and Google Scholar up to 1 June 2021. Study selection, data extraction and risk of bias were conducted independently by 2 authors. The primary outcome for efficacy was absolute LDL-reduction and for safety side-effects were reported. A random-effects meta-analysis and mixed-effects regression model was performed.

Results: 67 studies were included involving 28,266 patients using PCSK9-inhibitors in clinical setting, 44% women, mean age 60±4 years, 71% with CVD, 70% Familial Hypercholesterolemia (FH), 59%/41% using Evolocumab/Alirocumab, 59% statin co-medication, and 57% ezetimibe. Mean follow up was 8.9 ±6.5 months and mean adherence was 86.2% ±9.3.

Efficacy: The absolute reduction in LDL-cholesterol after initiation of PCSK9 inhibition was -2.25 mmol/L (p= <.0001; 40 studies; 13,416 participants) corresponding to a 54.6% LDL-cholesterol reduction. In the meta-regression, no influence of sex, statin-use, FH, or follow-up duration of PCSK9-inhibitor use was found for efficacy. Whereas baseline LDL-cholesterol was correlated with absolute LDL-reduction (β 0.5257, p<.0001). *Safety:* Side-effects were experienced by 26.2% ±22.4 of patients. The most common side-effects were flu-like symptoms (12.0% ±14.4), any pain or discomfort (8.5% ±8.2), myalgia (6.6% ±5.5), and injection site reactions (4.9% ±4.1). During follow-up 23.0% stopped and restarted their PCSK9-inhibitors. At the end of follow-up, 11.3% of the total population discontinued PCSK9-inhibitor of

which 47.8% due to side-



effects.

Conclusions: Real-world data of PCSK9-inhibitors show comparable efficacy and safety to trial data.

SE042

Topic: *ASA04 - CLINICAL VASCULAR DISEASE / ASA04-09 Lipid-lowering therapies*

UNCHANGED HAZARD RATIOS OF INCIDENT ACUTE MYOCARDIAL INFARCTION IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA DURING 2001-2017

SAAG SESSION 10: DO WE SUCCEED IN DYSLIPIDEMIA TREATMENT IN REAL WORLD?

Karianne Svendsen[1,2], Liv J. Mundal[3], Kirsten B. Holven[3,4], Martin P. Bogsrud[5], Jannicke Igland[6,7], Trond P. Leren[5], Kjetil Retterstøl[2,4]
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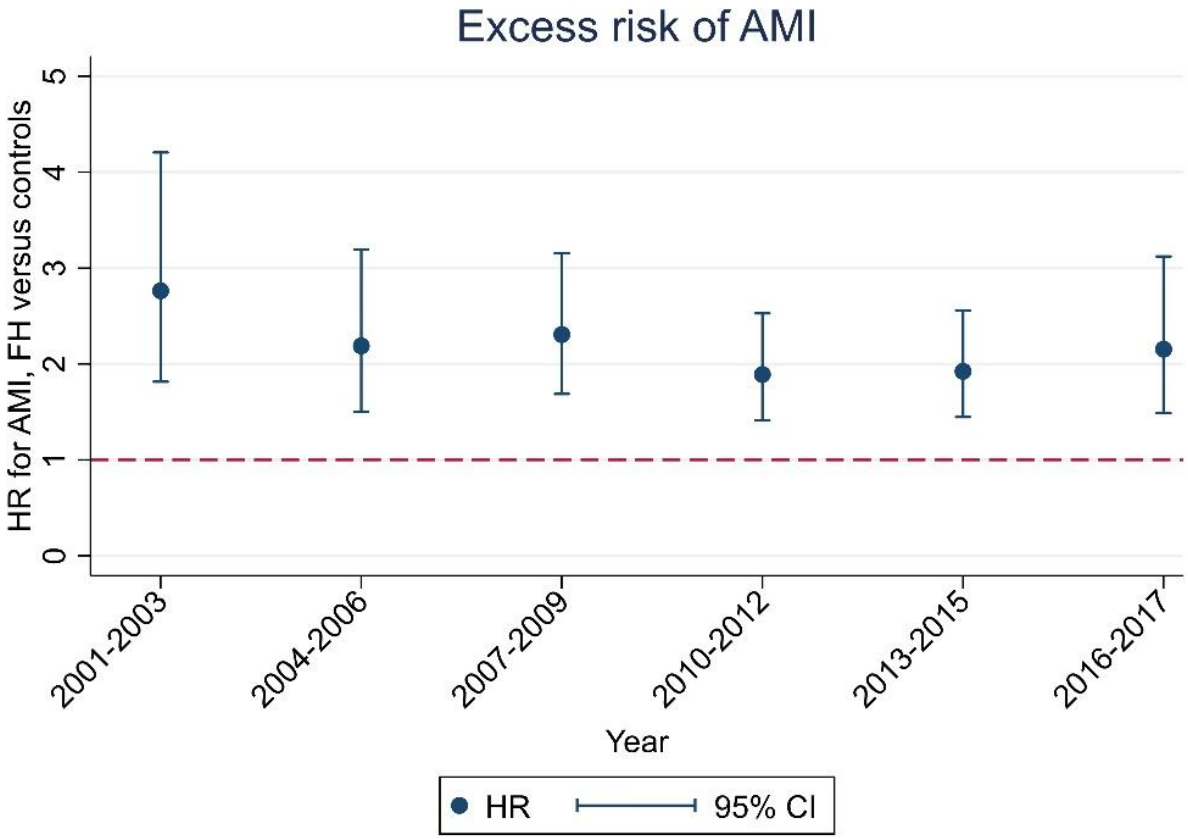
Background and Aims : Ideally, adequate treatment of familial hypercholesterolemia (FH) should reduce the risk of acute myocardial infarction (AMI) to that of the population in general. Therefore, we have studied the risk of AMI among Norwegian FH patients during 17 years with effective medication easily available

Methods: This is a prospective matched cohort study of 5,635 subjects with genetically verified FH and 112,589 age and sex matched controls. We used Norwegian Health registers to track all hospitalization for AMI from 1994-2017, but we subtracted the first 7 years (wash-out period) to be able to study incident AMI. Follow-up time in this study was therefore from 2001 until hospitalizations with AMI, death or December 31, 2017, whichever occurred first. An incident event of AMI was defined as a hospitalization with AMI as primary or secondary diagnosis or a death with AMI as an underlying cause without any prior hospitalizations with AMI. Using Cox proportional Hazards regression with stratification on matched case-set, HR comparing risk of AMI in FH versus controls was calculated.

Results: From 2001 to 2017 the HR of AMI was about 2 in Norwegian FH patients (all ages combined) compared to controls (Fig1). HR remained unchanged during the years. We will present age standardized

incidence rates at the conference.



Conclusions: There was no change in excess risk of AMI during 17 years observation in people with genetically verified FH compared to age and sex adjusted controls from the general population of Norway

SE043

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-05 Inherited dyslipidemias*

IDENTIFICATION, CHARACTERISTICS AND MANAGEMENT OF ADULTS WITH HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLAEMIA IN HIGH AND NON-HIGH INCOME COUNTRIES PARTICIPATING IN THE EAS FAMILIAL HYPERCHOLESTEROLAEMIA STUDIES COLLABORATION (FHSC)

SAAG SESSION 10: DO WE SUCCEED IN DYSLIPIDEMIA TREATMENT IN REAL WORLD?

Antonio J. Vallejo-Vaz[1,2,3], Christophe A. Stevens[4], Kanika I. Dharmayat[4], Alexander R. Lyons[4], Julia Brandts[3], Tomas Freiberger[5], G.K. Hovingh[6], John J. Kastelein[6], Pedro Mata[7], Frederick J. Raal[8], Raul Santos[9], Handrean Soran[10], Gerald F. Watts[11], Alberico L. Catapano[12], Kausik K. Ray[13]
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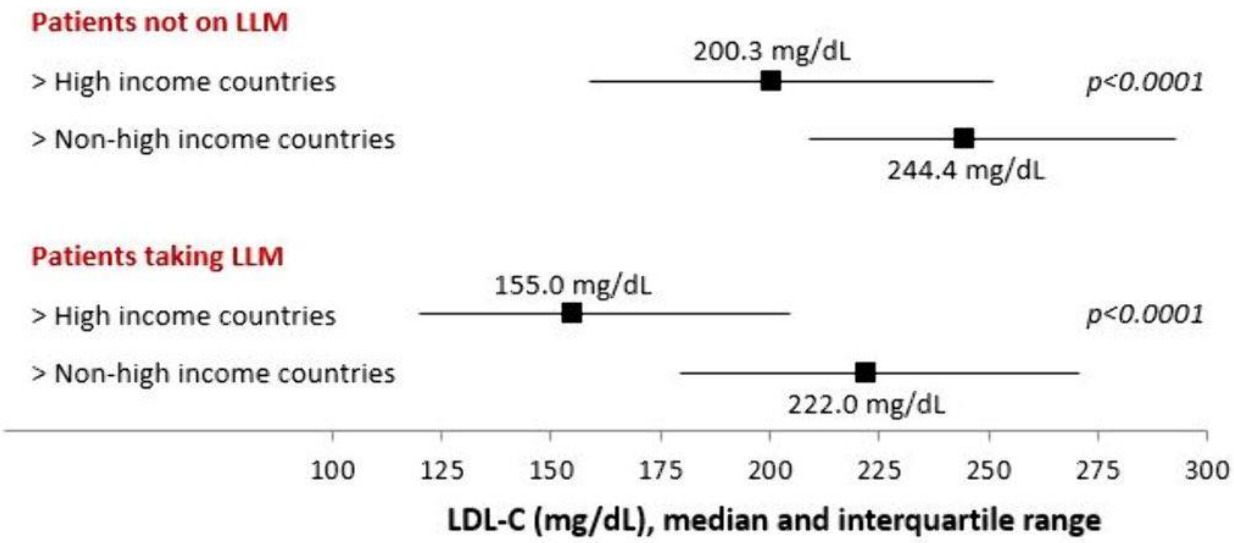
Background and Aims : Factors such as the availability and accesibility to different resources (e.g. genetic testing, effective lipid-lowering medications), access to healthcare and health policies in place (e.g. screening programmes) may impact on the time when FH patients are identified, their clinical presentation and how patients are treated, and it might ultimately result in health inequalities in FH care. In this study we examine potential disparities in the identification, characteristics and management of adults with heterozygous FH (HeFH) between high (HIC) versus non-high (non-HIC) income countries in the EAS FH Study Collaboration (FHSC) global registry.

Methods: We conducted a cross-sectional assessment of patients ≥18 years old with a clinical (probable or definite) and/or genetic diagnosis of HeFH at the time the patients were entered into the FHSC registry. Countries were grouped in HIC and non-HIC according to the 2021 World Bank definition of income categories.

Results: Among 37,972 patients included, 32,281 (85.0%) corresponded to HIC and 5,691 (15.0%) to non-HIC. A confirmed diagnosis of HeFH with a positive genetic testing occurred in 93.0% of cases from HIC vs. 54.9% of cases from non-HIC. Index cases represented a 30.4% and 53.4% of HIC and non-HIC cohorts, respectively. Characteristic of patients and LDL-cholesterol levels are shown in Table 1/ Figure 1.

Table 1.	High income Countries, all	Non-high income countries
Women	53.6%	57.2%
Age at registry entry, years	45.5 (33.7 – 57.9)	50.0 (39.0 – 59.8)
Age at FH diagnosis, years	44.0 (32.0 – 56.3)	48.0 (37.1 – 57.0)
Hypertension	14.7%	36.9%
Diabetes mellitus	4.2%	10.1%
Body mass index, kg/m^2	24.9 (22.4 – 27.9)	26.6 (23.9 – 30.0)
Coronary artery disease	13.9%	33.4%
Stroke	459 (1.8%)	108 (2.9%)

Figure 1.



Conclusions: HeFH adult patients from HIC, vs non-HIC, are more frequently diagnosed genetically and identified younger, with less cardiovascular risk factors or established cardiovascular disease, and with lower LDL-C levels. The higher identification of non-index cases in HIC suggests a greater implementation of cascade screening in HIC.

SE044

Topic: *ASA04 - CLINICAL VASCULAR DISEASE / ASA04-09 Lipid-lowering therapies*

BASELINE CHARACTERISTICS AND MANAGEMENT OF HIGH- AND VERY HIGH-RISK PATIENTS WITH HYPERCHOLESTEROLEMIA OR MIXED DYSLIPIDEMIA IN THE MULTINATIONAL OBSERVATIONAL SANTORINI STUDY

SAAG SESSION 10: DO WE SUCCEED IN DYSLIPIDEMIA TREATMENT IN REAL WORLD?

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Background and Aims : The 2019 ESC/EAS lipid guidelines recommended more intensive lipid goals. It is presently unknown what lipid-lowering treatments (LLT) are being used and what proportion of high and very high-risk patients achieve these new lower goals.

Methods: SANTORINI is a multinational observational study (NCT04271280) including adult patients with high and very high CV risk as assessed by the investigator, requiring LLT, and recruited from 14 European countries across primary and secondary care settings. Baseline characteristics, medical history, and LLT are reported.

Results: 9044 of 9606 recruited patients had available data through to August 2021. 29.2% of patients were classified by the investigator as high-risk and 70.8% as very high-risk. Among high-risk patients, the majority had evidence of atherosclerotic cardiovascular disease (ASCVD; Table 1). Mean (SD) LDL-C was 2.68 (1.29) and 2.30 (1.16) mmol/L for high- and very high-risk patients, respectively (Table 1). Despite LDL-C levels being above the recommended values, 21.9% of patients had no documented LLT and 54.1% received monotherapy (50.1% statins, 1.8% ezetimibe, 1.7% proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors, 0.6% other oral LLT). Combination LLT was used in 24.0% of patients, including 16.0% receiving statin plus ezetimibe, 4.5% taking a PCSK9 inhibitor plus oral LLT,

and 3.4% taking any other oral combination therapy (Table

Table 1. Baseline characteristics and cardiovascular risk factors by risk classification as reported by investigator

Characteristic	Overall (N=9044)	Risk classification as reported by investigator	
		High-risk (N=2637)	Very high-risk (N=6401)
Female, n (%)	2481 (27.4)	998 (37.8)	1481 (23.1)
Age, years, mean (SD)	65.3 (10.90)	63.5 (11.72)	66.0 (10.45)
ASCVD, n (%)			
Myocardial infarction	3780 (41.8)	429 (16.8)	3349 (52.3)
Stroke	584 (6.5)	102 (3.9)	482 (7.5)
Coronary artery disease	6088 (67.3)	839 (31.8)	5245 (81.9)
Unstable angina	999 (11.0)	131 (5.0)	868 (13.6)
Transient ischaemic attack	358 (4.0)	67 (2.5)	291 (4.5)
Peripheral arterial disease	1305 (14.4)	226 (8.6)	1079 (16.9)
Cerebrovascular disease	1027 (11.4)	200 (7.6)	827 (12.9)
Coronary artery bypass graft	1107 (12.2)	124 (4.7)	982 (15.3)
Percutaneous transluminal coronary angioplasty	4608 (50.9)	534 (20.2)	4073 (63.6)
Diabetes, n (%)	3038 (33.6)	882 (33.4)	2154 (33.6)
Diabetes with target organ damage)	610 (6.7)	125 (4.7)	485 (7.6)
Familial hypercholesterolemia, n (%)	893 (9.9)	413 (15.7)	480 (7.5)
LDL-C, mean (SD), mmol/L	2.41 (1.213)	2.68 (1.291)	2.30 (1.161)

Missing/not reported risk status, n=6. ASCVD, atherosclerotic cardiovascular disease; BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; SD, standard deviation

Table 2. Lipid lowering treatment by risk classification as reported by investigator

LLT, n (%)	Overall (N=9044)	Risk classification as reported by investigator	
		High-risk (N=2637)	Very high-risk (N=6401)
No LLT	1980 (21.9)	625 (23.7)	1351 (21.1)
Monotherapy			
Statin alone	4530 (50.1)	1431 (54.3)	3097 (48.4)
Ezetimibe alone	158 (1.8)	50 (1.9)	108 (1.7)
PCSK9i alone	150 (1.7)	32 (1.2)	118 (1.8)
Any other oral LLT alone	57 (0.6)	20 (0.8)	37 (0.6)
Combination therapy			
Statin + Ezetimibe	1446 (16.0)	306 (11.7)	1140 (17.8)
PCSK9i + other oral LLT	411 (4.5)	94 (3.6)	317 (4.9)
Any other oral combination therapy	311 (3.4)	78 (3.0)	233 (3.6)

Data from one patient were missing. LLT, lipid lowering therapy; PCSK9i, proprotein convertase subtilisin kexin 9 inhibitor

2).

Conclusions: The SANTORINI study shows suboptimal implementation of ESC/EAS 2019 guidelines on LDL-C, in particular low use of combination LLT. This means that a substantial proportion of patients will remain at high residual risk of ASCVD events.

SE045

Topic: *ASA01 - PATHOGENESIS OF ATHEROSCLEROSIS / ASA01-02 Smooth muscle cell biology*

SINGLE-CELL RNA-SEQ REVEALS A CROSSTALK BETWEEN HYALURONAN RECEPTOR LYVE-1-EXPRESSING MACROPHAGES AND VASCULAR SMOOTH MUSCLE CELLS

SAAG SESSION 11: HOW CELLS TALK? SMOOTH MUSCLE CELLS AND MACROPHAGES

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Background and Aims : Background: Atherosclerosis is a chronic inflammatory disease with macrophages as the most abundant hematopoietic cells present in plaques. Resident-like macrophages (res-like) were identified in the healthy and atherosclerotic aorta, however, what is their role in the lesional microenvironment is not completely understood.

Methods: Methods: A single-cell RNA sequencing analysis of CD45+ leukocytes of the atherosclerotic aorta of Apolipoprotein E-deficient (Apoe-/-) mice on a normal cholesterol diet (NCD) or a high cholesterol diet (HCD), respecting the site-specific predisposition to atherosclerosis was performed. A population of res-like macrophages expressing hyaluronan receptor LYVE-1 was investigated via flow cytometry, ELISA, immunofluorescence in human atherosclerotic plaques from carotid artery disease patients and co-culture experiments.

Results: Results: We identified 12 principal leukocyte clusters with distinct atherosclerosis disease-relevant gene expression signatures. LYVE-1+ res-like macrophages expressing highly C-C Motif Chemokine Ligand 24 (CCL24, eotaxin 2) expanded under hypercholesteremia in Apoe-/- mice and promoted VSMC phenotypic modulation to osteoblast/chondrocyte-like cells ex vivo in CCL24 dependent manner. Moreover, LYVE-1+CCL24+ macrophages are present in areas of vascular calcification in human atherosclerotic plaques while elevated systemic levels of CCL24 are associated with the occurrence of carotid artery disease (CAD) events in humans.

Conclusions: Conclusion: We generated a single-cell atlas illustrating the site-specific predisposition to atherosclerosis. We provide evidence that LYVE-1 res-like macrophages via secretion of CCL24 are potential mediators of VSMC transdifferentiation to osteogenic-like cells with a possible role in vascular calcification and a likely detrimental role in atherosclerotic plaque stability in human atherosclerosis.

SE046

Topic: *ASA01 - PATHOGENESIS OF ATHEROSCLEROSIS / ASA01-02 Smooth muscle cell biology*

EXPLORATION OF DUAL LET-7 MIMIC/STATIN THERAPY TARGETING VASCULAR SMOOTH MUSCLE CELL DYSFUNCTION IN ATHEROSCLEROSIS.

SAAG SESSION 11: HOW CELLS TALK? SMOOTH MUSCLE CELLS AND MACROPHAGES

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Background and Aims : Micro-RNA (miRNA) act as negative post-transcriptional regulators of gene expression and are involved in every stage of atherosclerotic plaque development. Statins remain the most common therapeutic agent for lipid lowering, and there is great interest in understanding the pleiotropic effects of statins as well as investigating drug combination therapies alongside statins. Here we investigated a dual statin/miRNA combination therapy approach to target human aortic SMC (HAoSMC) activation.

Methods: miRNA-mRNA network enrichment analysis was performed in human atherosclerotic plaque transcriptomics datasets using MiRNET 2.0. Primary HAoSMCs (Lonza Bioscience) were transfected with combinations of Let-7d mimic, miR-27a mimic and miR-155 antagomiR (20nmol/L;24h) and treated with pro-atherogenic stimuli (TNF-α, 10ng/ml;24h), and Atorvastatin (1μM;24h) or Lovastatin (1μM;24h). RNA-seq transcriptomics was performed by the Beijing Genomics Institute (DNBseq). All cell experiments were performed n=3-5 times.

Results: Analysis of miRNA-gene network interactions in human atherosclerosis transcriptomics datasets identified lead miRNA networks (miRs-27a, -33, -155, - 16 and Let-7d). Let-7d mimic significantly attenuated TNF-α-induced increase of IL-6, ICAM-1, VCAM-1, MCP1, CD68, MYOCD gene expression ($p<0.05$) in HAoSMCs. Statins (Atorvastatin, Lovastatin) significantly attenuated inflammatory gene expression and increased the expression of Let-7d in HAoSMCs ($p<0.05$). We next investigated dual Let-7d mimic/statin therapy in HAoSMCs treated with TNF-α. Our preliminary findings indicate that miRNA modulating strategies can enhance the effects of statins in HAoSMCs. RNA-seq studies elucidated transcriptome-wide responses to these therapies, identifying multiple inflammatory pathways modulate by these therapies.

Conclusions: Targeting the Let-7 network alongside statins can modulate HAoSMC activation and attenuate key inflammatory pathway signals.

SE047

Topic: *ASA01 - PATHOGENESIS OF ATHEROSCLEROSIS / ASA01-02 Smooth muscle cell biology*

HUMAN PRIMARY PLAQUE CELLS TO STUDY CELL FUNCTIONS AND THE BIOLOGY BEHIND SEX-DIFFERENCES IN ATHEROSCLEROSIS

SAAG SESSION 11: HOW CELLS TALK? SMOOTH MUSCLE CELLS AND MACROPHAGES

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Background and Aims : We previously showed that female key driver genes are involved in vascular smooth muscle cell plasticity in female atherosclerotic plaques. To further study mechanisms on how those sex-driven genes contribute to atherosclerotic disease they need to be first prioritized in a relevant in vitro model system. We hypothesize that human isolated plaque cells retain diseased key driver gene expression and activity and therefore can be used for plasticity experiments to prioritize and study the function of female key driver genes.

Methods: Conditioned outgrowth of cells from freshly obtained atherosclerotic lesions under optimized conditions were performed from 20 male/female, which underwent atherectomy surgeries. Using a combination of bulk and single-cell RNAseq, plaque cells have been deeply characterized. To investigate their lineage plasticity, we stimulated them with low-density-lipoproteins (LDL and oxidized LDL) to induce foam cell trans-differentiation.

Results: We found that plaque cells maintain a stable transcriptome over 10 passages of culture (~10 weeks) and resemble synthetic VSMCs when compared with human single-cells directly characterized from tissue. We identified female-specific key driver genes amongst the highest expressed genes (FN1, COL1A2, IGFBP7, SPARC, VCAN, CALD1). Moreover, after lipoproteins exposure, macrophages/foam cells specific markers (CD86, CD64, CD163, ABCA1, CD68 and LGALS3) were significantly upregulated, suggesting the capability of those cells to differentiate into other cell lineages.

Conclusions: Isolated plaque cells seem to retain the phenotypic and functional capabilities of synthetic VSMCs - what makes them suitable for further prioritization and mechanistic studies of female key driver genes involved in VSMC plasticity.

SE048

Topic: *ASA01 - PATHOGENESIS OF ATHEROSCLEROSIS / ASA01-03 Macrophages in lipid metabolism and atherosclerosis*

THE IMPACT OF RIPK1 KINASE INHIBITION ON ATHEROGENESIS

SAAG SESSION 11: HOW CELLS TALK? SMOOTH MUSCLE CELLS AND MACROPHAGES

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Background and Aims : RIPK1 (receptor-interacting serine/threonine-protein kinase 1) enzymatic activity drives both apoptosis and necroptosis, a regulated form of necrosis. Because necroptosis is involved in necrotic core development in atherosclerotic plaques, we investigated the effect of a RIPK1$^{S25D/S25D}$ mutation, which prevents the activation of RIPK1 kinase, on atherogenesis in *ApoE*$^{-/-}$ mice. Furthermore, we pharmacologically inhibited RIPK1 kinase activity by administering GSK'547 to *ApoE*$^{-/-}$ *Fbn1*$^{C1039G+/-}$ mice, a mouse model of advanced atherosclerosis.

Methods: *ApoE*$^{-/-}$ *Ripk1*$^{+/+}$ (n=16) and *ApoE*$^{-/-}$ *Ripk1*$^{S25D/S25D}$ (n=12) mice were fed a Western-type diet (WD) for 16 weeks to induce plaque formation. *ApoE*$^{-/-}$ *Fbn1*$^{C1039G+/-}$ mice received WD supplemented with GSK'547 (10 mg/kg BW/day, n=12-13/group) for 20 weeks to evaluate the effect of pharmacological RIPK1 kinase inhibition on atherogenesis.

Results: After 16 weeks WD, atherosclerotic plaques of *ApoE*$^{-/-}$ *Ripk1*$^{S25D/S25D}$ mice were significantly larger as compared to *ApoE*$^{-/-}$ *Ripk1*$^{+/+}$ mice (167±34 vs. 69±18 10^3 µm^2, P=0.01). Absolute cell numbers (350±34 vs. 154±33 nuclei) and deposition of glycosaminoglycans (Alcian blue: 31±6 vs. 14±4%, P=0.023) were increased in plaques from *ApoE*$^{-/-}$ *Ripk1*$^{S25D/S25D}$ mice while macrophage content (Mac3: 2.3±0.4 vs. 9.8±2.4%, P=0.012) was significantly decreased. Plaque apoptosis was not different between both groups. In contrast, pharmacological inhibition of RIPK1 kinase with GSK'547 in *ApoE*$^{-/-}$ *Fbn1*$^{C1039G+/-}$ mice did not significantly alter plaque size after 20 weeks WD, but induced apoptosis (TUNEL: 136±20 vs. 62±9 cells/mm^2, P=0.004).

Conclusions: Inhibition of RIPK1 kinase activity in *ApoE*$^{-/-}$ *Ripk1*$^{S25D/S25D}$ mice accelerated plaque progression, but this effect was not observed after pharmacological inhibition with GSK'547 in *ApoE*$^{-/-}$ *Fbn1*$^{C1039G+/-}$ mice.

SE049

Topic: *ASA01 - PATHOGENESIS OF ATHEROSCLEROSIS / ASA01-03 Macrophages in lipid metabolism and atherosclerosis*

MICROMANAGING ATHEROSCLEROSIS: MYELOID CELL-SPECIFIC MICRORNA-26B ATTENUATES ATHEROSCLEROSIS DEVELOPMENT

SAAG SESSION 11: HOW CELLS TALK? SMOOTH MUSCLE CELLS AND MACROPHAGES

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Background and Aims : Increasing evidence has shown that microRNAs (miRs) are fundamental players in atherosclerosis, but the exact role of various miRs remains elusive. Preliminary data demonstrated that miR-26b was the most highly expressed miR in human atherosclerotic plaques compared to healthy vessels. Therefore, we aimed to determine its cell-specific effects on atherosclerosis development.

Methods: For this, atherosclerotic plaque size/phenotype were analyzed in full-body and myeloid-specific (LysM-Cre) miR-26b-knockout (KO) *Apoe-/-* mice, via immunohistochemical/-fluorescent stainings. Phenotype and function of bone marrow-derived macrophages (BMDMs) were analyzed and lipid-nanoparticles served as vehicles for miR-26b mimics to restore miR-26b levels in KO BMDMs.

Results: Full-body miR-26b-KO resulted in a striking 3.5-fold increase in atherosclerotic lesion size (12w WTD). Consistent with a more advanced plaque phenotype, collagen, smooth muscle cell content and necrotic core area were all significantly increased in miR-26b-KO mice, whilst the relative macrophage content was reduced. Interestingly, miR-26b also already has very strong effects on early atherogenesis as full-body miR-26b-KO mice showed a remarkable 10-fold increase in plaque size after 4w WTD. Relative plaque size in the arches of myeloid miR-26b-KO mice was increased by 3-fold which coincided with a significantly increased collagen content. Further highlighting its myeloid-specific effects, miR-26b-KO BMDMs showed increased IL-6/TNFα secretion, which could be rescued by lipid-nanoparticles containing miR-26b mimics. Additionally, miR-26b-KO BMDMs showed a reduction in collagen breakdown.

Conclusions: Overall, our results clearly demonstrate an atheroprotective role of myeloid cell-specific miR-26b, mainly by suppressing inflammation and stimulating collagen breakdown, providing an important back-bone for future miR-26b focused research and potential new treatment options.

SE050

Topic: *ASA01 - PATHOGENESIS OF ATHEROSCLEROSIS / ASA01-06 Vascular biology of the arterial wall: Miscellaneous*

ASSOCIATION OF ENDOGENOUS SEX HORMONE LEVELS WITH PLAQUE COMPOSITION AND INSTABILITY IN MEN AND WOMEN WITH SEVERE CAROTID ATHEROSCLEROSIS

SAAG SESSION 12: NOVEL MECHANISMS IN PLAQUE STABILIZATION

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Background and Aims : Sex differences exist in plaque composition, where men are more likely to develop unstable plaques than women. Sex hormones affect vascular reactivity and inflammation, but it remains unclear if they are involved in plaque instability. Herein, we investigated the association between endogenous sex hormones and plaque composition/instability in men and women with severe carotid atherosclerosis.

Methods: Blood samples and plaque specimens were collected from patients with severe carotid atherosclerosis who underwent a carotid endarterectomy. Plaque composition and stability were examined according to gold-standard histological classifications. Liquid chromatography-tandem mass spectrometry-based methods were developed to quantify endogenous sex hormones, 17ß-estradiol (E_2), testosterone, androstenedione, and dehydroepiandrosterone, in sera samples.

Results: Clinical characteristics were similar among men (n=317) and post-menopausal women (n=143) with stable vs. unstable plaques (Table 1). Men had significantly greater proportion of unstable vs. stable plaques compared to women (men-unstable: 61.5%; women-unstable: 41.3%; P<0.001), characterized predominantly by a large lipid-rich core, hemorrhage, and high inflammatory cell infiltration. Men with unstable plaques had higher endogenous testosterone levels than men with stable plaques (P=0.004). Testosterone was inversely associated with plaque fibrosis levels in men and women (P<0.001), while directly associated with greater lipid core size, and presence of foam cells, neovascularization, and plaque and cap inflammation only in men (P<0.050). Endogenous E_2 levels were inversely associated with plaque and cap inflammation in men but not in women

(P<0.010).

Table 1. Sex-specific differences in patient clinical characteristics in relation to plaque stability

Clinical Characteristics	Women (n=143)		Men (n=317)		P-value
	With Stable Plaques (n=84)	With Unstable Plaques (n=59)	With Stable Plaques (n=122)	With Unstable Plaques (n=195)	
Age, y	71.8±8.1	70.0±9.5	71.0±9.1	70.9±9.3	0.690
BMI, kg/m^2	27.8±5.2	26.3±4.4	26.7±3.7	27.5±4.5	0.200
Ever smoker, %	65.1	73.7	71.9	78.8	0.114
CAD, %	32.1	28.8	46.7	41.0	0.056
Cerebrovascular Symptomatology, %	79.8	84.7	72.1	79.5	0.218
Carotid Artery Stenosis, 50-79%/80-99%	29.6/70.4	22.0/78.0	37.0/63.0	30.5/69.5	0.231
SBP, mmHg	141±22	137±21	138±18	138±19	0.743
DBP, mmHg	72±10	72±12	73±10	74±10	0.483
Hypertension, %	82.1	84.7	88.5	83.1	0.537
Anti-hypertensive medication, %	95.7	94.0	96.3	92.6	0.580
Glucose, mmol/L	6.00 [5.30-7.26]	6.36 [5.50-7.66]	5.90 [5.30-7.25]	6.38 [5.50-7.66]	0.199
T2DM, %	32.5	23.7	39.3	33.3	0.218
Anti-hyperglycemic medication, %	100.0	92.9	95.8	87.7	0.149
Hypercholesterolemia, %	79.8	84.7	84.4	82.1	0.805
Statin use, %	76.2	83.1	86.1	74.4	0.067
hsCRP, mg/L	1.91 [0.80-5.00]	1.60 [0.60-3.76]	1.65 [0.93-4.18]	1.90 [0.75-4.30]	0.951

Values are either represented as mean ± standard deviation or median [interquartile range] (for continuous data) or as percentages (for nominal data).
BMI indicates body mass index; CAD, coronary artery disease; SBP, systolic blood pressure; DBP, diastolic blood pressure; T2DM, type 2 diabetes mellitus; hsCRP, high-sensitivity C-reactive protein.
P-value indicates comparison among all groups: women with stable plaques, women with unstable plaques, men with stable plaques, men with unstable plaques (analysis was performed by one-way ANOVA, Kruskal-Wallis, or Chi-square (χ^2) test, as appropriate).

Conclusions: Our findings suggest that higher testosterone and lower E_2 levels may play a role in the development of unstable plaque composition in men with carotid atherosclerotic disease.

SE051

Topic: *ASA01 - PATHOGENESIS OF ATHEROSCLEROSIS / ASA01-06 Vascular biology of the arterial wall: Miscellaneous*

FOLLICLE-STIMULATING HORMONE-RECEPTOR A NEW MARKER FOR ATHEROSCLEROTIC PLAQUES

SAAG SESSION 12: NOVEL MECHANISMS IN PLAQUE STABILIZATION

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Background and Aims : Experimental evidence suggests that the follicle-stimulating hormone (FSH) may act directly on endothelial cells (ECs) inducing atherosclerosis development. However, the expression of FSH-receptor in human atherosclerosis lesions was not demonstrated. We conducted a study to assess the expression of the FSH-receptor in atherosclerotic plaques.

Methods: We used immunohistochemical techniques involving the FSHR-specific monoclonal antibody 323 that recognizes a FSHR-epitope in tissue samples from 50 patients with atherosclerotic plaques located in the carotid, coronary, and saphenous arteries. Immunoelectron microscopy was used to detect FSHR in ApoE-KO/hFSHR-KI mouse aortic atherosclerotic plaques.

Results: In early atherosclerotic plaques a strong signal for FSHR-protein was detected only in the arterial endothelial cells and in the vasa vasorum located in the adventitia. In advanced atherosclerotic plaques a strong signal for FSHR was also detected in ECs of blood neovessels located in the intima. A heterogeneous staining pattern was observed associated with M1-macrophages, M1-macrophage-derived foam cells, and giant cells. By contrast, FSHR was not expressed in M2-macrophages, M2-macrophage-derived foam cells, smooth muscle cells, and lymphocytes, other components of atherosclerotic plaques. The normal human thoracica interna and its vasa vasorum did not express FSHR. Aortic endothelial cells covering the atherosclerotic plaques in ApoE-KO/hFSHR-KI mice were FSHR-positive. Immunoelectron microscopy with the use of anti-hFSHR323 antibody-colloidal gold particles indicated that FSHR is exposed on the luminal endothelial surface and can bind and internalize circulating ligands.

Conclusions: FSHR is selectively expressed in atherosclerotic plaques. FSHR ligands could be used for screening, diagnosis, and therapy of atherosclerosis.

SE052

Topic: *ASA01 - PATHOGENESIS OF ATHEROSCLEROSIS / ASA01-08 Platelets, thrombosis and atherosclerosis*

APAC TREATMENT LIMITS COLLAR-INDUCED CAROTID ATHEROSCLEROTIC PLAQUE DEVELOPMENT IN APOE-/- MICE

SAAG SESSION 12: NOVEL MECHANISMS IN PLAQUE STABILIZATION

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Background and Aims : Mimics of mast cell-derived heparin proteoglycans (HEP-PG) can be tailored to molecules carrying both antiplatelet and anticoagulant properties. These dual antiplatelet and anticoagulant (APAC) constructs can also shield adhesion molecules such as P-selectin and VCAM-1 expressed by endothelial cells upon atherosclerosis development. We hypothesize that via this way, APAC prevents macrophage accumulation and lesion development. In this study, we therefore determined the efficacy of APAC in inhibiting atherosclerosis.

Methods: Male western-type diet fed apoE$^{-/-}$ mice were equipped with perivascular carotid artery collars to induce atherosclerosis. In this collar model, mRNA expression of adhesion molecules such as ICAM-1, VCAM-1, P-Selectin but also of Platelet Factor 4 (PF4) are significantly upregulated upon lesion development (all $P<0.05$ at 2 weeks after collar placement compared with control arteries). From lesion initiation, mice were treated with 0.2 mg/kg APAC or vehicle control (i.v, 3x per week, n=12-14 per group) for 2.5 weeks. At 5 weeks after collar placement, mice were sacrificed.

Results: APAC treatment did not affect body weight or plasma total cholesterol levels of the mice during the experiment. Interestingly, carotid artery plaque size was reduced by over 50% upon APAC treatment (APAC: $50\pm10*10^3$ versus controls: $102\pm13*10^3$ μm^2; $P<0.01$). This observation was aligned with reduced plaque macrophage area (APAC: $20\pm5*10^3$ versus controls: $33\pm5*10^3$ μm^2) and collagen content (APAC: $13\pm4*10^3$ versus controls: $28\pm6*10^3$ μm^2; $P<0.05$).

Conclusions: We here show that APAC effectively inhibits atherosclerotic lesion development when administered during lesion initiation and may have potential as therapeutic agent to prevent atherosclerosis.

SE053

Topic: *ASA01 - PATHOGENESIS OF ATHEROSCLEROSIS / ASA01-08 Platelets, thrombosis and atherosclerosis*

PLATELET SOLUBLE GUANYLYL CYCLASE DEFICIENCY PROMOTES ATHEROSCLEROSIS IN MICE

SAAG SESSION 12: NOVEL MECHANISMS IN PLAQUE STABILIZATION

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Background and Aims : Variants in genes encoding the soluble guanylyl cyclase (sGC) are associated with coronary artery disease (CAD) risk. Platelets are strongly affected by impaired sGC function, while their role in atherosclerosis remains incompletely understood. Here we sought to investigate the contribution of platelet sGC to atherosclerosis and the therapeutic potential of targeting sGC in atherosclerosis.

Methods: We genetically deleted sGC in platelets of atherosclerosis-prone *Ldlr*$^{-/-}$ mice and performed histology, flow cytometry, intravital microscopy and *in vitro* assays including human sGC risk allele carrier platelets. Furthermore, atherosclerosis-prone *Ldlr*$^{-/-}$ mice were treated with an oral sGC stimulator or vehicle, and examined by histology, flow cytometry and adoptive transfer experiments.

Results: Pf4-*Cre*$^{+}$*Gucy1b1*$^{flox/flox}$*Ldlr*$^{-/-}$ mice displayed enhanced leukocyte adhesion to atherosclerotic plaques, more numerous inflammatory leukocytes and larger plaque sizes in aortic tissue in comparison with their litter mates. *In vitro*, supernatant from activated, sGC-deficient platelets promoted leukocyte adhesion to endothelial cells via increasing activation of endothelial cells. We identified reduced angiopoietin-1 release by Pf4-*Cre*$^{+}$*Gucy1b1*$^{flox/flox}$ and human sGC risk allele carrier platelets to be responsible for enhanced activation of endothelial cells and subsequent leukocyte adhesion. Pharmacological sGC stimulation increased platelet angiopoietin-1 release *in vitro* and reduced recruitment of adoptively transferred leukocytes, while atherosclerotic plaque formation and vascular inflammation were reduced.

Conclusions: Loss of sGC in platelets contributes to atherosclerotic plaque formation via reduced release of the soluble factor angiopoietin-1 and, subsequently, enhanced leukocyte recruitment. Pharmacological sGC stimulation might represent a novel therapeutic strategy to prevent and treat CAD.

SE054

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-11 Liver metabolism and steatosis*

THE ENDOSOMAL SORTING PROTEIN VPS35 CONTROLS LIPID HOMEOSTASIS THROUGH REGULATING HEPATIC LYSOSOMAL FUNCTION

SAAG SESSION 13: THE CULPRIT? FATTY LIVER

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Background and Aims : The lysosome plays a key role in lipid metabolism, as loss of lysosomal function can lead to aberrant accumulation of lipid species, including cholesterol esters as seen in Lysosomal Acid Lipase (LAL) deficiency. A mutation in *VPS35*, which encodes for an endosomal sorting protein, is associated with impaired lysosomal function in neurons and correlated with Parkinson's disease. Here, we investigated whether VPS35 is also required for hepatic lysosomal function to control lipid homeostasis.

Methods: VPS35-deficient (KO) Hepa1-6 cells were generated with CRISPR/Cas9 technology and were used to study lysosomal function. Hepatic VPS35-deficient mice (*Vps35*^{HKO}) were generated using the Cre-Lox system. Mice were fed either a chow or a high-fat high cholesterol (HFC) diet and the level of hepatic lipid accumulation was determined. The hepatic expression of lysosomal markers and enzymes were analyzed by immunoblotting or proteomics. In addition, gene expression analyses were performed to study metabolic pathways, including lipid metabolism.

Results: Hepatic loss of VPS35 resulted in hypercholesterolemia and hepatic cholesterol ester accumulation, which was exacerbated by HFC-diet feeding. In contrast, plasma and hepatic triglyceride levels were markedly reduced in *Vps35*^{HKO} mice. The increase in hepatic cholesterol esters was associated with liver inflammation and macrovesicular steatosis. In addition, VPS35 deficiency reduced the protein levels and activity of LAL in murine hepatocytes.

Conclusions: Here we found that hepatic ablation of VPS35 copies the phenotype of hepatic LAL-deficient mice, and show that VPS35 is a critical regulator of lysosomal function in hepatocytes, and hence lipid homeostasis.

SE055

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-11 Liver metabolism and steatosis*

ROLE OF LYSOSOMAL ACID LIPASE IN PPAR-MEDIATED REDUCTION OF LIPID ACCUMULATION IN HEPATOCYTES

SAAG SESSION 13: THE CULPRIT? FATTY LIVER

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Background and Aims : Lysosomal acid lipase (LAL) catalyzes the hydrolysis of cholesteryl esters and triglycerides in the lysosomal compartment. Recently, it was shown that NAFLD patients can develop an acquired LAL deficiency which is rescued by PPAR-alpha activation. Aim of the study was to investigate the role of other isoform-specific and dual PPAR agonists on lipid accumulation and LAL activity using an in vitro model of steatosis.

Methods: To induce lipid accumulation, HepG2 cells were loaded with free fatty acids (FAs) and incubated with selective agonists of PPAR alpha (fenofibric acid), gamma (pioglitazone) and delta (seladelpar) receptors or with dual alpha/gamma (saroglitazar) and alpha/delta (elafibranor) agonists. LAL activity and expression, lipid accumulation and the activation of autophagy were assessed.

Results: Pioglitazone, seladelpar and saroglitazar were more effective than fenofibric acid in stimulating LAL activity in FA-loaded hepatocytes. All the tested agonists significantly reduced lipid accumulation, but the effect was completely lost when LAL was blocked by a specific inhibitor. PPAR agonists promoted TFEB expression, with consequent lysosomal biogenesis and activation of autophagy, as shown by the expression of p62 and LC3.

Conclusions: The ability of PPAR-alpha agonists to rescue defective LAL in hepatocytes was shared by other isoform-specific and dual PPAR agonists. LAL activation plays a key role in PPAR-mediated reduction of lipid accumulation by catalyzing the hydrolysis of intracellular lipids routed to the lysosomes by autophagy. These data suggest that the pharmacological modulation of LAL should be explored in the management of steatosis.

SE056

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-11 Liver metabolism and steatosis*

VALIDATION OF NETWORK MEDICINE INTERACTOMES: FROM PATIENTS TO A HUMAN HEPATOCYTE-LIKE MODEL

SAAG SESSION 13: THE CULPRIT? FATTY LIVER

Francesca Grassi[1], Daniele Dall'Olio[2], Osman Ahmed[3], Claudia Sala[2], Matteo Bersanelli[4], Camilla Pramfalk[5], Paolo Garagnani[3,6], Enrico Petrillo[7], Joseph Loscalzo[7], Matteo Pedrelli[3], Paolo Parini[3]
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Background and Aims : AIM: Atherosclerotic cardiovascular disease results from a complex pathophysiology. Network Medicine identifies underlying pathophenotypes of complex diseases, or the effects of their treatments, as modules of interactions (interactomes). Using a unique human hepatocyte-like model, we aimed to validate effects on the novel genes identified by hepatic multi-source interactomes in patients treated with simvastatin and/or ezetimibe.

Methods: METHODS: SOAT2-only-HepG2 cells were treated with atorvastatin (5µM) and ezetimibe (25µM), alone or in combination, for 16 hours. Gene expressions were analyzed by qPCR and compared to the hepatic expression of genes known to be affected in patients (n=27) treated with simvastatin (80 mg/day) and ezetimibe (10 mg/day), alone or in combination (*Stockholm Study*). Multi-source interactomes from the patients were constructed using the Dr Loscalzo's Human PPI Personal Protein I and integrated with transcriptome, methylome, and plasma biochemical and lipoprotein parameters.

Results: RESULTS: Patient's interactomes revealed new treatments effects on seed genes, such as stratifin (*SFN*), which is involved in Akt/mTOR and p53/TP53 signaling pathways, and predicted genes, such as transmembrane BAX inhibitor motif containing 6 (*TMBIM6*), which is involved in apoptosis. Treatment of SOAT2-only-HepG2 cells showed that atorvastatin significantly increased *SFN* expression (0.66 Log_2-fold), whereas ezetimibe alone or together with atorvastatin increased the expression of *TMBIM6* (0.53 and 0.79 Log_2-fold, respectively).

Conclusions: CONCLUSIONS: SOAT2-only-HepG2 cell model recreates the effects on genes observed in the Stockholm Study and its interrogation via a network medicine approach offers a highly credible resource for exploring mechanisms of lipid lowering drug actions and for identifying potential novel drug targets.

SE057

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-11 Liver metabolism and steatosis*

LOWER BLOOD GLUCOSE AND DISRUPTED GLUCAGON SIGNALING IN MICE WITH LIVER-SPECIFIC ABLATION OF THE HEPATOCYTE NUCLEAR FACTOR 4A (HNF4A) GENE.

SAAG SESSION 13: THE CULPRIT? FATTY LIVER

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Background and Aims : *Hnf4a* gene ablation in mouse liver causes hepatic steatosis, perturbations in HDL structure and function and affects many pathways and genes related to glucose metabolism. Our aim was to investigate the role of liver HNF4A in glucose homeostasis.

Methods: Serum and tissue samples were obtained from *Alb-Cre;Hnf4a$^{fl/fl}$* (H4LivKO) mice and their littermate *Hnf4a$^{fl/fl}$* controls. Binding of HNF4A to DNA was assessed by chromatin immunoprecipitation (ChIP) assays. Fasting glucose and insulin, glucose tolerance, insulin tolerance and glucagon challenge tests were performed by standard procedures. Gene expression analysis was performed by quantitative real time PCR (qRT-PCR).

Results: H4LivKO mice presented lower blood levels of fasting glucose, improved glucose tolerance, increased serum insulin and lactate levels and reduced response to glucagon challenge test compared to their control littermates. The expression of the gene encoding the glucagon receptor (*Gcgr*) was markedly reduced in H4LivKO liver and ChIP assays revealed specific binding of HNF4A to the *Gcgr* promoter. Glucose administration in the drinking water of H4LivKO mice beginning at four weeks of age resulted in an impressive extension of survival beyond 28 weeks of age compared to 6-8 weeks in mice that did not receive glucose.

Conclusions: Our results reveal a novel role of liver HNF4A in controlling blood glucose levels via regulation of the glucagon receptor. In combination with the steatotic phenotype, our results suggest that H4LivKO mice could serve as a valuable model for studying glucose homeostasis in the context of non-alcoholic fatty liver disease (NAFLD).

SE058

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-11 Liver metabolism and steatosis*

ATORVASTATIN ATTENUATES DIET-INDUCED NON-ALCOHOLIC STEATOHEPATITIS IN APOE*3-LEIDEN MICE BY REDUCING HEPATIC INFLAMMATION

SAAG SESSION 13: THE CULPRIT? FATTY LIVER

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Background and Aims : Non-alcoholic fatty liver disease (NAFLD) is considered the hepatic manifestation of the metabolic syndrome, and due to the worldwide obesity epidemic, prevalence of both NAFLD and the more severe form non-alcoholic steatohepatitis (NASH) is rapidly rising. Patients with metabolic syndrome are prescribed statins to prevent development of cardiovascular disease due to a disturbed lipoprotein metabolism. Although the effects of statins on cardiovascular disease are relatively well described, data on their effects on NASH are lacking. Here, we evaluated the effects of atorvastatin on NASH and liver fibrosis development and inflammatory parameters.

Methods: ApoE*3-Leiden mice were fed a Westernized diet (WTD) with (n=16) or without (n=16) atorvastatin admixture for 32 weeks. Plasma parameters and inflammatory markers were evaluated and hearts and livers were histologically and biochemically examined at study endpoint.

Results: Atorvastatin significantly reduced the WTD-induced increase in plasma cholesterol levels (-43%, $p<0.01$) and thereby significantly attenuated development of atherosclerosis (lesion area -47%, $p<0.01$). Atorvastatin significantly reduced hepatic steatosis (-22%, $p<0.01$) and induced a robust decrease of hepatic inflammation (-80%, $p<0.01$) and fibrosis (-92%, $p<0.001$) compared to mice that received the WTD only. Interestingly, atorvastatin almost fully blunted the formation and accumulation of hepatic cholesterol crystals (-78%, $p<0.05$), structures that drive inflammation via inflammasome activation. Analyses on inflammatory markers substantiated this effect and revealed significant reductions in infiltration of hepatic macrophages, neutrophils and monocytes together with lower levels of pro-inflammatory cytokines.

Conclusions: Atorvastatin attenuates NASH development by reducing intrahepatic cholesterol crystal and cholesterol ester levels, hepatic steatosis, inflammation and fibrosis.

SE059

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-06 Cholesterol efflux and reverse cholesterol transport*

ACHILLES TENDON XANTHOMA TICKINESS ASSOCIATES TO SERUM LIPOPROTEIN FUNCTIONS ALTERATIONS

SAAG SESSION 14: GET RID OF IT: CHOLESTEROL EFFLUX AND REVERSE CHOLESTEROL TRANSPORT

Maria Pia Adorni[1], Marta Biolo[2], Marcella Palumbo[3], Francesca Zimetti[3], Paolo Scarinzi[4], Sabina Zambon[2], Franco Bernini[3], Alberto Zambon[2]
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Background and Aims : Achilles tendon xanthoma (ATX) formation involves the uptake of modified LDL by macrophages within the tendon similarly to that occurring in the atheroma. Macrophage cholesterol homeostasis strictly depends on serum lipoprotein functions, including the HDL capacity to promote cell cholesterol efflux (cholesterol efflux capacity, CEC) and the serum capacity to promote cell cholesterol accumulation (cholesterol loading capacity, CLC). In this study, we explored the serum lipoproteins functions comparing FH patients with ATX to those without ATX.

Methods: HDL-CEC and serum CLC have been evaluated in a cohort of 45 FH patients included in the LIPIGEN study, 16 of which did not present ATX and 29 presented ATX detected by physical examination and/or bilateral AT ultrasound. Total HDL-CEC and its contributors, ABCA1 and ABCG1 HDL-CEC and HDL-CEC by aqueous diffusion (AD) were determined by a cell-based radioisotopic technique. Serum CLC was evaluated fluorimetrically in human macrophages.

Results: No differences in total HDL-CEC were observed between the groups. AD HDL-CEC tended to be reduced in ATX presenting patients compared to subjects without ATX . ABCA1 HDL-CEC was higher (+18.6%), while ABCG1 HDL-CEC was reduced (-11%) in patients with ATX compared to no ATX subjects, independently of HDL-c levels. Serum CLC increased by 14% in patients with ATX compared to those without, despite no change in LDL-c levels. Both ABCG1 CEC and serum CLC inversely correlated with ATX thickness**.**

Conclusions: Serum lipoprotein functions, beyond the well reported role in atherosclerosis, seems to be involved also in the mechanisms underlying Achille tendon xanthoma formation and expansion.

SE060

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-06 Cholesterol efflux and reverse cholesterol transport*

CEREBROSPINAL FLUID AND SERUM HDL CHOLESTEROL EFFLUX CAPACITY IS IMPAIRED IN NEURODEGENERATIVE DISORDERS

SAAG SESSION 14: GET RID OF IT: CHOLESTEROL EFFLUX AND REVERSE CHOLESTEROL TRANSPORT

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Background and Aims : Brain cholesterol transport occurs through particles similar to serum HDL, that have been identified in human cerebrospinal fluid (CSF). We functionally characterized CSF and serum of Alzheimer's disease (AD) and nonAD-dementia patients, by evaluating HDL cholesterol efflux capacity (CEC).

Methods: CSF and serum from AD (n=36), nonAD-dementia (n=13) and control subjects (n=14) were collected by lumbar puncture and blood withdrawal, respectively. CEC was evaluated by a radioisotopic cell-based technique. Human cell models of astrocytes, in the absence or presence of beta amyloid (Aß), have been used to evaluate CSF CEC, while ABCG1-overexpressing CHO and J774 macrophages were used for serum CEC.

Results: CSF CEC in astrocytes was reduced in AD and nonAD-dementia patients compared to controls (-40%, p=0.0193; -38%; p=0.0308, respectively). CSF CEC of AD patients was further lowered in Aß-treated astrocytes (-10%; p<0.001). Serum HDL total efflux from macrophages was similar among the groups, while ABCA1-CEC was reduced in AD and nonAD-dementia compared to control subjects (-19.5%, p=0.0151 and -28.4%; p=0.0015, respectively). ABCG1-CEC was lower only in AD patients compared to controls (-19.5%; p=0.0153). Serum HDL ABCG1-CEC evaluated at baseline directly correlated with the MMSE score at follow-up (r=0.47; p=0.03).

Conclusions: Neurodegenerative disorders are associated with brain HDL-cholesterol transport alterations, that can be further worsened in the presence of Aß. These alterations are also detectable for serum HDL, correlating with the degree of cognitive decline. Further studies will be necessary to establish whether CSF or serum CEC may represent a novel biomarker or a pharmacological target.

SE061

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-05 Inherited dyslipidemias*

CHOLESTYRAMINE PERINATAL TREATMENT OF APOE DEFICIENT MICE REDUCES ATHEROSCLEROTIC PLAQUES DEVELOPMENT IN ADULT OFFSPRING.

SAAG SESSION 14: GET RID OF IT: CHOLESTEROL EFFLUX AND REVERSE CHOLESTEROL TRANSPORT

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Background and Aims : We have shown that ApoE-/- mice born to hypercholesterolemic ApoE-/- mothers develop more atherosclerotic plaques than ApoE-/- mice born to normocholesterolemic ApoE+/-. We aimed to investigate the effect of treatment with a cholesterol-lowering drug in ApoE-/- mice during gestation only or during gestation and lactation on the development of atherosclerosis in adult ApoE-/- offspring.

Methods: Three groups of ApoE-/- females were fed either control diet (CTR), a diet with 3% cholestyramine during gestation (CTY-G) or during gestation and lactation (CTY-GL). At 25 weeks of age, males and females offspring were sacrificed and plasma cholesterol, triglycerides and bile acids concentrations were determined. Atherosclerotic plaques in the aortic roots as well as gene expression in liver were quantified.

Results: A significant reduction in atherosclerotic plaque area was observed in male progeny of CTY-G and CTY-GL, at 600µm from the heart (p= 0.03 and p=0.01 respectively), as well as at 800 µm (p=0.05). Plaque regression is also observed in females at 600 µm for CTY-G (p=0,009). Neither the cholesterolemia nor triglyceridemia showed any significant difference between groups. In male and female CTY-GL offspring, significant increase in the plasma bile acids pools was noted (p=0.004 and p=0.028 respectively) which is due to higher concentration of tauro-conjugated bile acids (p=0.0165 and p=0,031 respectively). Among analyzed gene expression, *LXRa* decreased in male CTY-G and CTY-GL (p=0.019).

Conclusions: The protective effect of perinatal treatment with cholestyramine against atherosclerosis development in offspring is independent of their cholesterol levels whereas bile acids' pools increase could reflect the implication of reverse cholesterol transport.

SE062

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-06 Cholesterol efflux and reverse cholesterol transport*

RELATIONSHIP BETWEEN CIRCULATING ADIPOKINES, CHOLESTEROL EFFLUX CAPACITY, AND POST-SURGICAL OUTCOMES IN PATIENTS WITH SEVERE CAROTID ATHEROSCLEROSIS

SAAG SESSION 14: GET RID OF IT: CHOLESTEROL EFFLUX AND REVERSE CHOLESTEROL TRANSPORT

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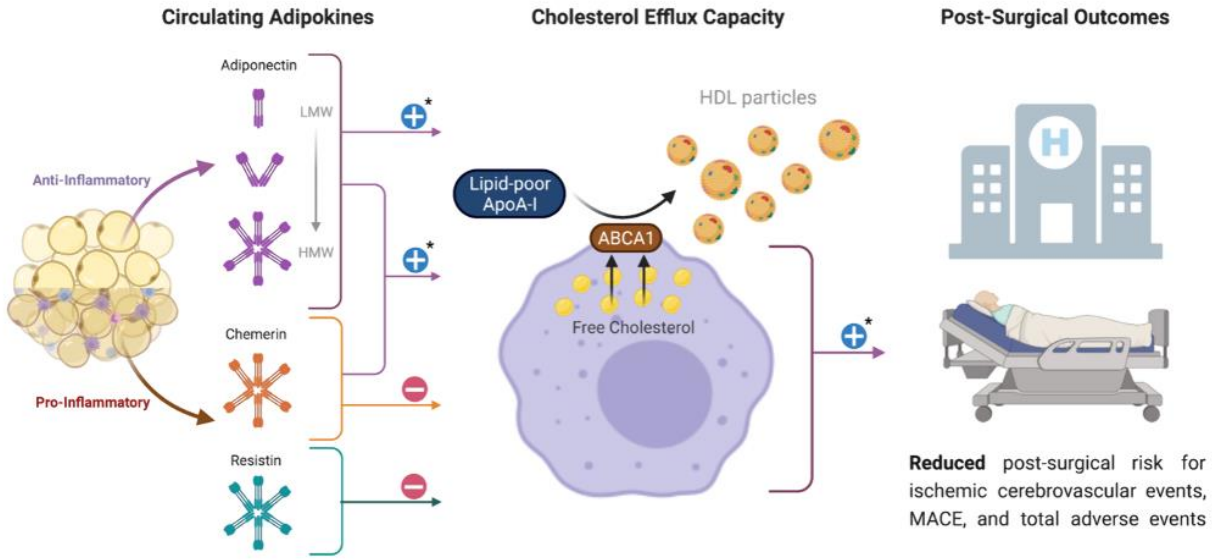
Background and Aims : Cholesterol efflux capacity (CEC) as a measure of HDL functionality is inversely associated with increased risk for cardiovascular events and mortality, and advanced plaque morphology. Adipokines, adipose tissue-derived factors, influence systemic lipoprotein metabolism and regulate vascular function. Herein, we investigated the association between CEC and circulating adipokines (anti-inflammatory adiponectin, pro-inflammatory chemerin and resistin) in patients with severe carotid atherosclerosis and evaluated their impact on post-surgical outcomes.

Methods: Fasting blood samples were collected pre-operatively from patients with severe carotid atherosclerosis who underwent a carotid endarterectomy (CEA) and used for measurement of 1) plasma total and high-molecular weight (HMW) adiponectin, chemerin, and resistin, and 2) cholesterol efflux assays in J774 macrophage-like cells. Five-year post-surgical outcomes were obtained through medical chart review.

Results: Subjects (n=285; mean age of 70.1±9.4 years; 67.0% male) had various comorbidities (hypercholesterolemia [85.3%], hypertension [83.5%], type 2 diabetes [34.5%]). CEC was independently and positively associated with total (ß[95% CI]; 0.216[0.134-0.298]) and HMW adiponectin (0.107[0.037-0.176]) but not with chemerin or resistin. Total adiponectin accounted for 8.3% of the variance in CEC. Interaction regression models demonstrated a significant interaction between adiponectin and chemerin in increasing CEC. With each unit increase in CEC there was a 92.9% decrease in the odds of having an ischemic cerebrovascular event 5 years post-CEA (0.071[0.007-

0.714]).



Conclusions: Circulating adiponectin had a strong independent association with increased CEC in patients with severe carotid atherosclerosis, while high CEC was associated with more favourable post-surgical outcomes. These findings reflect the importance of adipose tissue health in influencing CEC levels and atherosclerotic cardiovascular disease risk.

SE063

Topic: *ASA01 - PATHOGENESIS OF ATHEROSCLEROSIS / ASA01-07 Imaging technology*

ASSESSMENT OF THE IMPROVEMENT IN PERFUSION AFTER ENDOVASCULAR INTERVENTION BY HYPERSPECTRAL IMAGING.

SAAG SESSION 15: PREVENTION AND TREATMENT OF CVD

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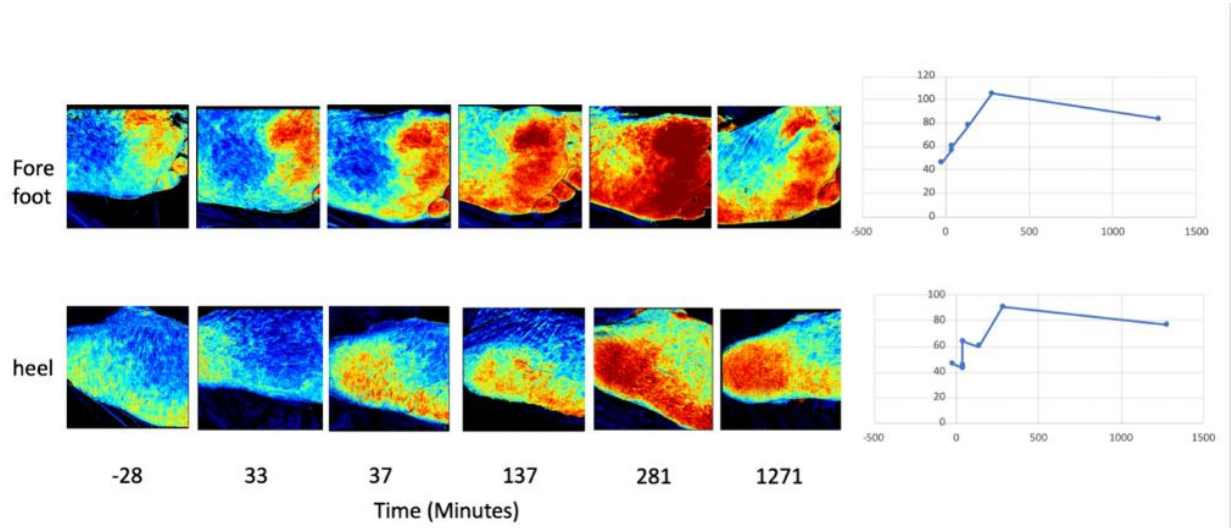
Background and Aims : The Hyperview® is a camera that applies hyperspectral imaging (HSI) to project local concentrations of oxyhemoblobin (OXY), deoxyhemoglobin (DEOXY) and O_2-saturation (O2-SAT) in a map. The aim of this study is to assess the immediate time course of changes in patients undergoing intervention for PAD by using this device.

Methods: The plantar region of the involved foot was imaged before and after endovascular intervention in patients with PAD. Pts included in this study underwent single angioplasty ± stent of an iliac or SFA lesion.

Results:



7 pts underwent intervention and assessed by HSI. Representative OXY images of the forefoot and heel prior to and immediately after an isolated iliac artery intervention are shown with a plot of the relative units. Enhanced perfusion appears around 30 min and peaks by 4hrs.

Conclusions: This study provides insight into the time course of improvement in foot perfusion following intervention. The Hyperview® is an easy, fast and noninvasive instrument to assess regional improvement in blood flow which may aid in determing the success of the procedure or the need for further intervention.

SE064

Topic: *ASA04 - CLINICAL VASCULAR DISEASE / ASA04-12 Prevention and treatment of cardiovasculardisease; miscellaneous*

PREVALENCE OF ATHEROSCLEROTIC CARDIOVASCULAR DISEASE STRATIFIED BY LOW-DENSITY-LIPOPROTEIN CHOLESTEROL AND ASSOCIATED TREATMENT PATTERNS WITHIN THE FOUR NATIONS OF THE UNITED KINGDOM: A ROUTINE DATABASE STUDY.

SAAG SESSION 15: PREVENTION AND TREATMENT OF CVD

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Background and Aims : To estimate the prevalence of atherosclerotic cardiovascular disease (ASCVD) and associated lipid-lowering therapy (LLT) and low-density-lipoprotein cholesterol (LDL-C) levels in the United Kingdom (UK).

Methods: The study was conducted in the Clinical Practice Research Datalink (CPRD) GOLD database; a longitudinal UK primary-care database. Patients ≥18 years registered on the midpoint date (30-06-2018) formed the denominator. ASCVD patients were selected by a diagnosis recorded in the database prior to the midpoint date. LDL-C values recorded three years prior to the midpoint date and LLT prescribed six months prior were detailed. The prevalence of ASCVD, overall and stratified by LDL-C thresholds, was calculated within each nation, and applied to UK population estimates.

Results: 210,343 of 2,771,418 eligible adult patients in CRPD GOLD had a diagnosis of ASCVD, equating to an adjusted UK prevalence of 6.82% (95% CI 6.79%-6.85%) and an extrapolated ASCVD population of 3,571,393 (see table). Prevalence ranged from 6.58% (6.53%-6.63%) in England to 8.26% (8.15%-8.38%) in Northern Ireland. Only 133,618 (63.5%) ASCVD patients had an LDL-C measurement recorded. This varied from 52.2% in Scotland to 80.8% in Northern Ireland. Of those with LDL-C recorded, 117,163 (87.7%) had an LDL-C ≥1.4mmol/L. Overall, 153,209 (72.8%) ASCVD patients were treated with LLT; however, only 90,634 (77.4%)) patients with an LDL-C ≥1.4mmol/L were treated with

LLT.

Table. Patient numbers, prevalence estimates and extrapolated population sizes for atherosclerotic cardiovascular disease overall and stratified according to low-density lipid cholesterol testing and low-density lipid cholesterol thresholds by UK nation

	England	N. Ireland	Scotland	Wales	Total	United Kingdom (adjusted)[1]
ASCVD patients	58,306	17,075	79,189	55,773	210,343	188,948
Prevalence (95% CI)	6.58% (6.53%-6.63%)	8.26% (8.15%-8.38%)	8.04% (7.99%-8.09%)	8.04% (7.98%-8.10%)	7.59% (7.56%-7.62%)	6.82% (6.79%-6.85%)
Treated with LLT	41,746 (71.6%)	13,051 (76.4%)	58,033 (73.3%)	40,379 (72.4%)	153,209 (72.8%)	
Extrapolated	2,895,830	119,261	354,556	201,727	3,975,799	3,571,393
With LD-C recorded						
Patients n, (%[2])	33,598 (57.6%)	13,795 (80.8%)	41,357 (52.2%)	44,868 (80.4%)	133,618 (63.5%)	111,764 (59.2%)
Prevalence (95% CI)	3.79% (3.75%-3.83%)	6.68% (6.57%-6.78%)	4.20% (4.16%-4.24%)	6.47% (6.41%-6.53%)	4.82% (4.80%-4.85%)	4.03% (4.01%-4.06%)
Treated with LLT	26,407 (78.6%)	11,439 (82.9%)	33,124 (80.1%)	35,482 (79.1%)	106,452 (79.7%)	
Extrapolated	1,668,680	96,368	185,169	162,284	2,525,581	2,112,501
With LDL-C ≥ 1.4 mmol/L[4]						
Patients n, (%[2])	29,873 (88.9%)	11,290 (81.8%)	36,560 (88.4%)	39,440 (87.9%)	117,163 (87.7%)	98,875 (88.5%)
Prevalence (95% CI)	3.37% (3.33%-3.41%)	5.46% (5.37%-5.56%)	3.71% (3.68%-3.75%)	5.69% (5.63%-5.74%)	4.23% (4.20%-4.25%)	3.57% (3.55%-3.59%)
Treated with LLT	22,841 (76.5%)	9,016 (79.9%)	28,541 (78.1%)	30,236 (76.7%)	90,634 (77.4%)	
Extrapolated	1,483,674	78,868	163,652	142,651	2,214,557	1,868,886
With LDL-C ≥ 2.6 mmol/L[5]						
Patients n, (%[2])	11,320 (33.7%)	3,213 (23.3%)	13,027 (31.5%)	14,616 (32.6%)	42,176 (31.6%)	36,815 (32.9%)
Prevalence (95% CI)	1.28% (1.25%-1.30%)	1.56% (1.50%-1.61%)	1.32% (1.30%-1.35%)	2.11% (2.07%-2.14%)	1.52% (1.51%-1.54%)	1.33% (1.31%-1.34%)
Treated with LLT	6,364 (56.2%)	1,819 (56.6%)	7,431 (57.0%)	8,170 (55.9%)	23,784 (56.4%)	
Extrapolated	562,220	22,445	58,326	52,865	797,190	695,356

[1]Estimate adjusted to account for the different population structure of the Clinical Practice Research Datalink GOLD database to the United Kingdom.
[2] Proportion of all ASCVD patients.
[3] Proportion of all ASCVD patients with LDL-C recorded.
[4] Threshold based on European Society of Cardiology/ European Atherosclerosis Society (ESC/EAS) guidelines see: Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, et al. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. Eur Heart J. 2020;41(1):111-88.
[5] Threshold based on Inclisiran Technology Assessment see: National Institute for Health and Care Excellence. TA733: Inclisiran for treating primary hypercholesterolaemia or mixed dyslipidaemia. Available at: https://www.nice.org.uk/guidance/ta733 (last accessed 12/11/2021). 2021

LDL-C – Low-density lipoprotein cholesterol
LLT – Lipid-lowering therapy

Conclusions: Estimated ASCVD prevalence in the UK was 6.82%. LDL-C for a significant proportion of ASCVD patients was above guideline recommendations, yet, despite the association of elevated LDL-C with cardiovascular events, many were untreated with LLT.

SE065

Topic: *ASA04 - CLINICAL VASCULAR DISEASE / ASA04-14 Other*

AN INTEGRATED E-HEALTH SUPPORT OF SHARED DECISION MAKING AND SELF-MANAGEMENT (POWER2DM) FOR PATIENTS WITH DIABETES MELLITUS AND THEIR HEALTHCARE PROFESSIONALS

SAAG SESSION 15: PREVENTION AND TREATMENT OF CVD

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Background and Aims : Diabetes mellitus accelerates the development and progression of atherosclerotic disease, especially when glucose levels are not well regulated. Several e-health systems aim to support patients in diabetes self-management (DSM), but lack to acknowledge its complexity. We developed and field-tested an e-health system integrating medical, psychological, and behavioral aspects and connected wearables to support patients and healthcare professionals in DSM (POWER2DM).

Methods: Patients with type 1 (T1DM) and type 2 diabetes (T2DM) were recruited from hospital outpatient diabetes clinics (Netherlands/Spain) and randomized to POWER2DM or usual care (UC) for 37 weeks. Mixed models were used to assess change in HbA1c, quality of life (QoL) and DSM and differences between POWER2DM and UC.

Results: In total 226 patients were included (T1DM: n=108, T2DM: n=118). In the POWER2DM group HbA1c decreased from 60.6±14.7 mmol/mol (7.7±1.3%) to 56.7±12.1 mmol/mol (7.3±1.1%)($p<0.001$), compared to no change in UC (between-group difference:-2.6 mmol/mol (-0.24%), $p=0.008$). The improvement in HbA1c was mainly observed in patients with T2DM in the POWER2DM group (-6.9 mmol/mol (-0.63%), $p<0.001$). QoL improved in patients with T1DM in the POWER2DM group compared to UC (baseline: 15.7±3.8, end: 16.3±3.5, between-group difference: $p=0.047$), but not in patients with T2DM. DSM improved in both patients with T1DM (7.3±1.2 to 7.7±1.2, $p<0.005$) and T2DM (6.5±1.3 to 6.7±1.3, $p<0.01$) within the POWER2DM group, without a significant difference between POWER2DM and UC.

Conclusions: POWER2DM improves HbA1c compared to UC in patients with T2DM and improves QoL in patients with T1DM. DSM tended to improve in all patients with diabetes, without a significant between-group difference.

SE066

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-06 Gender and cardiovascular risk*

IMPACT OF PREECLAMPSIA ON CARDIOVASCULAR RISK FACTORS IN MOTHERS AND NEWBORNS

SAAG SESSION 15: PREVENTION AND TREATMENT OF CVD

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Background and Aims : The increased risk of cardiovascular disease and mortality in later life of women with preeclamptic pregnancies is well-known. The impact of preeclampsia on the offspring is, however, still uncertain. The aim of the present study was to investigate the impact of preeclampsia on both mother and offspring.

Methods: For this purpose, we used the Copenhagen General Population Study comprising 59,571 women of which 1,365 had a diagnosis of preeclampsia to investigate the association between preeclampsia and risk of cardiovascular diseases. Further, we used the Copenhagen Baby Heart Study comprising more than 13,000 umbilical cord blood samples and assessed the impact of preeclampsia on atherogenic lipid traits in cord blood.

Results: Multivariable adjusted hazard ratios for preeclampsia versus no preeclampsia (95% CI) were 1.31 (1.00-1.72) for IHD, 1.44 (1.02-2.02) for ICVD, and 1.39 (1.11-1.74) for composite IHD and ICVD. Concentrations of non-HDL cholesterol, total cholesterol, calculated LDL cholesterol, directly measured LDL cholesterol, apoB, and triglycerides in cord blood increased stepwise from no preeclampsia (n=11,221) to mild/moderate preeclampsia (n=253) to severe preeclampsia (n=104) (p for trends <0.0001 for non-HDL cholesterol, total cholesterol and LDL cholesterol; p for trend=0.0002 for apoB; p for trend=0.003 for triglycerides).

Conclusions: Women with preeclampsia had increased risk of future cardiovascular disease and lipid traits in umbilical cord blood of their offspring were elevated. This indicates that preeclampsia affects lipid metabolism during fetal life and potentially contributes to an increased risk of future cardiovascular disease in offspring of mothers with preeclampsia.

SE067

Topic: *ASA04 - CLINICAL VASCULAR DISEASE / ASA04-09 Lipid-lowering therapies*

LDL-CHOLESTEROL GOAL ATTAINMENT THROUGH OPTIMAL IMPLEMENTATION OF THE 2019 ESC/EAS DYSLIPIDEMIA TREATMENT ALGORITHM IN EUROPEAN PATIENTS WITH/WITHOUT ATHEROSCLEROTIC CARDIOVASCULAR DISEASE: SIMULATION STUDY FROM DA VINCI

SAAG SESSION 15: PREVENTION AND TREATMENT OF CVD

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Background and Aims : In the European-wide DA VINCI study, only 33% of patients attained their risk-based 2019 ESC/EAS guideline LDL-C goals. We aimed to simulate the impact of the step-wise implementation of the ESC/EAS lipid-lowering treatment (LLT) pathway on LDL-C goal attainment across risk categories.

Methods: DA VINCI was an 18-country cross-sectional study of LLT in routine clinical management. We included 1594, 409, 424 and 55 very high, high, moderate and low-risk patients on stable LLT. Based on published efficacy estimates, the Monte Carlo method was used to simulate the proportion of patients who would achieve risk-based LDL-C goals, first through optimisation of statin intensity, then through addition of ezetimibe, and finally through addition of PCSK9 inhibitors (PCSK9i).

Results: In patients without atherosclerotic cardiovascular disease (ASCVD), statin optimisation would be expected to result in 22.4% of patients with low, 32.4% moderate, 25.0% high and 10.4% very high-risk attaining LDL-C goals (Table). Adding ezetimibe would lead to 44.2%, 64.3%, 55.6% and 32.2% attaining goals, respectively. Additional treatment of very high risk patients with PCSK9i would be predicted to result in 88.1% of patients achieving goal. Among ASCVD patients, only 12.0% would be likely to achieve LDL-C goals through statin optimisation, with 42.1% achieving goal with add-on ezetimibe, and 93.2% with adjunct

PCSK9i.

Table: Simulation of LDL-C goal attainment through optimal implementation of the 2019 ESC/EAS dyslipidemia guidelines**

	Patients without ASCVD					Patients with ASCVD			
	Low risk (N=55)	Moderate risk (N=424)	High risk (N=409)	Very high risk (N=74)	Total (N=962)	Not recurrent (N=1470)	Recurrent (N=50)	Total (N=1520)	Overall (N=2482)
Achieved 2019 LDL-C goal (optimisation of statin therapy)*	22.4%	32.4%	25.0%	10.4%	27.0%	12.4%	1.3%	12.0%	17.8%
Achieved 2019 LDL-C goal (addition of ezetimibe)	44.2%	64.3%	55.6%	32.2%	57.0%	43.0%	15.8%	42.1%	47.9%
Achieved 2019 LDL-C goal (addition of PSCK9 inhibitor)	NA	NA	NA	88.1%	NA	93.4%	84.6%	93.2%	NA

*Uptitration of statin (for patients not already on ezetimibe and not already on the highest dose of statin available).

**For a full overview of the LDL-C treatment goals in the 2019 ESC/EAS dyslipidemia guidelines, see https://academic.oup.com/view-large/207091448 (Mach et al. EHJ 2020; 41: 111–188).

NA: not applicable; according to the 2019 ESC/EAS dyslipidemia guidelines, PCSK9 inhibitors are only recommended for very high-risk patients.

The 2482 subjects included in this analysis are those subjects who had not achieved their risk-based ESC/EAS 2019 LDL-C goals in DA VINCI. Patients were also required to be stabilised on a known intensity of statin and/or ezetimibe. Patients already receiving a PSCK9 inhibitor were excluded.

Conclusions: Over half of patients without ASCVD would attain 2019 ESC/EAS LDL-C goals by optimising statins and adding ezetimibe. However, for very high-risk patients, including those with ASCVD, use of PCSK9i combined with oral LLTs would achieve LDL-C goals in ~90% of patients.

SE068

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-04 Adipose tissue homeostasis*

NOVEL ROLES OF PCSK6 IN ADIPOSE TISSUE HOMEOSTASIS

SAAG SESSION 16: NOVEL STANDPOINTS OF LIPOPROTEIN SIGNIFICANCE

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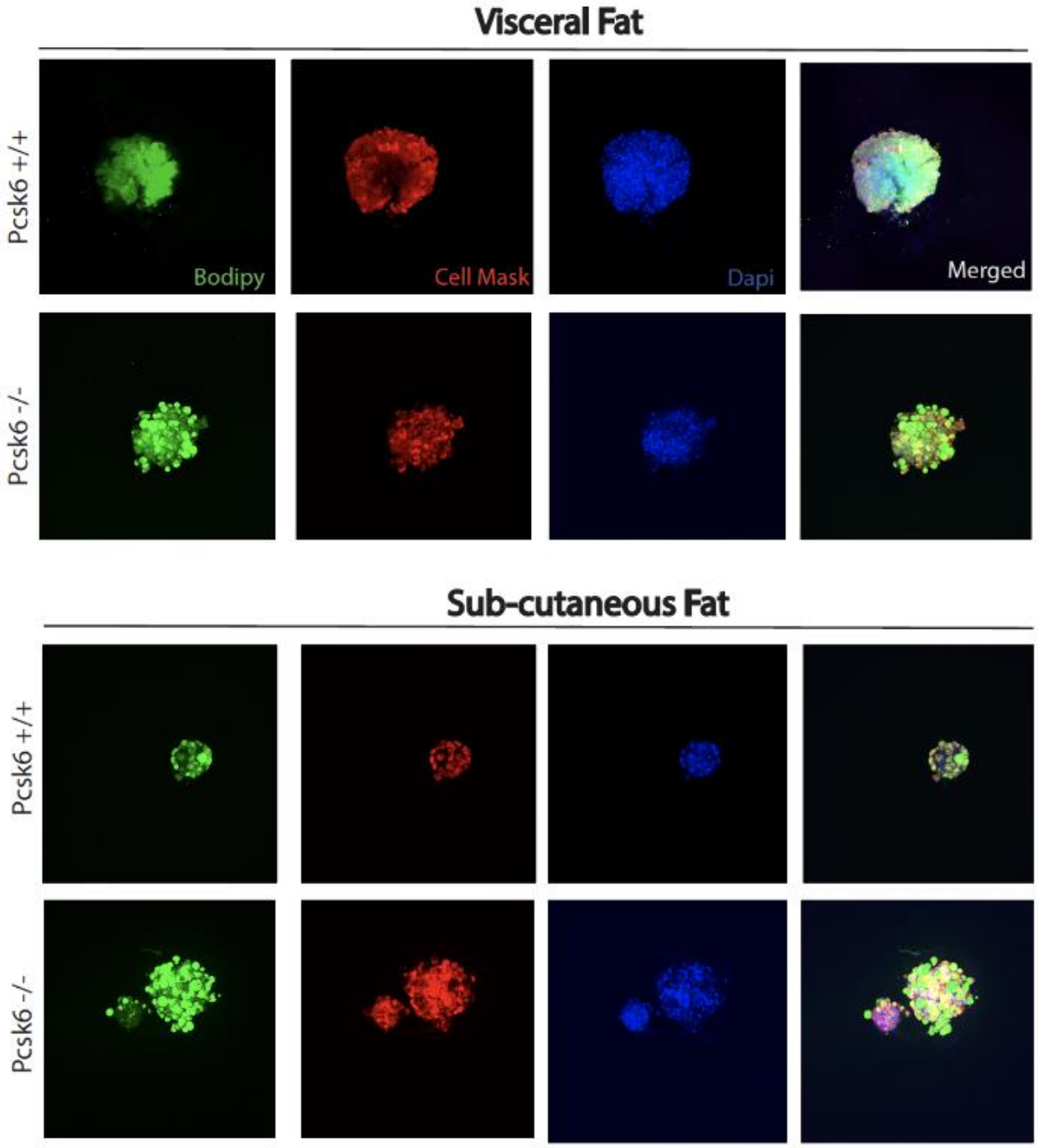
Background and Aims : We have discovered that PCSK6 modulates vascular smooth muscle cell remodeling in atherosclerosis and participates in plaque (in)stability via activation of matrix metalloproteinases, various mitogens and growth factors. In addition, PCSK6 processes two triglyceride lipase family members, endothelial lipase and lipoprotein lipase (LPL) with a possible impact on plasma lipoprotein levels and the adipose tissue. We aimed to investigate the role of PCSK6 in the adipose tissue related lipid metabolism using *Pcsk6-/-* mice.

Methods: Visceral and sub-cutaneous fat was collected from *Pcsk6-/-* and *Pcsk6* +/+ (control) mice followed by gene expression analysis of lipid metabolism related genes (n=5-10 mice per group). The stromal vascular fraction from the fat pads was isolated and pre-adipocytes were cultured and differentiated into mature spheroids. At D30, lipolysis assay, adiponectin measurement and immunofluorescence stainings were performed.

Results: *Pcsk6-/-* were found to be heavier than control mice. Gene expression analysis demonstrated an upregulation of *Adiopq*, *Lpl*, *Pparg* and *Fabp4* in the subcutaneous fat of *Pcsk6 -/-* mice compared to *Pcsk6 +/+, indicating* enhanced adipogenic differentiation. Immunofluorescence analysis of adipocyte spheroids generated from both subcutaneous and visceral fat of *Pcsk6-/-* mice demonstrated adipocytes with hypertrophied morphology with larger droplet sizes in both fat depots. Subcutaneous spheroids also showed increased lipolysis compared to controls. Adiponectin levels were significantly higher in the *Pcsk6 -/-* mice vs. controls in both subcutaneous and visceral fat.

Conclusions: Our preliminary data demonstrate that PCSK6 plays important roles in the adipose tissue differentiation and homeostasis which remains to be further

investigated.



SE069

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-16 Other*

NON-HDL-CHOLESTEROL IS A BETTER MARKER OF ATHEROGENIC DYSLIPIDEMIA IN OBSTRUCTIVE SLEEP APNEA

SAAG SESSION 16: NOVEL STANDPOINTS OF LIPOPROTEIN SIGNIFICANCE

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Background and Aims : Obstructive sleep apnea (OSA) is independently associated with dyslipidemia. LDL-cholesterol is accepted as a major independent risk factor for cardiovascular disease. However, non-HDL-cholesterol is a better marker of atherogenic dyslipidemia and recommended as a target of lipid lowering therapy. We aimed to assess the prevalence of atherogenic dyslipidemia, and relationship between OSA severity and serum LDL-cholesterol and non-HDL cholesterol levels in OSA patients.

Methods: We retrospectively evaluated treatment naïve 2361 subjects admitted to the sleep laboratory of a university hospital for polysomnography. All subjects' lipid profile including total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, and non-HDL-cholesterol were measured.

Results: Out of 2361 patients (mean-age 49.6±1.9 years; 68.9% male, apnea-hypopnea index 37± 28/h), 185 (7.8%) had no OSA and 2176 (92.2%) had OSA. Atherogenic dyslipidemia prevalence was high (57.66%) in OSA patients, and increased in severe OSA compared to other groups ($p < 0.05$). Though total and LDL-cholesterol did not differ between those with and without OSA, non-HDL-cholesterol ($p = 0.020$), and triglycerides ($p=0.001$) were higher and HDL-cholesterol levels ($p=0.018$) were lower in OSA patients than non-OSA. Non-HDL-cholesterol was significantly correlated with OSA severity ($p < 0.001$) and hypoxia parameters ($p < 0.01$), whereas LDL-cholesterol showed no correlation.

Conclusions: Atherogenic dyslipidemia is highly prevalent and non-HDL-cholesterol levels are significantly increased, predominantly in severe OSA patients. Non-HDL-cholesterol but not LDL-cholesterol, is significantly correlated with OSA severity and hypoxia parameters. Therefore, it could be better to use non-HDL-cholesterol as a marker of atherosclerotic cardiovascular risk in OSA patients.

SE070

Topic: *ASA04 - CLINICAL VASCULAR DISEASE / ASA04-12 Prevention and treatment of cardiovasculardisease; miscellaneous*

ELEVATED REMNANT CHOLESTEROL AND 2-FOLD MORTALITY FROM CARDIOVASCULAR AND OTHER CAUSES, BUT NOT FROM CANCER

SAAG SESSION 16: NOVEL STANDPOINTS OF LIPOPROTEIN SIGNIFICANCE

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Background and Aims : Cholesterol held in triglyceride-rich lipoproteins, also called remnant cholesterol, is being increasingly acknowledged as an important causal risk factor for atherosclerosis, atherosclerotic cardiovascular disease risk and even all-cause mortality; however, the association with cause-specific mortality is unknown. We tested the hypothesis that elevated remnant cholesterol is associated with increased mortality from cardiovascular disease, cancer, and other causes in a contemporary population-based cohort.

Methods: We included 87,201 individuals aged 20-69 years from the Copenhagen General Population Study 2003–2015 examination with remnant cholesterol calculated from a standard lipid-profile. During up to 13 years of follow-up, 687 individuals died from cardiovascular disease, 1,595 from cancer, and 856 from other causes. Cause of death was obtained from the national Danish Causes of Death Registry.

Results: Multivariable adjusted hazard ratios for cause-specific mortality in individuals with remnant cholesterol ≥1 mmol/L (≥39 mg/dL) compared to individuals with remnant cholesterol <0.5 mmol/L (<19 mg/dL) were 2.0 (95% confidence interval: 1.2-3.3) for cardiovascular disease, 1.0 (0.7-1.4) for cancer, and 1.9 (1.2-3.0) for other causes. Corresponding hazard ratios for the strongest associated sub-categories of cause-specific mortality were 4.1 (1.5-11) for ischemic heart disease, 8.2 (1.9-34) for infectious diseases, 18 (2.0-175) for hematologic diseases, and 10 (2.1-51) for endocrinological diseases.

Conclusions: Remnant cholesterol above 1 mmol/L (39 mg/dL) is associated with 2-fold mortality from cardiovascular and other causes, but not from cancer.

SE071

Topic: *ASA04 - CLINICAL VASCULAR DISEASE / ASA04-14 Other*

ASSOCIATION BETWEEN LIPOPROTEIN(A) CONCENTRATIONS AND ATHEROSCLEROTIC CARDIOVASCULAR DISEASE RISK IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA: AN ANALYSIS FROM THE HELLAS-FH

SAAG SESSION 16: NOVEL STANDPOINTS OF LIPOPROTEIN SIGNIFICANCE

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Background and Aims : Lipoprotein(a) [Lp(a)] is an independent risk factor for atherosclerotic cardiovascular disease (ASCVD) in the general population. However, such a role in patients with familial hypercholesterolemia (FH) is less documented. The purpose of this study was to evaluate the association between Lp(a) concentrations and ASCVD prevalence in adult patients with FH.

Methods: This was a cross-sectional study from the Hellenic Familial Hypercholesterolemia Registry (HELLAS-FH). Patients were categorized into 3 tertiles according to Lp(a) levels.

Results: A total of 541 adult patients (249 males) with heterozygous FH (HeFH) were included (mean age 48.5±15.0 years at registration, 40.8±15.9 years at diagnosis). Median (interquartile range) Lp(a) concentrations in the 1st, 2nd and 3rd Lp(a) tertile were 6.4 (3.0-9.7), 22.4 (16.0-29.1) and 77.0 (55.0-102.0) mg/dL, respectively. There was no difference in lipid profile across Lp(a) tertiles. The overall prevalence of CVD was 9.4% in the first, 16.1% in the second and 20.6% in the third tertile (p=0.012 among tertiles). This was also the case for premature ASCVD, with prevalence rates of 8.5%, 13.4% and 19.8%, respectively (p=0.010 among tertiles). A trend for increasing prevalence of coronary artery disease (8.3%, 12.2% and 16.1%, respectively; p=0.076 among tertiles) was also observed. No difference in the prevalence of stroke and peripheral artery disease was found across tertiles.

Conclusions: Elevated Lp(a) concentrations are significantly associated with increased prevalence of ASCVD in patients with HeFH.

SE072

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-13 Other*

PROTECTIVE EFFECTS OF PCSK9 DEFICIENCY OR INHIBITION ON FOOD ALLERGY DISEASE

SAAG SESSION 17: THINK OUTSIDE THE BOX: ON COGNITION AND PCSK9

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Background and Aims : Beyond its canonical role on LDL receptor and cholesterol homeostasis, concordant studies have suggested that PCSK9 is involved in several inflammatory responses. The present study aims to decipher the role of PCSK9 on food allergy.

Methods: Wheat gliadin food allergy (FA) was induced (Bouchaud et al., 2015) on 5 weeks-old mice fed with a gliadin-free diet. Briefly, at day 0, 10, 20, mice were sensitized by an intraperitoneal injection of vehicle (PBS) or deamidated gliadin (DG; 10mg with alum adjuvant). At day 27 and 34, mice received an oral gavage of water or 20mg DG and we respectively measured after these 2 challenges the intestinal transit time and ear swelling. The impact of PCSK9 on FA was investigated on 1) *Pcsk9*$^{+/+}$ and *Pcsk9*$^{-/-}$ mice 2) control and intestinal-specific *Pcsk9*$^{-/-}$mice, 3) vehicle or anti-PCSK9 monoclonal antibodies (mAb) treated BALB/c mice.

Results: FA significantly increased intestinal transit time (+ 71%, P<0,0001) and ear thickening in *Pcsk9*$^{+/+}$ mice compared to vehicle treated *Pcsk9*$^{+/+}$ mice (-2,22 µm vs 16,4 µm, P<0,0001). In similar conditions, no significant changes in these parameters were observed between control and FA induced *Pcsk9*$^{-/-}$ mice suggesting that full PCSK9 deficiency protects against FA symptoms. If we did not retrieve this protection in intestinal *Pcsk9*$^{-/-}$ mice, the inhibition of circulating PCSK9 with anti-PCSK9 antibodies in Balb/c mice did mimic the anti-allergic effects observed in full *Pcsk9*$^{-/-}$ mice.

Conclusions: Our data describes a new role for PCSK9 by demonstrating that the absence or inhibition of circulating PCSK9 protects mice against gliadin induced FA symptoms.

SE073

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-08 Novel risk factors and biomarkers*

SERUM AND CEREBROSPINAL FLUID CONCENTRATIONS OF PCSK9 AND HYDROXYSTEROLS IN PATIENTS WITH COGNITIVE IMPAIRMENT

SAAG SESSION 17: THINK OUTSIDE THE BOX: ON COGNITION AND PCSK9

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Background and Aims : Altered cerebral cholesterol homeostasis is associated with Alzheimer's Disease (AD). We previously demonstrated higher cerebrospinal fluid (CSF) PCSK9 levels in AD patients compared to controls. This study evaluated the concentrations of PCSK9 in CSF/serum of patients with different degrees of cognitive impairment. We further measured CSF/serum concentrations of the cholesterol metabolites 24-, 25- and 27-hydroxycholesterol (OHC).

Methods: Patients with AD (AD; n=27), with mild cognitive impairment (MCI) that converted to AD at follow-up (MCI-AD; n=28) and with stable MCI (MCI; n=28) were recruited. Serum/CSF PCSK9 levels were quantified through ELISA assay, while 24-25- and 27-OHC through LC-MS/MS.

Results: PCSK9 CSF concentration was similar among the three groups, but higher if compared to those of control subjects previously analyzed ($p<0.0001$ vs all). Within AD subjects, apoE Ɛ4 carriers presented higher PCSK9 concentration compared to non-carriers (+38%; $p=0.04$). In AD patients, a positive correlation between serum and CSF PCSK9 levels was found ($r=0.521$, $p=0.004$). CSF and serum 24-OHC, 25-OHC and 27-OHC levels were similar in the three groups. An inverse association was found between CSF PCSK9 and 27-OHC levels in CSF ($r=-0.238$; $p=0.047$) and serum ($r=-0.228$; $p=0.05$). PCSK9 CSF levels of apoE Ɛ4 carriers inversely correlated with 24-OHC ($r=-0.340$; $p=0.036$).

Conclusions: Our data suggest that PCSK9 levels may be increased since the early phases of the disease. In addition, the correlation between CSF/serum PCSK9 in AD suggests an increased blood-brain barrier permeability. The correlation between CSF PCSK9 levels and OHC implies a link with sterol metabolism that requires further investigation.

SE074

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-09 Epidemiology of socioeconomic and psychosocial risk factors*

CHRONIC STRESS MAY LEAD TO PCSK9 INCREASES AND DYSLIPIDEMIA THROUGH IFNG-CONNECTED PATHWAYS IN A CROSS-SECTIONAL STUDY OF AFRICAN AMERICANS AT-RISK FOR CVD

SAAG SESSION 17: THINK OUTSIDE THE BOX: ON COGNITION AND PCSK9

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Background and Aims : Proprotein convertase subtilisin/kexin type 9 (PCSK9) is linked to dyslipidemia and CVD outcomes, yet the relationship between chronic stress and PCSK9 is not well known. We evaluated connections between psychosocial and environmental stressors (PSES), cytokines, PCSK9, and lipoprotein levels.

Methods: D.C. Cardiovascular Health and Needs Assessment participants were recruited to NIH for clinical labs. Amygdalar activity (AmygA), as a marker of chronic PSES, was determined by ^{18}FDG-PET/CT. Plasma cytokines and PCSK9 were measured using ELISA-based techniques. Regression modeling was performed to identify associations between AmygA, cytokines, PCSK9, and lipids while adjusting for ASCVD 10-year risk score and BMI.

Results: The participants were 60 African Americans (mean age 61±11 years, 93% female) at risk for ASCVD. AmygA associated with PCSK9 (β=0.38, p=0.005) and IFNγ (β=0.47, p<0.001), and IFNγ mediated 46.1% of the AmygA and PCSK9 relationship. Subsequently, PCSK9 associated with Apo-A1 (β=0.44, p=0.001) and HDL (β=0.55, p<0.001), and IFNγ associated with Apo-A1 (β=0.45, p=0.001) and HDL (β=0.48, p<0.001). No associations were identified with Apo-B or LDL.

Conclusions: Results demonstrate links between chronic stress-related neural activity and IFNγ and PCSK9, which then associate with Apo-A1 and HDL. The PCSK9 and HDL connection is intriguing as recent work highlighted HDL transport by PCSK9. Together these findings provide a potential mechanism where chronic stress interacts with PCSK9 and IFNγ to drive HDL dysfunction. Findings also suggest IFNγ may be a target in stress-related dyslipidemia, but longitudinal studies are needed to further investigate these patterns.

SE075

Topic: *ASA04 - CLINICAL VASCULAR DISEASE / ASA04-14 Other*

DELIRIUM IS ASSOCIATED WITH THE NETS COMPONENT DSDNA IN SERUM AND CEREBROSPINAL FLUID

SAAG SESSION 17: THINK OUTSIDE THE BOX: ON COGNITION AND PCSK9

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Background and Aims : Delirium is an acute disruption of cognition, attention and awareness, commonly presenting during acute illness. The pathophysiology of delirium is largely unknown, but neuroinflammation is believed to play a role. We explored if serum and cerebrospinal fluid (CSF) markers of neutrophil extracellular traps (NETs) were associated with delirium.

Methods: Hip fracture patients (n= 457) were enrolled at Oslo University Hospital, Oslo, Norway. Delirium was assessed daily, pre- and postoperatively. Cognitively healthy subjects (n= 32) were included as controls. Double-stranded deoxyribonucleic acid (dsDNA) was quantified by a fluorescent nucleic acid stain in serum and CSF. Myeloperoxidase-deoxyribonucleic acid (MPO-DNA) and citrullinated histone 3 (H3Cit) were analyzed by ELISA in serum.

Results: All the NETs markers in serum were significantly higher in delirium compared with cognitively healthy controls ($p< 0.005$, all). dsDNA were significantly higher both in serum and CSF in hip fracture patients with delirium (n= 220) vs no delirium [577 (515, 640) vs 507 (458, 573) ng/ml, $p< 0.0005$] and [69 (58, 88) vs 63 (53, 79) ng/ml, $p= 0.002$], respectively, and only in delirium patients without pre-existing dementia. No significant differences were found for MPO-DNA and H3Cit in patients with delirium vs no delirium. Levels of dsDNA in serum and CSF were not correlated.

Conclusions: Delirium was associated with significantly higher levels of the NETs-related component dsDNA, both in the systemic circulation and CSF. Our results may indicate a role for the innate immune system and NETs in the pathophysiology of delirium.

SE076

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-13 Genomics, GWAS and population genetics; Mendelian randomization*

GENETIC CONTROL OF BODY WEIGHT BY THE HUMAN BRAIN PROTEOME

SAAG SESSION 17: THINK OUTSIDE THE BOX: ON COGNITION AND PCSK9

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Background and Aims : Genome-wide association studies (GWAS) have identified hundreds of genetic variants linked with body weight but the biological relevance of most of them remains unexplored. Given the critical role of the brain in body weight regulation, we set out to explore whether genetic variants associated with body mass index (BMI) could influence brain protein concentrations.

Methods: We included two GWAS on 5686 brain protein concentrations measured by mass spectrometry, from the dorsolateral prefrontal cortex of 330 and 140 participants, respectively. We combined these data with a GWAS on body mass index from 681,275 individuals of European ancestry. We performed Mendelian randomization (MR) and genetic colocalization analyses to identify proteins influenced by BMI genetic loci. A proteome-wide MR study was also performed to identify new proteins linked with BMI.

Results: We mapped 22 BMI loci to brain protein concentrations (colocalization posterior probability >0.8). The proteome-wide MR approach identified 32 brain proteins, including 11 proteins already identified in the genome-wide mapping phase ($p<0.05/5382=9.3e-06$ and colocalization posterior probability >0.8). Among the 45 proteins, 10 had cis-regulated mRNA levels that colocalized with brain protein concentrations and BMI. Multi-trait colocalization identified dietary habits, especially sugar intake, as potential mediators between brain proteins concentrations and BMI.

Conclusions: We have identified 45 dorsolateral prefrontal cortex proteins that may be involved in the control of body weight. These results support that the role of the brain in regulating body weight might be more critical than previously estimated.

SE077

Topic: *ASA01 - PATHOGENESIS OF ATHEROSCLEROSIS / ASA01-01 Endothelial cell function and biology*

SUPERVISED IN SILICO RECONSTRUCTION OF ENDOMT IN HUMAN ATHEROSCLEROTIC PLAQUES IDENTIFIES CRUCIAL CELL POPULATIONS AND PROCESSES.

SAAG SESSION 18: THE CRUCIALITY OF ENDOTHELIAL CELLS

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Background and Aims : In atherosclerosis, endothelial cells can differentiate into mesenchymal cells by a process called EndoMT. In humans, histological studies suggested the presence of EndoMT in atherosclerotic lesions. However, in contrast to murine models, where EndoMT can be studied with lineage tracing techniques, the molecular underpinnings of endoMT in human lesions are not yet known. Consequently, the molecular markers of EndoMT are typically represented by pathways and gene sets curated from genes associated with end-stage mesenchymal cells and do not fully capture this critical process. Here we combine the in vitro EndoMT models with single-cell transcriptomic datasets of human atherosclerotic lesions to reconstruct the EndoMT process and identify markers and crucial molecular mechanisms.

Methods: First, we stimulated HCAEC cells with TNF-α and TFG-β to study the transcriptomic changes over different time points (0, 2, 4 ,8, 12, 24, 48 and 72h) and curated a molecular fingerprint of genes upregulated during EndoMT.

Results: Next, We could demonstrate that this reference set of genes was upregulated in Cdh5-CreERT2/Rosa-eYFP/apoE-/- lineage traced scRNA-seq data along the imputed trajectory from EC to mesenchymal cells (R = 0.85). Finally, we used this reference set to reconstruct the same lineage across multiple subpopulations of ECs and SMCs in human carotid scRNA-seq data (n = 46) and revealed crucial cell populations and molecular pathways involved in EndoMT.

Conclusions: By combining RNA-seq data from *in vitro* EndoMT models with single-cell transcriptomic datasets of human atherosclerotic lesions, we uncovered a central hub of human cells that are highly plastic and that point towards cells undergoing EndoMT.

SE078

Topic: *ASA01 - PATHOGENESIS OF ATHEROSCLEROSIS / ASA01-01 Endothelial cell function and biology*

HIGH-INTENSITY STATINS COMBINED WITH EICOSAPENTAENOIC ACID (EPA) IMPROVES ENDOTHELIAL FUNCTION DURING EXPOSURE TO OXIDIZED LDL

SAAG SESSION 18: THE CRUCIALITY OF ENDOTHELIAL CELLS

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Background and Aims : Endothelial cell (EC) dysfunction leads to reduced nitric oxide (NO) bioavailability and increased cytotoxic peroxynitrite ($ONOO^-$). Loss of NO promotes inflammation and impairs vasodilation. Eicosapentaenoic acid (EPA) administered as icosapent ethyl (IPE) reduced cardiovascular (CV) events in statin-treated patients (REDUCE-IT). We tested the effects of high-intensity statins and EPA in ECs exposed to oxidized LDL (oxLDL).

Methods: Human umbilical vein ECs (HUVECs) were pretreated with 20 mg/dL oxLDL for 20 min, then treated with atorvastatin (active metabolite, ATM) and rosuvastatin (rosuva) at 1.0μM ± EPA (10μM) for 1 hr. Cells were stimulated with calcium and assayed for the $NO/ONOO^-$ release ratio using porphyrinic nanosensors.

Results: ECs challenged with oxLDL showed a 60% reduction in NO release compared with vehicle (386±29 to 156±18 nM, $p<0.001$) concomitant with a 38% increase in $ONOO^-$ release (205±31 to 283±16 nM, $p<0.001$), resulting in a 71% decrease in the $NO/ONOO^-$ release ratio (1.89±0.32 to 0.55±0.07, $p<0.001$). ECs treated with ATM had an improved $NO/ONOO^-$ release ratio (53%) that increased (216%, $p<0.01$) when combined with EPA. Similarly, ECs treated with rosuva had a larger $NO/ONOO^-$ release ratio (38%) that increased (131%, $p<0.05$) with EPA. EPA combined with either statin significantly decreased $ONOO^-$ more than statin alone ($p<0.01$).

Conclusions: EPA enhanced NO bioavailability in combination with a highly effective statins under disease-relevant conditions. The ability of EPA/statins to limit EC dysfunction may contribute to its benefits in high-risk CV patients.

SE079

Topic: *ASA01 - PATHOGENESIS OF ATHEROSCLEROSIS / ASA01-01 Endothelial cell function and biology*

ENDOTHELIAL CELL SPECIFIC DEFICIENCY OF CD40 STABILIZES MURINE ATHEROSCLEROTIC LESIONS BY IMPAIRING INFLAMMATORY CELL RECRUITMENT

SAAG SESSION 18: THE CRUCIALITY OF ENDOTHELIAL CELLS

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Background and Aims : Phenotypes of atherosclerotic plaques that favor plaque instability, subsequent rupture, and acute cardiovascular complications are driven by local inflammation of the vessel wall. The co-stimulatory CD40L–CD40 dyad exerts a critical role in atherosclerosis. The cell-type specific contribution of both molecules, however, remains elusive. Here, we evaluated the contribution of CD40 expressed on endothelial cells (ECs) in a mouse model of atherosclerosis.

Methods: $CD40^{fl/fl}Apoe^{-/-}Bmx^{Cre}$mice and littermate controls received tamoxifen at 8 weeks and consumed a chow diet for additional 25 weeks. Metabolic and inflammatory phenotypes were characterized by plasma analysis, intravital microscopy, flow cytometry, qPCR, ELISA, quantification of cytokines, and histology. Human plaques samples were obtained from patients with symptomatic carotid artery stenosis. Dynamic adhesion assays were conducted on HUVECs in fluorescence microscopy.

Results: Atherosclerotic plaques of $Apoe^{-/-}$mice and humans displayed increased expression of CD40 on ECs compared with controls. EC-specific deletion of CD40 ameliorated plaque lipid deposition and lesional macrophage accumulation, and increased intimal smooth muscle cell and collagen content, while atherosclerotic lesion size did not change. Leukocyte adhesion to the vessel wall was impaired in iEC-CD40-deficient mice as demonstrated by intravital microscopy. In accord, expression of VCAM-1 and ICAM-1 in the vascular endothelium declined after deletion of CD40. In vitro, antibody-mediated inhibition of human endothelial CD40 abrogated monocyte adhesion on ECs.

Conclusions: Endothelial deficiency of CD40 in mice promotes structural features associated with a stable plaque phenotype in humans and decreases leukocyte adhesion. These results suggest that endothelial-expressed CD40 contributes to inflammatory cell migration and consecutive plaque formation in atherogenesis.

SE080

Topic: *ASA01 - PATHOGENESIS OF ATHEROSCLEROSIS / ASA01-01 Endothelial cell function and biology*

OMIC CHARACTERIZATION OF THE ENDOTHELIAL MODEL MIMICKING GLUCOSE VARIABILITY

SAAG SESSION 18: THE CRUCIALITY OF ENDOTHELIAL CELLS

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Background and Aims : Glycemic variability (GV), a complex phenomenon affecting subjects with diabetes, is one of the main contributors to the risk to develop both acute and long-term complications in type 1 (T1D) and type 2 (T2D) subjects. The characteristic cellular phenotype induced by GV is scarcely defined. Aims of this study are: 1) to describe a global pattern about the regulation of the major proteins differentially expressed in a cellular (human endothelial cells) model of GV and 2) the role of microRNAs on the pathways activated during the *in-vitro*long-term exposures to GV.

Methods: We utilized human endothelial cells exposed for 21 days to high and oscillating glucose concentration (25 mM and 5-25 mM, respectively). The proteomic analysis were performed using 2D-DIGE and MALDI/TOF. microRNA discovery was performed by Megaplex RT primers Human Pool A.

Results: We identified a dataset of proteins and micro-RNAs differentially regulated by the oscillating and high glucose conditions. In particular, microarray of immunoprecipitated target bound to miR-146a-5p identified the main mRNA transcripts in this model. We found a protein identified both in proteomic and microarray experiments, vimentin, which is the principal protein involved in the activation of fibrotic pathways. Immunofluorescence analysis showed that the increased expression of vimentin is due to silencing of miR-146a-5p.

Conclusions: Our set of data constitutes the “Proof of Principle” about the ability of the omic-approaches (proteomic analyses coupled with miRNome) to reveal potential biomarkers for the GV model. Future perspective is to draw the GV cellular signature along with associated functions and mechanisms.

SE081

Topic: *ASA01 - PATHOGENESIS OF ATHEROSCLEROSIS / ASA01-01 Endothelial cell function and biology*

GENETICALLY ELEVATED LDL CHOLESTEROL BURDEN RESULTS INTO REDUCED PROGENITOR CELLS WITH IMPAIRED ENDOTHELIAL FORMING POTENTIAL

SAAG SESSION 18: THE CRUCIALITY OF ENDOTHELIAL CELLS

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Background and Aims : Genetically determined Familial Hypercholesterolemia (FH) leads to lifelong elevated LDL cholesterol (LDL-C) burden and premature vascular endothelial dysfunction. We previously demonstrated that genetically determined FH results into premature hematopoietic cellular aging and we now aim to evaluate whether Endothelial Progenitor Cells (EPCs), in charge of the endothelial formation, display features of premature impairment.

Methods: EPCs were taken by 33 Heterozygous FH (Next Generation Sequencing on LDLR) (95% on LDL-C lowering treatment), by 10 clinically defined but not genetically confirmed FH (“CD-FH”, 11% on LDL-C treatment) and by 13 normocholesterolemic subjects (“controls”, none on treatment). EPCs were characterized for: (i) blood count (Flow-Cytometry); (ii) ex vivo number of Endothelial Colony Forming Units (“EC-CFUs”); (iii) proliferation index (Cell-trace Violet).

Results: Blood EPCs were reduced in HeFH versus controls (37±53 vs 225±506 cells/mL, p=0,050). Of note, EPCs number was comparable between CD-FH and controls (50±43 vs 225±506 cells/mL, p=0,687). After three days of peripheral blood cells culture, EC-CFUs of HeFH were less versus that of controls (7±2,27 vs 20±7,72 EC-CFU/well, p=0,04). Vice versa, EC-CFUs from CD-FH and controls were comparable (5±1,37 vs 20±7,72 EC-CFU/well, p=0,152). These differences were not mirrored by different proliferation indexes which, from cell seeding up to the third day of culture of EC-CFU, was numerically but not significantly reduced in HeFH vs controls (25,05±15,13% vs 32,73±18,40%).

Conclusions: A low number of EPCs is present in subjects with genetically elevated LDL-C burden, also with ongoing LDL-C lowering treatment. In depth analyses are warranted to unravel developmental aspects of this cell compartment in FH.

SE082

Topic: *ASA01 - PATHOGENESIS OF ATHEROSCLEROSIS / ASA01-04 Inflammation, immunity and infection in atherosclerosis*

CIRCULATING MICROVESICLES IN ASSOCIATION WITH THE NLRP3 INFLAMMASOME IN CORONARY THROMBI FROM STEMI PATIENTS

SAAG SESSION 19: CONTROLLING INFLAMMATION IN ATHEROSCLEROSIS

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Background and Aims : Circulating microvesicles (MVs) are membrane-enclosed particles shed from activated cells. The NLRP3-inflammasome and the interleukin 6 (IL-6)-pathways are essential in cardiovascular disease. Knowledge of how inflammasome-related inflammation impacts secretion of MVs is limited. We aimed to investigate whether MVs in plasma associate with genes encoding inflammasome-signaling in coronary thrombi. Moreover, any relationships between inflammasome-activation, phosphatidylserine (PS) externalization, determined through Annexin V (AV^+) labelling, and the degree of myocardial injury, assessed by peak troponin T (TnT), were analyzed.

Methods: Aspirated intracoronary thrombi and blood samples from STEMI patients (n= 33) were investigated. mRNA of NLRP3, caspase-1, interleukin-1β (IL-1β), interleukin-18 (IL-18), IL-6, soluble IL-6-receptor (sIL-6R), and glycoprotein-130 (gp130) were isolated from the thrombi and relatively quantified by RT-PCR. The MVs were analyzed by flow cytometry and integrity ensured by use of the membrane dye carboxyfluorescein diacetate succinimidyl ester (CFSE).

Results: Total AV^+ MVs, mainly reflecting hypercoagulability, were positively correlated to NLRP3 gene-expression (r= 0.545, p= 0.009). A similar pattern was seen for platelet, endothelial and leukocyte derived MVs, separately. The majority of the MVs were AV^-(96%). Total- and AV^- MVs inversely correlated to IL-1β (r= -0.399 and -0.438, respectively, p< 0.05, both) and gp130 (r= -0.457 and -0.502, respectively, p< 0.05, both). No correlations between MVs and TnT were observed.

Conclusions: Our findings indicate a relationship between NLRP3-inflammasome in coronary thrombi and procoagulant AV^+ MVs in STEMI-patients. The inverse associations between AV^- MVs and gene expression of inflammasome activation may indicate an immuno-dampening role of this subpopulation.

SE083

Topic: *ASA01 - PATHOGENESIS OF ATHEROSCLEROSIS / ASA01-04 Inflammation, immunity and infection in atherosclerosis*

DUAL ROLE PLAYED BY NKT CELLS IN THE DEVELOPMENT OF ATHEROSCLEROSIS: EVIDENCE FROM META-ANALYSIS OF PRE-CLINICAL STUDIES

SAAG SESSION 19: CONTROLLING INFLAMMATION IN ATHEROSCLEROSIS

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Background and Aims : NKT cells play crucial role in regulating early inflammation in atherosclerosis. Broadly, there are two subsets, invariant (iNKT) and variant (vNKT), where iNKTs are thought to be pro-atherosclerotic, yet uncertainty remains.

Methods: We performed a pre-clinical meta-analysis to understand the true role of NKT cells in atherosclerosis. Twenty studies comprising 550 mice were selected for the meta-analysis. A random-effect model was employed to estimate the effect size. Additionally, we also fed high-fat-diet (HFD) to ApoE-null mice for 5 weeks, followed by identification of NKT lymphocytes from liver, spleen and blood with antibodies and α-GalCer loaded CD1d tetramer and flow cytometric analysis.

Results: Our analysis showed that whole NKT cells are increased in atheroprone mice after HFD (SMD 2.62, CI 1.47-3.77, $p<0.01$). Absence of NKT or iNKT cells (Fig. A) individually, in the mouse, leads to reduction (NKT [SMD -1.35, CI 2.19- -0.52, $p<0.01$]; iNKT [SMD -0.70, CI -1.96-0.55, $p<0.01$]), and activation of iNKT remarkably increases the lesion area (SMD 1.89, CI 0.84-2.95, $p<0.01$). Contrastingly, unlike NKT cells number increase, the iNKT numbers are reduced with the atherosclerosis (Fig. B). Our experimental results also reveal the reduction of iNKT cells in 5 weeks of HFD to ApoE-null mice (Liver-28%, Spleen-13%, Blood-

58%).

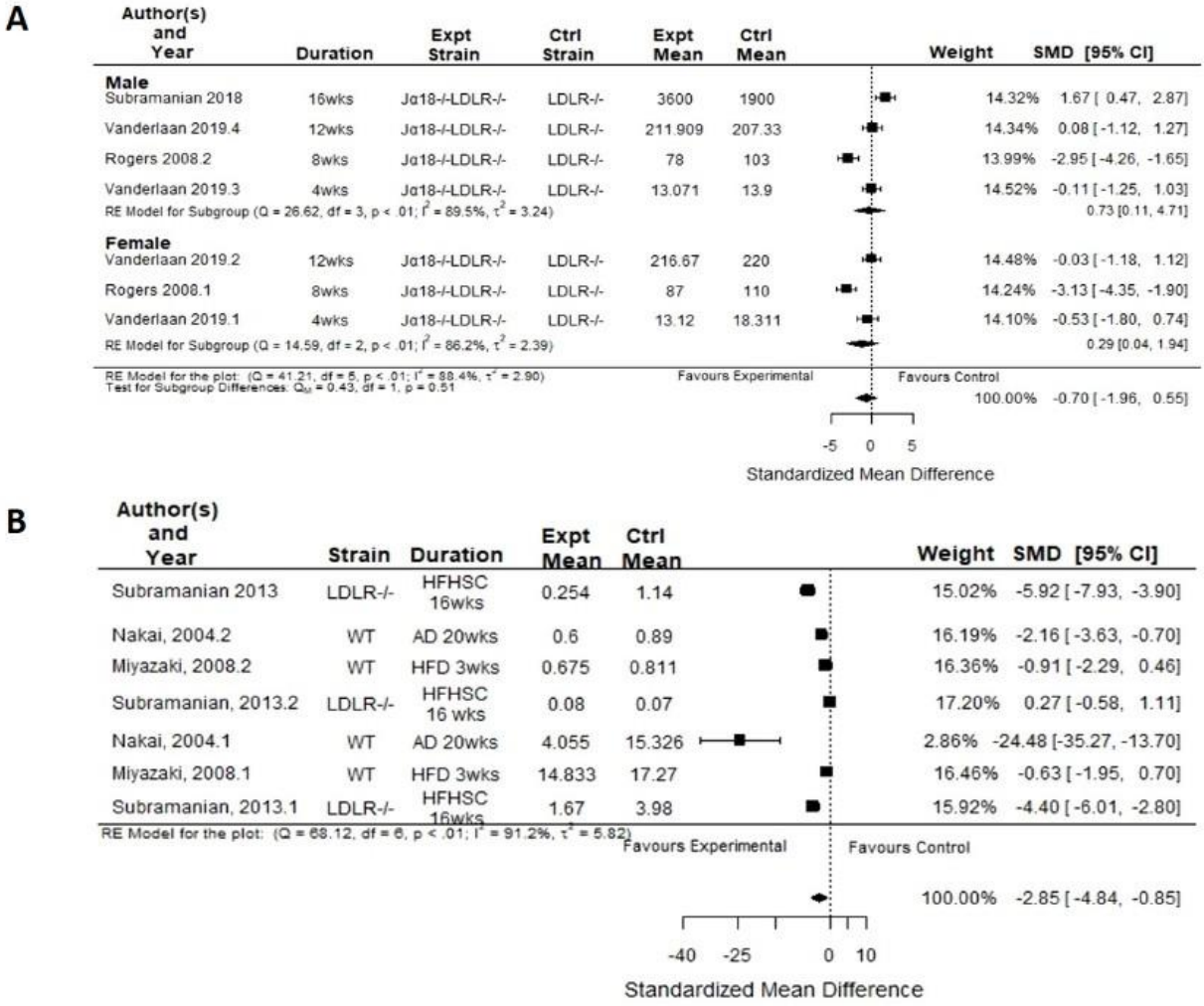

Figure. Forest Plots for **A.** Lesion area in the absence of iNKT cells in HFD fed Jα18-/-LDLR-/- and LDLR-/- mice, **B.** iNKT cell numbers in mice fed with atherogenic diet compared with chow fed mice.

Conclusions: This is the first meta-analysis on NKT cells and its subsets in preclinical atherosclerotic models. Based on this analysis and our data, we conclude that both NKT and iNKT cells are pro-atherosclerotic in nature, however, a gap still remains in the understanding of interplay of both subsets of NKT cells in atherosclerosis.

SE084

Topic: *ASA01 - PATHOGENESIS OF ATHEROSCLEROSIS / ASA01-04 Inflammation, immunity and infection in atherosclerosis*

IL-1RAP BLOCKADE REDUCES ATHEROSCLEROSIS AND LIMITS PLAQUE INFLAMMATION

SAAG SESSION 19: CONTROLLING INFLAMMATION IN ATHEROSCLEROSIS

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Background and Aims : Aim: The interleukin-1 receptor accessory protein (IL-1RAP) is required for signaling of several IL-1 family cytokines, including IL-1α and IL-1β which have been shown to promote plaque progression and inflammation, as well as IL-33 and IL-36. In this study we investigated the effects of a novel non-depleting blocking anti-IL-1RAP antibody on plaque burden and plaque inflammation.

Methods: Methods: *Apoe*$^{-/-}$ mice fed a high cholesterol diet were treated with either anti-IL-1RAP antibody (clone: 3A9) or isotype control IgG for six weeks (n=14/group). After total 10 weeks of diet, mice were terminated and organs were isolated. Digested aortas, lymphoid tissues, and blood were analyzed by flow cytometry and subvalvular plaques were analyzed by histology.

Results: Results: Anti-IL-1RAP treatment led to a 20% mean reduction in plaque size and volume (p<0.05) compared to isotype control mice. Histological and flow cytometry analysis of aortas demonstrated reduced plaque inflammation in anti-IL-1RAP treated mice, as evident by reductions in numbers of plaque macrophages, T cells, and neutrophils. No changes were observed in levels of splenic regulatory T cells or IFN-γ-producing Th1 cells, nor in levels of circulating monocytes or neutrophils. However, levels of splenic Th17 cells were reduced by IL-1RAP blockade.

Conclusions: Conclusions: Disruption of IL-1RAP signaling using a novel anti-IL-1RAP antibody reduces plaque burden and plaque inflammation in atherosclerotic mice. Our findings support further investigation into IL-1RAP blockade as a therapeutic strategy for limiting plaque inflammation in patients. Ongoing studies are investigating the expression patterns of IL-1RAP in human plaques and atheroprotective mechanisms of IL-1RAP blockade.

SE085

Topic: *ASA01 - PATHOGENESIS OF ATHEROSCLEROSIS / ASA01-04 Inflammation, immunity and infection in atherosclerosis*

PLASMA INTERLEUKIN-6 PREDICTS CAROTID PLAQUE SEVERITY, VULNERABILITY, AND PROGRESSION IN THE CARDIOVASCULAR HEALTH STUDY

SAAG SESSION 19: CONTROLLING INFLAMMATION IN ATHEROSCLEROSIS

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Background and Aims : We investigated if plasma IL-6 levels predict carotid plaque severity, vulnerability, and progression in the Cardiovascular Health Study.

Methods: Duplex carotid ultrasound was performed at baseline and 5 years. Plaque severity was scored 0 to 5 based on NASCET grade of stenosis. Plaque vulnerability at baseline was the presence of irregular, ulcerated or echolucent plaques. Plaque progression at 5 years was a ≥1 point increase in stenosis severity. Relationship of baseline plasma IL-6 levels with plaque characteristics was modeled using multivariable linear (severity) or logistic (vulnerability and progression) regression. Risk factors of atherosclerosis were included as independent variables. Stepwise backward elimination was used with p>0.05 for variable removal. We computed optimism-adjusted odds ratios using heuristic shrinkage method and performed internal validation with 100 bootstrap samples.

Results: In 4334 participants with complete data (58.9% women, mean age: 72.7 ± 5.1 years), the prevalence of mild, moderate, and severe carotid stenosis was 72%, 3%, and 0.7%. There were 1267 (29.2%) participants with vulnerable plaque and 1474 (34.0%) with plaque progression. Log IL-6 predicted plaque severity (β = 0.09, p=0.04), vulnerability (OR = 1.22, 95% CI: 1.06-1.40, p=0.006) and progression (OR = 1.44, 95% CI: 1.23-1.69, p<0.001). Associations remained significant in optimism-adjusted models. In participants with >50% predicted probability of progression, mean log IL-6 was 0.54 corresponding to 2.0 pg/mL. Dichotomizing IL-6 levels did not affect the performance of regression models.

Conclusions: Plasma IL-6 predicts carotid plaque severity, vulnerability, and progression. The 2.0 pg/mL cut-off could help select individuals that could benefit from anti-IL-6 drugs for stroke prevention.

SE086

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-01 Epidemiology of cardiovascular diseases and risk factors*

DOSE-RESPONSE RELATIONSHIP BETWEEN PHYSICAL ACTIVITY AND THE INCIDENCE OF PERIPHERAL ARTERY DISEASE IN GENERAL POPULATION: INSIGHTS FROM THE NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY

SAAG SESSION 20: FROM BENCH TO BEDSIDE IN ATHEROSCLEROSIS

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Background and Aims : Peripheral artery disease (PAD) is more unrecognized compared to other cardiovascular diseases, and yet is the most common cause of tissue loss, infection and major amputation. It is reported that more than 200 million people have PAD worldwide. More than half of the patients diagnosed with PAD are asymptomatic. Despite the advanced nature of medical systems in many countries, the health hazard and economic burden of PAD remain high due to the requirement for frequent intervention.Thus, determining the aetiology of PAD and taking effective measures are very important to reduce the burden of this condition. Ankle brachial index (ABI) is the ratio of ankle to arm systolic pressure and a valid non-invasive measure that predicts for PAD as well as other cardiovascular events. Though there is good evidence for higher physical activity (PA) levels reducing risk of cardiovascular disease and exercise therapy is recommended for patients with intermittent claudication in latest European Society of Cardiology guideline for PAD, little is known about the association between the quantity, intensity of PA and the incidence of PAD. Besides, most of the studies included in the guideline are based on patients with intermittent claudication. The situation for general population is not clear and the dose-response relationship between PA and the incidence of PAD has not been reported before. This study aimed to determine whether there is a dose-response relationship between PA and the incidence of PAD in American adults by analyzing the data from National Health and Nutrition Examination Survey (NHANES) from 1999-2004.

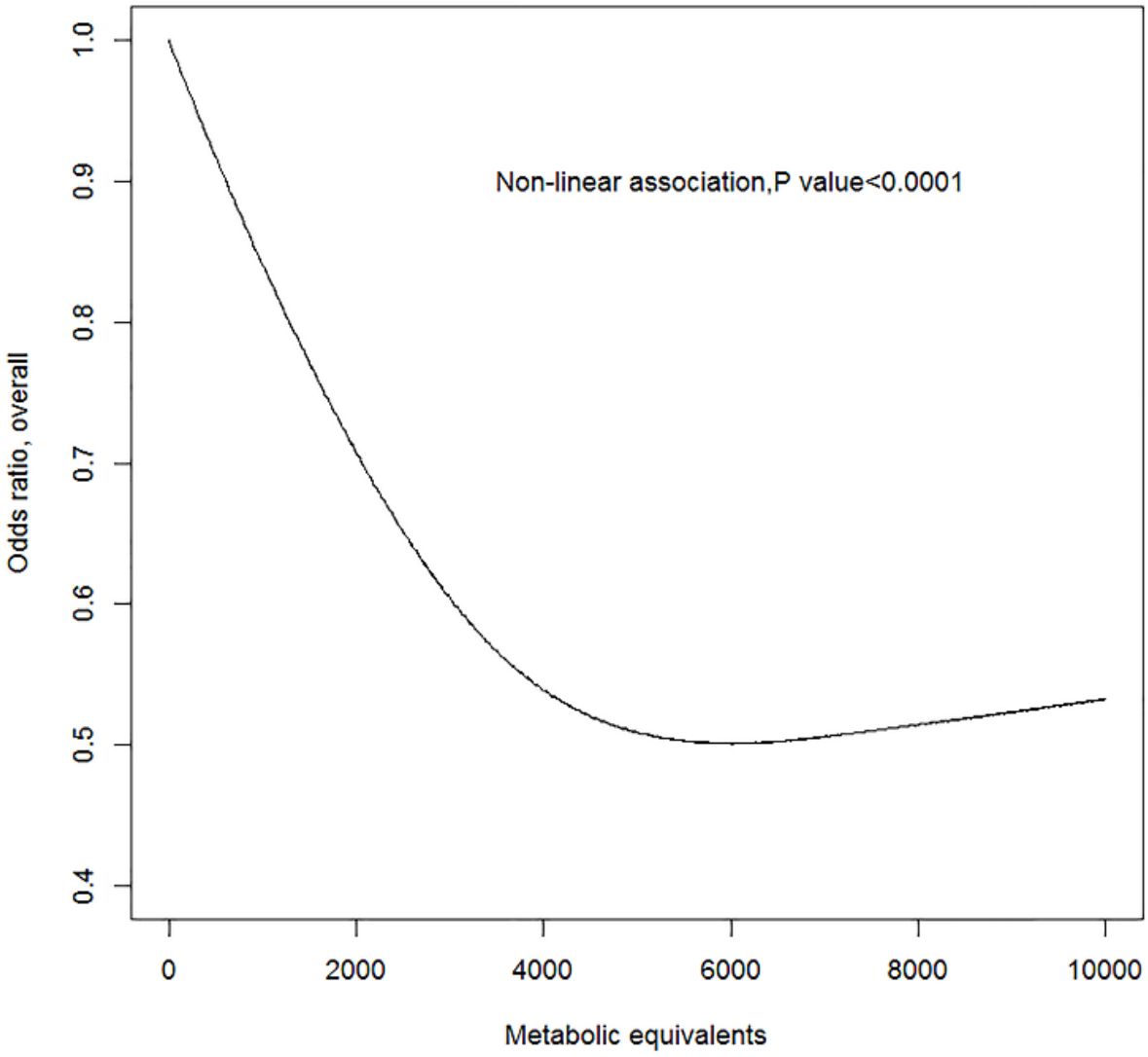
Methods: The data of this study was obtained from the NHANES 1999-2004. In present study, subjects with missing values of ABI, blood glucose, total cholesterol and other interested covariates were excluded from the analyses. To minimize the potential confounding from prior morbidity, we excluded participants with low cardiovascular fitness level. Finally, 1370 participants older than 40 years with complete date were included in our analysis. The available data had been weighed demographically, and NHANES study protocol was approved by the National Center for Health Statistics ethics review board with all participants providing written informed consent. Participants' ABI were measured by trained technicians and PAD was defined as ABI≦0.9. PA was obtained with a standard questionnaire and metabolic equivalents (MET) were used to quantify the PA level. The questionnaire collected the type, frequency (number of days per month) and duration (amount of time spent on a typical day) of PA in the past 30 days for a minimum of 10 min. PA was calculated by multiplying the number of days by the mean duration by the recommended MET, and then summed the values to obtain a value of total PA. Logistic regression was used to assess the association between PA and incidence of PAD, and the dose-response relationship was analyzed with RCS.

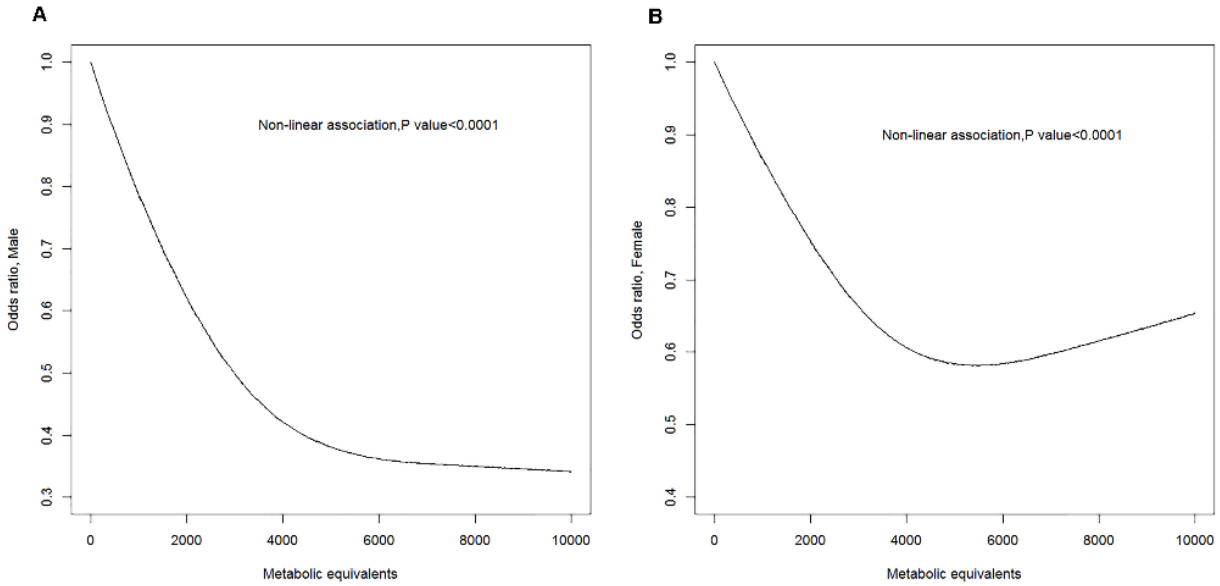
Results: PAD was present in 6.2% participants: 5.6% of males and 6.9% of females. After adjusting for potential confounders, compared with the first quartile (Q1) of MET, the odds ratios (ORs) of PAD for those with Q2, Q3 and Q4 of MET were 0.688 [95% confidence interval (CI)=0.684-0.692], 0.463 (95% CI=0.460-0.466), 0.816 (95% CI=0.812-0.821), respectively (all p<0.001). The RCS regression showed that PA was related to the incidence of PAD in a non-linear manner (p for non-linearity<0.0001). For females, the prevalence of PAD decreased as physical activity increased, reaching the minimum for

activity at approximately 5800 MET-min month^{-1} (OR=0.425, 95% CI=0.424-0.426), and for males, no pleatu was found in this study.

Baseline characteristics of included participants				
Characteristic	Non-PAD	PAD	Z/x^2	*P*
N (participants)	1285	85		
MET, median (IQR)	3652.5(5825)	2100(4095)	-3.090	0.002
Left ABI, median (IQR)	1.14(0.13)	0.87(0.18)	-12.687	<0.001
Right ABI, median (IQR)	1.14(0.14)	0.84(0.17)	-14.358	<0.001
Sex (%)			0.983	0.321
Female	594(46.23)	44(51.76)		
Male	691(53.77)	41(48.24)		
Age (%)			70.343	<0.001
40-64	854(66.46)	28(32.94)		
65-74	255(19.84)	21(24.71)		
75-84	154(11.98)	26(30.59)		
≥85	22(1.72)	10(11.76)		
Smoking (%)			8.216	0.004
No	614(47.78)	27(31.76)		
Yes	671(52.22)	58(68.24)		
Glucose, plama, mg/dL, median (IQR)	99.1(17.10)	105.2(19.80)	3.025	0.003

Odds ratio (OR) and 95% CIs of the MET quartiles for PAD.						
Variable	Univariate		Model A		Model B	
	OR(95% CI)	*P*-value	OR(95% CI)	*P*-value	OR(95% CI)	*P*-value
MET quartiles						
Q1	1		1		1	
Q2	0.618(0.614-0.621)	<0.001	0.636(0.632-0.639)	<0.001	0.688(0.684-0.692)	<0.001
Q3	0.451(0.449-0.454)	<0.001	0.437(0.434-0.439)	<0.001	0.463(0.460-0.466)	<0.001
Q4	0.597(0.594-0.600)	<0.001	0.735(0.731-0.739)	<0.001	0.816(0.812-0.821)	<0.001
P for trend		<0.001				


Non-linear association,P value<0.0001
Odds ratio, overall
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0
2000
4000
6000
8000
10000
Metabolic equivalents



Conclusions: The strengths of our study include the inclusion of a representative population from a nationwide survey on non-institutionalized people. Logistic regression and RCS models were used to evaluate the relationship between PA and incidence of PAD, and stratification was performed for gender. By using RCS model, dose-response relationship was ideally evaluated without jumping directly from one interval to another, avoiding the subjectivity of traditional regression methods. In this study, we innovatively identified a mirrored “J-shaped” relationship between PA and PAD risk by the RCS method, especially for females (Figure 2), revealing that neither too low nor high PA may be optimal for PAD prevention. The determination of these non-linear dose-response relationship helps public health leaders to develop medical policies. The finding of this paper also inspires further basic research on the causal mechanisms underlying the “J-shaped” trajectory. There is a significant inverse dose-response relationship between PA and the incidence of PAD. When PA increased to 5800 MET-min month, the prevalence of PAD in overall population decreased by around 50%, while increases in PA may benefit males but reduces the protective effect of PA in females.

SE087

Topic: *ASA01 - PATHOGENESIS OF ATHEROSCLEROSIS / ASA01-04 Inflammation, immunity and infection in atherosclerosis*

M1 AND M2 MONOCYTES AS MARKERS OF ADVANCED CAROTID ATHEROSCLEROSIS IN MIDDLE-AGED PATIENTS

SAAG SESSION 20: FROM BENCH TO BEDSIDE IN ATHEROSCLEROSIS

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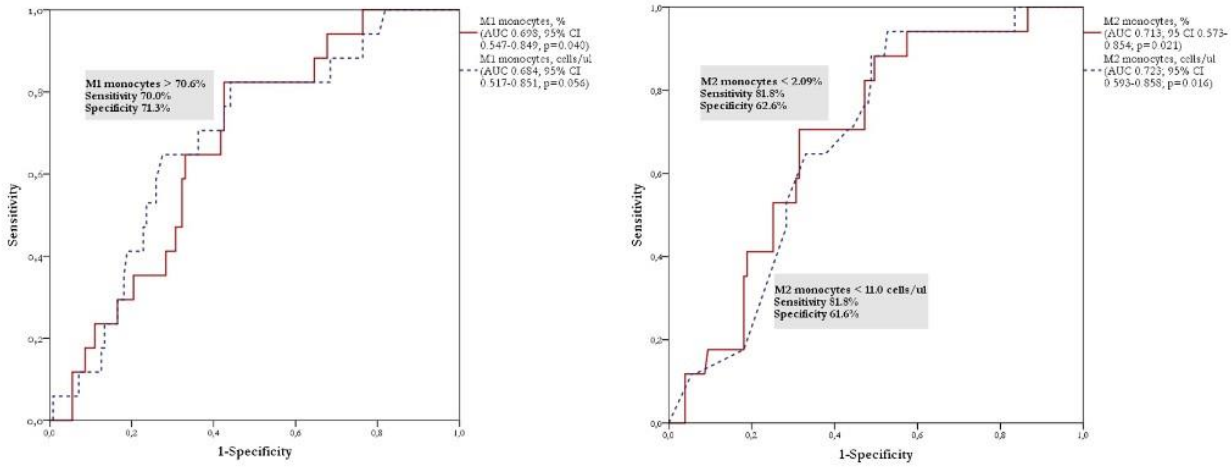
Background and Aims : Monocytes play a critical role in promoting chronic inflammation during all stages of atherosclerosis. Immunophenotyping of monocytes in the systemic circulation and in atherosclerotic plaque has been recognized by several clinical studies as a promising approach to finding new biomarkers of atherosclerosis. The aim of this study was to investigate the diagnostic value of M1 and M2 monocytes in relation to the presence of advanced carotid atherosclerosis.

Methods: The study included patients aged 40–64 years who underwent a screening duplex ultrasound scanning. The presence of plaque was assessed according to Mannheim consensus. Advanced carotid atherosclerosis was defined as the presence of carotid plaques with a maximum thickness ≥ 2.5 mm, which is classified as grade 3 in the American Society of Echocardiography classification. Monocyte subpopulations were phenotyped by flow cytometry, based on CD68 and CD163 expression (M1 monocytes – $CD14^{+}CD68^{+}CD163^{-}$; M2 monocytes $CD14^{+}CD163^{+}CD68^{-}$).

Results: The study included 150 patients, median age was 48.5 (43.0; 56.0) years. 15 (10.0%) patients had plaques with a maximum thickness ≥ 2.5 mm. Furthermore, an ROC analysis was performed to determine the possible diagnostic value of M1 and M2 monocyte in relation to the presence of carotid plaques ≥ 2.5 mm and to find optimal cut-off values (Figure 1).



Conclusions: The relative count of circulating M1 and the relative and absolute count of M2 monocytes have demonstrated diagnostic value in relation to the detection of advanced carotid atherosclerosis. The diagnostic value of identifying M2 monocytes was higher than that of measuring M1 monocytes.

SE088

Topic: *ASA01 - PATHOGENESIS OF ATHEROSCLEROSIS / ASA01-12 Other*

COMBINED SELENIUM AND COENZYME Q10 INTERVENTION PREVENTS LEUKOCYTE TELOMERE ATTRITION, WITH ASSOCIATION TO CARDIOVASCULAR MORTALITY – A SECONDARY ANALYSIS OF A RANDOMIZED CLINICAL TRIAL

SAAG SESSION 20: FROM BENCH TO BEDSIDE IN ATHEROSCLEROSIS

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Background and Aims : Leukocyte telomere length (LTL), a marker of ageing, has been associated with cardiovascular (CV) disease. Any influence of Selenium (Se) and Coenzyme Q10 (CoQ10) on LTL is not known. We aimed to explore the variance in LTL after such supplementation with potential impact on CV mortality.

Methods: This is a sub-study of a double-blind placebo-controlled randomized trial*. Swedish elderly citizens (n=118) were included. Intervention period was 4 years, with 10-years follow-up time for recording of CV mortality, defined as fatal myocardial infarction, cerebrovascular lesions, cardiac arrhythmias, heart failure and aortic aneurysms. LTL was relatively quantified with real-time PCR at baseline and after 42 months.

Results: Mean age (SD) in the active treatment group (n=67) was 77.1 (3.3), vs. 77.5 (3.4) years in the placebo group (n=51); 35 (52%) and 30 (59%), respectively, were women. The two populations were clinically balanced. After 42 months, less shortened LTL was observed in the active treatment compared to placebo group (0.0185 vs. 0.129, respectively, p=0.017). Significant difference between groups could be demonstrated by analysing individual changes from inclusion to 42 months (p<0.001). Subjects suffering CV death after 42 months presented with significantly shorter LTL (SD) at 42 months; 0.791 (0.19) compared to survivors, 0.941 (0.279) (p=0.014), and the difference in LTL change during the intervention period according to CV mortality and survival was significant (p=0.034).

Conclusions: Supplementation of Se and CoQ10 for 4 years inhibited LTL attrition in elderly significantly. Decline in LTL during intervention associated with future CV mortality. * *Clinicaltrials.gov NCT01443780*

SE089

Topic: *ASA01 - PATHOGENESIS OF ATHEROSCLEROSIS / ASA01-12 Other*

GLUTAMINASE-DEPENDENT METABOLISM, INCIDENT CARDIOVASCULAR DISEASE, AND PROGRESSION OF CAROTID PLAQUE.

SAAG SESSION 20: FROM BENCH TO BEDSIDE IN ATHEROSCLEROSIS

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Background and Aims : Glutamine (Gln) is a plasmatic amino acid and a strong inverse association has been observed between its plasma level and cardiometabolic traits. The glutaminase 2 (Gls2) in the liver is the main enzyme that metabolize glutamine into glutamate and some studies have recently revealed that SNPs in Gls2 were the major determinant of the plasma glutamine levels in humans. Altogether, these findings raise the question of the relevance of the hepatic glutaminolysis to the glutamine to glutamate ratio and the consequence it may have on atherosclerosis. We aim to better clarify the human relevance of the plasma glutamine levels to cardiovascular diseases and use preclinical models to understand the role of Gls2 in cardiometabolic outcomes.

Methods: To enable this, we have made a knocking down (KD) of Gls2 in the liver of some ApoE $^{-/-}$ mice compared to a control group. We have observed the plasmatic parameters, and tissues histology after 12 weeks of High Fat diet (HFD).

Results: We report that the KD of Gls2 during a HFD increase the plasma triglycerides and the volume atherosclerotic plaques. The plasma glutamine to glutamate ratio is also decreased at the beginning and a monocytosis is observed. Moreover, the plasma glutamine to glutamate ratio measured is significantly disturbed in high risk cardiovascular patients.

Conclusions: These findings demonstrate that the hepatic glutaminolysis can increase atherosclerosis development, by modifying the plasma glutamine to glutamate ratio and other parameters as triglycerides or monocytosis, and could be a new pathway to explore to identify novel therapeutic opportunities.

SE090

Topic: *ASA04 - CLINICAL VASCULAR DISEASE / ASA04-12 Prevention and treatment of cardiovasculardisease; miscellaneous*

RESIDUAL RISK OF MAJOR ADVERSE CARDIOVASCULAR AND LIMB EVENTS AMONG DIFFERENT ATHEROSCLEROTIC CARDIOVASCULAR DISEASE PHENOTYPES RECEIVING GUIDELINE-RECOMMENDED ANTIPLATELET MONOTHERAPY: THE RESRISK STUDY

SAAG SESSION 20: FROM BENCH TO BEDSIDE IN ATHEROSCLEROSIS

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Background and Aims : To quantify the long-term residual atherothrombotic risk among a routine care cohort with coronary artery disease (CAD), ischaemic stroke (IS) or peripheral artery disease (PAD) on guideline-recommended antiplatelet monotherapy (APMT).

Methods: Retrospective cohort study (2010-2020) using the United Kingdom Clinical Practice Research Datalink linked to Hospital Episode Statistics, including patients aged ≥18 years on recommended APMT according to ESC guidelines and NICE (aspirin for CAD/stroke, clopidogrel for PAD), diagnosed with CAD, IS or PAD prior to initiating APMT. Main outcomes included incidence rates (including recurrent events) and event-free survival of major adverse cardiovascular events (MACE) for CAD/IS/PAD patients, and major adverse limb events (MALE) in PAD, as well as cardiovascular hospitalisations.

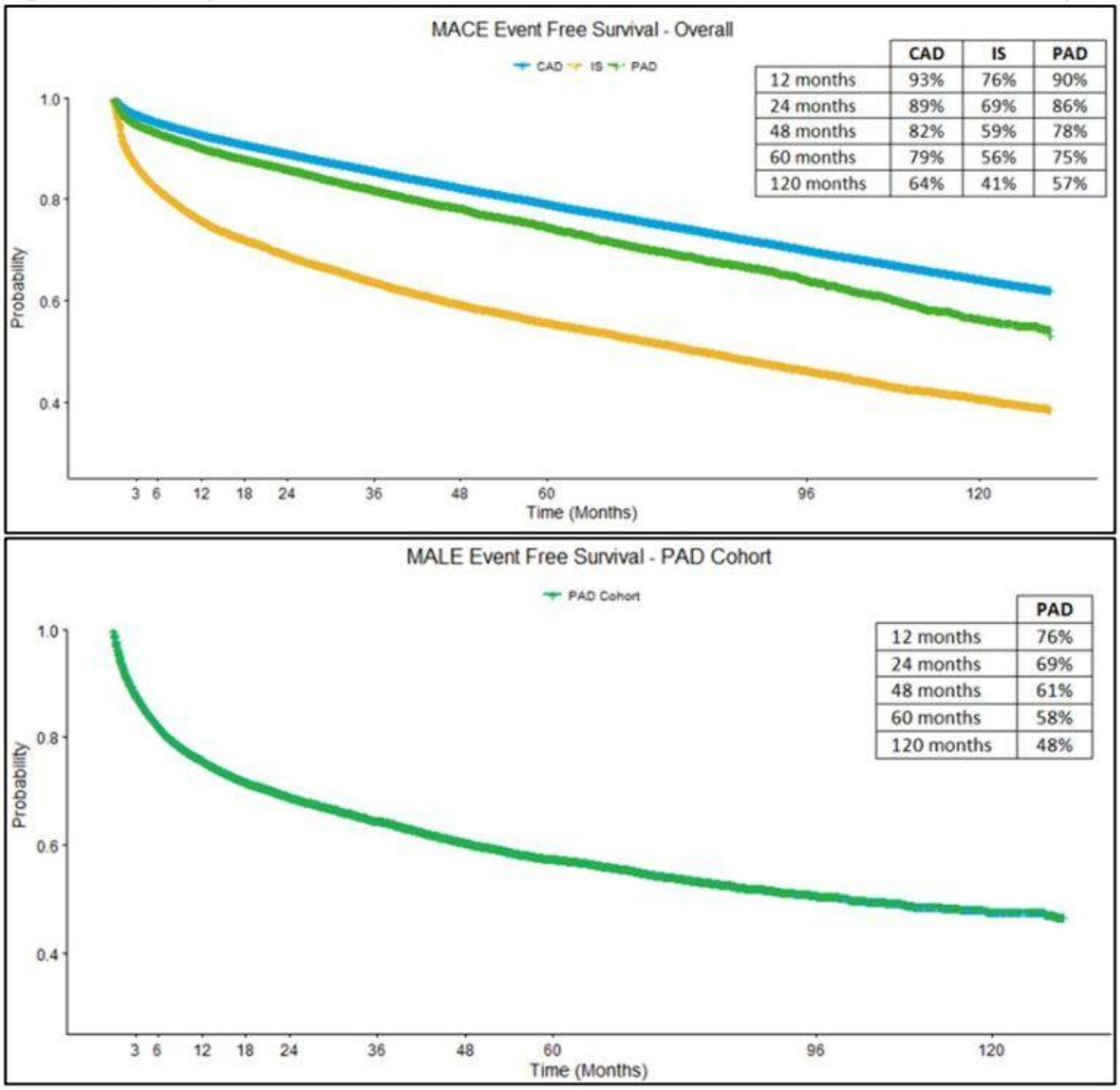
Results: 266,478 CAD, 14,788 IS and 13,162 PAD patients were included (median follow-up: 89.9, 75.9 and 42.4 months, respectively). Baseline characteristics are shown in the Table. MACE incidence rates were higher in the IS cohort (268.7 [95%CI 265.3–272.0] per 1000 person-years) than in CAD (92.9 [92.5–93.4] or PAD (97.2 [94.6–99.8]). IS patients had a lower probability of being “MACE event-free” over time (Figure 1A). MALE incidence rate in PAD patients was 195.9 (95%CI 192.2–199.6) per 1000 person-years. Figure 1B shows MALE event-free survival analysis. PAD patients had the highest rate of cardiovascular hospitalisation and shortest time to first hospitalization.

Table. Baseline characteristics of participants

	CAD cohort n = 266,478	Ischemic stroke cohort n =14,788	PAD cohort n =13,162
Males	62.3%	52.5%	56.7%
Age, years	71.3 (11.2)	71.3 (13.0)	70.6 (11.8)
Hypertension	53.8%	59.5%	56.1%
Diabetes	32.9%	25.8%	50.3%
Dyslipidaemia	56.0%	38.5%	43.2%
Current smokers	22.7%	23.6%	40.4%
Heart Failure	12.7%	4.7%	7.0%
Chronic kidney disease	25.0%	22.5%	23.8%
Body Mass Index, kg/m^2	28.7 (5.6)	27.9 (5.7)	28.3 (6.2)
Medications	-	-	-
- **Statins**	87.5%	81.1%	81.6%
- **Ezetimibe**	5.5%	3.6%	3.6%
- **ACEI/ARB**	66.3%	57.6%	52.9%
- **Beta-blockers**	58.2%	19.3%	18.1%
- **Calcium channel blockers**	35.9%	33.8%	38.6%

Data shown as relative frequencies (%) for categorical variables and as mean (SD) for quantitative variables. ACEI/ARB: angiotensin-converting enzyme inhibitors/angiotensin receptor blockers.

Figure 1A-B. Kaplan-Meier Curves for MACE and MALE event-free survival analysis.



	CAD	IS	PAD
12 months	93%	76%	90%
24 months	89%	69%	86%
48 months	82%	59%	78%
60 months	79%	56%	75%
120 months	64%	41%	57%

	PAD
12 months	76%
24 months	69%
48 months	61%
60 months	58%
120 months	48%

Conclusions: Among a contemporary cohort with atherosclerotic cardiovascular disease, residual atherothrombotic risk remains high despite being on guideline-recommended APMT. Recent evidence-based therapies have emerged that may attenuate residual risk in these individuals, including novel drugs adjunct to antiplatelet therapies.

SE091

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-04 Lipoprotein receptors*

IMPACT OF DYSLIPIDEMIA ON PERIPHERAL NEUROPATHY

SAAG SESSION 21: NEW TREATMENT ERA FOR DIABETES

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Background and Aims : Diabetes mellitus (DM) can damage the peripheral nervous system leading to diabetic neuropathy (DN). There is a limited correlation between glycemic control and DN but accumulating evidence indicate that dyslipidemia is a risk factor for DN. We hypothesize that dysregulation of plasma lipids metabolism can impact the onset of DN.

Methods: We characterized neuropathy in *Ldlr* KO (n=6) and *ApoE* KO (n=6) (models of dyslipidemia) compared to C57BL/6 (WT) mice (n=10) using sensory and motor tests (Von Frey, Hargreaves, pinprick, brush test and rotarod test) at 10 and 24 weeks of age, as well as skin innervation analysis. Sciatic nerves of WT mice were tested for the expression of major lipoprotein receptors using western blot and immunohistochemistry. The functional role of these receptors was analyzed by ex-vivo nerve incubation with labeled LDL and VLDL.

Results: We did not find any significant sensory and motor deficit in LDLR KO and ApoE KO compared to WT mice. However Schwann cells of the mouse sciatic nerves expressed functional lipoprotein receptors: LDLR, VLDLR and LRP1.

Conclusions: Dyslipidemic mice did not present sensory or motor abnormalities, however the expression of functional lipoprotein receptors in Schwann cells indicate that they could play a critical role in lipid induced damage under pathological conditions such as T2D. In subsequent studies, we will analyze the impact of LDLR and ApoE deficiency on DN onset in a mouse mode of induced neuropathy.

SE092

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-03 Diabetes, insulin sensitivity and resistance*

DIABETES STATUS AND RISK OF PREMATURE ATHEROSCLEROTIC CARDIOVASCULAR DISEASE: A STUDY FROM APPROXIMATELY 1 MILLION YOUNG ADULTS

SAAG SESSION 21: NEW TREATMENT ERA FOR DIABETES

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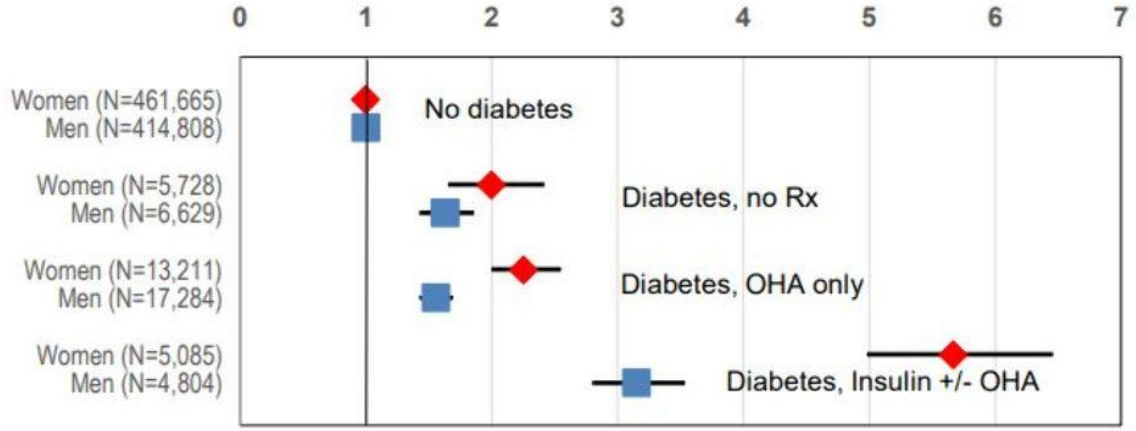
Background and Aims : Risk of premature atherosclerotic cardiovascular disease (ASCVD) attributable to diabetes is poorly understood. We evaluated the impact of diabetes on future risk of ASCVD in young men and women.

Methods: This observational cohort study included young adults (ages 30-55 years) without established ASCVD (as of January 1, 2006) who were members of an integrated healthcare delivery system in Northern California. Adjusted multivariate models were specified to estimate risk ratios (RRs) for incident ASCVD events by diabetes status: no diabetes (reference) versus diabetes with no treatment, with oral hypoglycemic (OH) only and with OH plus insulin. Incident ASCVD events were defined as a composite of nonfatal myocardial infarction, ischemic stroke, or coronary heart disease death through December 31, 2015.

Results: A total of 929,214 individuals met the selection criteria. Mean age was 43.9 (±7.3) years, 52.3% were women, and the median follow-up was 10 years. 6.5% of men and 4.9% of women had diabetes at baseline. In models adjusted for demographics (age, race) and traditional risk factors (hypertension, LDL-cholesterol, HDL-cholesterol, total cholesterol, smoking), patients with diabetes using insulin had > 3x greater risk among men and > 5x the risk among women, compared to those without diabetes (figure).

Conclusions:

Risk of ASCVD by Diabetes and Treatment Status among 30-55 year old Men and Women



There was a graded level of increased risk of premature ASCVD by diabetes status, with especially

higher risk for those who used insulin. Earlier screening and preventive therapy may be indicated in this highest risk subset of younger patients with diabetes.

SE093

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-03 Diabetes, insulin sensitivity and resistance*

UNCONTROLLED VS CONTROLLED TYPE 2 DIABETES AND INCREASED RISK OF 30-DAYS ALL-CAUSE MORTALITY IN COVID-19 PATIENTS

SAAG SESSION 21: NEW TREATMENT ERA FOR DIABETES

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Background and Aims : Based on current evidence, type 2 diabetes increases the risk for severe illness from COVID-19. The aim of the study was to evaluate whether the history of type 2 diabetes prior to COVID-19 was associated with the increased risk of 30-days all-cause mortality and whether uncontrolled diabetes mitigates this risk.

Methods: This is a prospective multi-center observational cohort study with 30-days outcomes of 5680 consecutive SARS-CoV-2 PCR-positive patients older than 18 years, 612 of them had type 2 diabetes. Patients received treatment at the distribution centers, of whom 1183 patients were in COVID-19 clinics of Tashkent, Uzbekistan (June 1–September 30, 2020). Diabetes was defined by a hemoglobin HbA1C ≥ 6.5%, history of physician-based diagnosis, or use of anti-diabetic medications. The association of risk factors and 30-days all-cause mortality was assessed using multivariate logistic regression to examine how variables predict an outcome in the main analysis.

Results: 30-days all-cause mortality was in 112 patients in the whole cohort. After adjusting for age, sex, history of myocardial infarction, obesity, hypertension, COPD, asthma, lifestyle risk factors, type 2 diabetes showed a significant difference in all-cause mortality. Having uncontrolled type 2 diabetes increased the odds of 30-days all-cause mortality 4.78-fold (95%CI [3.36–8.21]), $p<0.001$. Having controlled diabetes increased the odds 2.35-fold (95%CI [1.84–3.19]), $p<0.001$.

Conclusions: Controlled Type 2 diabetes increased COVID-19 mortality 2.35-fold compare to those who did not have diabetes prior to COVID-19. Uncontrolled versus controlled diabetes doubles the risk for 3o-days all-cause mortality. Management of diabetes is important for preventive measures to decrease the more severe course of COVID-19.

SE094

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-03 Diabetes, insulin sensitivity and resistance*

COMBINATION THERAPY WITH GLP-1 ANALOGUES AND SGTL2 INHIBITORS IN OBESE PATIENTS WITH DM2: CURRENT DATA AND FUTURE CONSIDERATIONS

SAAG SESSION 21: NEW TREATMENT ERA FOR DIABETES

Alexandra Sianni, Styliani Lagou, Euaggelia Mougkaraki, Lydia Archontouli, Nikolaos Daviotis, Vasileios Giannakopoulos, Eleni Marinaki, Nikolaos Fytrakis, Dimitrios Sakkas, Archontoula Fragkou
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Background and Aims : The combination of GLP-1 analogues and SGTL2 inhibitors beyond lowering glucose levels, has multiple beneficial actions in cardiovascular system. To evaluate the effect of the combination of GLP-1 and SGTL2 in body weight and BMI of obese patients with DM2.

Methods: Methods We enrolled 150 obese patients (mean BMI=36 ±4) with DM2. 73.3% were women mean aged 58 ± 5 years and 26.6% were men mean aged 62 ± 6 years. All patients were treated with oral agents. We switched their medication to metformin plus GLP-1 and SGLT2. Fasting glucose, HbA1c, total body weight, and BMI were measured at 3, 6, 9 and 12 months.

Results: Results Mean HbA1c was 10 ± 1.8, 9 ± 1.3, 8.7 ± 1 and 7 ± 0.8 at 3, 6, 9 and 12 months respectively (p = 0.030). Fasting glucose was 185 ± 29mg/dl at 3 months, 168 ± 22mg/dl at 6 months, 156 ± 20mg/dl at 9 months and 145 ± 18mg/dl at 12 months (p = 0.0045). Body weight decreased from 125 ± 18 kg (3 months), to 108 ± 14 kg (6 months), 100 ± 8 kg (9 months) and 98 ± 8 kg (12 months, p = 0,042). BMI had a similar reduction (BMI was 42 ± 7.2 at 3 months, 40 ± 6.8 at 6 months, 38 ± 6.4 at 9 months and 35 ± 5 at 12 months, p = 0.043).

Conclusions: Conclusion Obese patients with DM2 may benefit from combination therapy with metformin plus GLP-1 and SGLT2.

SE095

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-01 Epidemiology of cardiovascular diseases and risk factors*

A MULTIVARIABLE MENDELIAN RANDOMIZATION ANALYSIS DISENTANGLING THE CAUSAL RELATIONS BETWEEN ABDOMINAL OBESITY, NON-ALCOHOLIC FATTY LIVER DISEASE AND CARDIOMETABOLIC DISEASES

SAAG SESSION 22: BIG DATA OF CVD RISK AND TREATMENTS

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Background and Aims : Obesity and especially abdominal obesity are closely linked to non-alcoholic fatty liver disease. These traits are also associated with type 2 diabetes and coronary artery disease. We used multivariate Mendelian randomization (MVMR) to disentangle these correlated causal factors.

Methods: We included publicly available genome-wide association study (GWAS) meta-analysis of NAFLD (8434 cases and 770,180 control), type 2 diabetes (74,124 cases and 824,006 controls), coronary artery disease (122,733 cases and 424,528 controls) waist circumference (n = 462,166) and body mass index (N = 461,460). We used MVMR to determine whether obesity (defined using body mass index [BMI]) and abdominal obesity (defined using waist circumference) were causally associated with NAFLD.

Results: In univariable Mendelian randomization analyses, both BMI and waist circumference were associated with NAFLD. NAFLD was not associated with obesity or abdominal obesity. In MVMR analyses, waist circumference was associated with NAFLD when accounting for BMI (OR per 1-standard deviation increase = 2.56 95% CI: 1.39-4.69, p=2.4e-03), but BMI was not associated with NAFLD when accounting for waist circumference (0.81 95% CI: 0.5-1.31, p =3.9e-01). Abdominal obesity was a strong, independent and causal contributor to non-alcoholic fatty liver disease, type 2 diabetes and coronary artery disease.

Conclusions: This study underscore interventions targeting abdominal obesity rather than body weight per se for the prevention and management of cardiometabolic diseases.

SE096

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-01 Epidemiology of cardiovascular diseases and risk factors*

IMPACT OF DIET ON 10-YEAR ABSOLUTE CARDIOVASCULAR RISK: A PROSPECTIVE POPULATION-BASED COHORT STUDY

SAAG SESSION 22: BIG DATA OF CVD RISK AND TREATMENTS

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Background and Aims : The burden of diet on cardiovascular risk is substantial. Current dietary guidelines are not sufficiently implemented and effective strategies to encourage people to change and maintain healthy diets are lacking. The impact of incorporating dietary assessment into 10-year absolute risk charts for cardiovascular disease is unknown.

Methods: Using a prospective cohort design including 94,321 individuals from the general population, we generated 10-year absolute risk scores for fatal and non-fatal cardiovascular disease and for ischemic vascular disease according to groups based on adherence to dietary guidelines.

Results: Non-adherence to dietary guidelines was associated with an atherogenic lipid and inflammatory profile. In both sexes, 10-year absolute risk of ischemic vascular disease increased with increasing age, increasing systolic blood pressure, and decreasing adherence to dietary guidelines. The highest 10-year absolute risk of ischemic vascular disease of 38% was observed in smoking men aged 65-69 with very low adherence to dietary guidelines and systolic blood pressure between 160-179. The corresponding value for women was 26%. Risk charts using non-HDL cholesterol instead of dietary assessment yielded similar estimates.

Conclusions: Incorporation of dietary assessment into 10-year absolute risk charts may contribute to a more effective prevention strategy as it may improve individual motivation to change dietary habits and therefore advance personalized prevention. These risk charts may also be useful in regions of the world where access to laboratory facilities is limited. Improved implementation of national dietary guidelines must be a cornerstone for future prevention of cardiovascular disease in both younger and older individuals.

SE097

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-01 Epidemiology of cardiovascular diseases and risk factors*

META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS COMPARING EFFECT OF LIPID-LOWERING THERAPIES ON C-REACTIVE PROTEIN LEVELS

SAAG SESSION 22: BIG DATA OF CVD RISK AND TREATMENTS

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Background and Aims : Cardiovascular disease is widely recognized to have an inflammatory background. Yet, the role of inflammation is not clear. Our study aimed to assess the anti-inflammatory effect of lipid-lowering drugs on C-reactive protein (CRP) and lipids reduction.

Methods: We conducted a meta-analysis according to the PRISMA guidelines. PubMed, Web of Science, EMBASE, Cochrane Library, and ClinicalTrial.gov were searched from inception to June 2021. Inclusion criteria were: (1) randomized controlled trials (RCTs) in human, phase II, III or IV; (2) English language; (3) reporting the effects on CRP levels; (4) with intervention duration more than 3 weeks; (5) and sample size (for each arm) over than 100 subjects. Pooled estimates were assessed by a random-effects model. Between-study heterogeneity was tested by Cochrane's Q test and measured with the I^2 statistics.

Results: Overall, 11 RCTs were included for statins (46,499 participants), 5 trials for PCSK9 inhibitors [PCSK9i] (47,709 participants) and 5 trials for ezetimibe (15,505 participants). An additional 1.10 mg/L (95%CI, -1.11 to -1.10) and 0.66 mg/L (95%CI, -0.68 to -0.64) absolute reduction of CRP concentration was observed for statins and ezetimibe respectively. However, CRP level was slightly increased by 0.06 mg/L (95%CI, 0.06 to 0.06) for PCSK9i. For LDL-C levels, there was a significant decrease of 51.28 mg/dL for statins, 62.28 mg/dL for PCSK9i and 17.28 mg/dL for ezetimibe. TG levels were reduced by 19.85 mg/dL, 26.70 mg/dL, and 13.32 mg/dL, respectively.

Conclusions: In conclusion, statin and ezetimibe administration reduces serum CRP concentration while patients treated with PCSK9i experience no significant changes in CRP levels.

SE098

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-01 Epidemiology of cardiovascular diseases and risk factors*

RESULTS FROM THE NATIONWIDE FRENCH REGISTRY ODIACOR. A CALL TO OPTIMIZE RISK FACTORS CONTROL AND REACH THE TARGETS IN CORONARY DIABETIC PATIENTS.

SAAG SESSION 22: BIG DATA OF CVD RISK AND TREATMENTS

Pierre Sabouret[1], Jean-Claude Dib[2], Ali Sharareh[2], Francois Dievart[2], Olivier Hoffman[2], Jean-Pierre Huberman[2], Gilles Montalescot[1], Nicolas Lellouche[3]
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Background and Aims : Background: Recent international guidelines modified cardiovascular risk factors management and highlighted the importance to reach risk factors target. However, real world data are sparse regarding risk factors care in this specific population. Objective: To determine the current clinical profiles, risk factors control and management in private practice of coronary diabetic patients in France.

Methods: The Observatoire des patients DIAbétiques CORonariens (ODIACOR) Registry collected data on consecutive diabetic patients with documented CAD on risk factors (RF),treatments, awareness of risk factors control and goals.
574 consecutive patients aged 18 years or older were included. They had either a CCS or an ACS. The survey was performed between March 2019 and December 2020.
Baseline clinical characteristics, prevalence of RF, medications, RF control, collaboration between MD, patient's knowledge were collected.

Results: Coronary diabetic French patients have a high prevalence of hypertension (81.8%) and hypercholesterolemia (72.4%). 16% had a polyvascular disease. Prevalence of overweight (39.8%), obesity (26.6%) were high. Prescriptions of anti diabetic agents were suboptimal, as Metformin, GLP1RA, Insulin, DPP4i, and sulfonylurea were prescribed in 64%, 11%, 28%, 20,2% and 19,5% of cases. Despite many patients received statins (92.1 %) and antiplatelet agents (78.6%). RF control was poor with only 37% with a LDL-C below 70 mg/dL, 50% with a HBPM below 135/85 mmHg, current smokers were 14,4%, a glycated haemoglobin > 7% for 38% .

Conclusions: Conclusion: in this contemporary french cohort of CAD patients, we report major discrepancies between guidelines and treament's strategies. The control of major risk factors remain suboptimal, advocating efforts to obtain a better implementation of European guidelines.

SE099

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-01 Epidemiology of cardiovascular diseases and risk factors*

IMPACT OF SCORE2 ON CARDIOVASCULAR RISK RECLASSIFICATION AND PREVALENCE OF LDL-CHOLESTEROL CONTROL ACCORDING TO MOST RECENT EQUATIONS IN A WIDE HYPERTENSIVE POPULATION

SAAG SESSION 22: BIG DATA OF CVD RISK AND TREATMENTS

Riccardo Sarzani[1,2], Federico Giulietti[1,2], Massimiliano Allevi[1], Paolo Turri[1], Silvia Sarnari[1], Serena Re[1], Francesco Spannella[1,2]
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Background and Aims : Recently, an updated cardiovascular risk (CVR) model (SCORE2) and several apparently more accurate equations than Friedewald to calculate low-density lipoprotein cholesterol (LDLc) have been proposed and validated. Our work aimed to evaluate the impact of both SCORE2 on CVR stratification and of new formulas on prevalence of LDLc control in a wide hypertensive population.

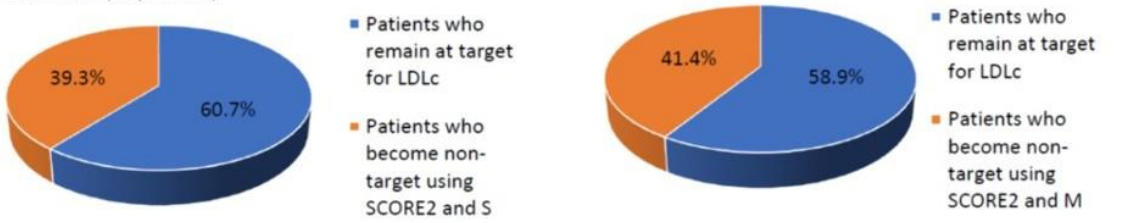
Methods: Cross-sectional study on 1192 consecutive hypertensive outpatients. LDLc was calculated using the Friedewald formula (F), the modified Friedewald formula proposed by Martin SS. (M) and the equation proposed by Sampson M. (S). SCORE and SCORE2 have been used for individual CVR stratification. LDLc control was defined according to the 2019 ESC/EAS Dyslipidemia Guidelines

Results: According to SCORE2, there is a significant re-classification of the individual CVR ($p<0.001$). The 57.6% and the 4.5% of patients with low-moderate risk at SCORE moved to high risk and very high risk according to SCORE2, respectively, while the 4.0% and the 47.6% of patients with high risk according to SCORE moved to low-moderate risk and very high risk according to SCORE2, respectively. Within patients at target for LDLc according to SCORE and F, the 39.3% and the 41.1% was not at target according to SCORE2 and S or M, respectively (all

p<0.001).

Figure 1. Individual CV risk from SCORE to SCORE2

Figure 2. Prevalence of patients not at target for LDLc according to SCORE2 and S or M with those at target for LDLc according to SCORE and F (all p<0.001)



Conclusions: We found non-negligible differences in CVR reclassification and LDLc control in our hypertensive population, after the application of SCORE2 and new LDLc equations. Our findings show how the application of these new tools may significantly affect the management of dyslipidemia and therefore the CV prevention in clinical practice

SE100

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-02 Epidemiology of dyslipidemias*

NONOPTIMAL LIPID LEVELS INCREASE THE RISK OF THE PROGRESSION OF CORONARY ARTERY CALCIFICATION IN ASYMPTOMATIC YOUNG ADULTS: RESULTS FROM THE KOICA REGISTRY

SAAG SESSION 22: BIG DATA OF CVD RISK AND TREATMENTS

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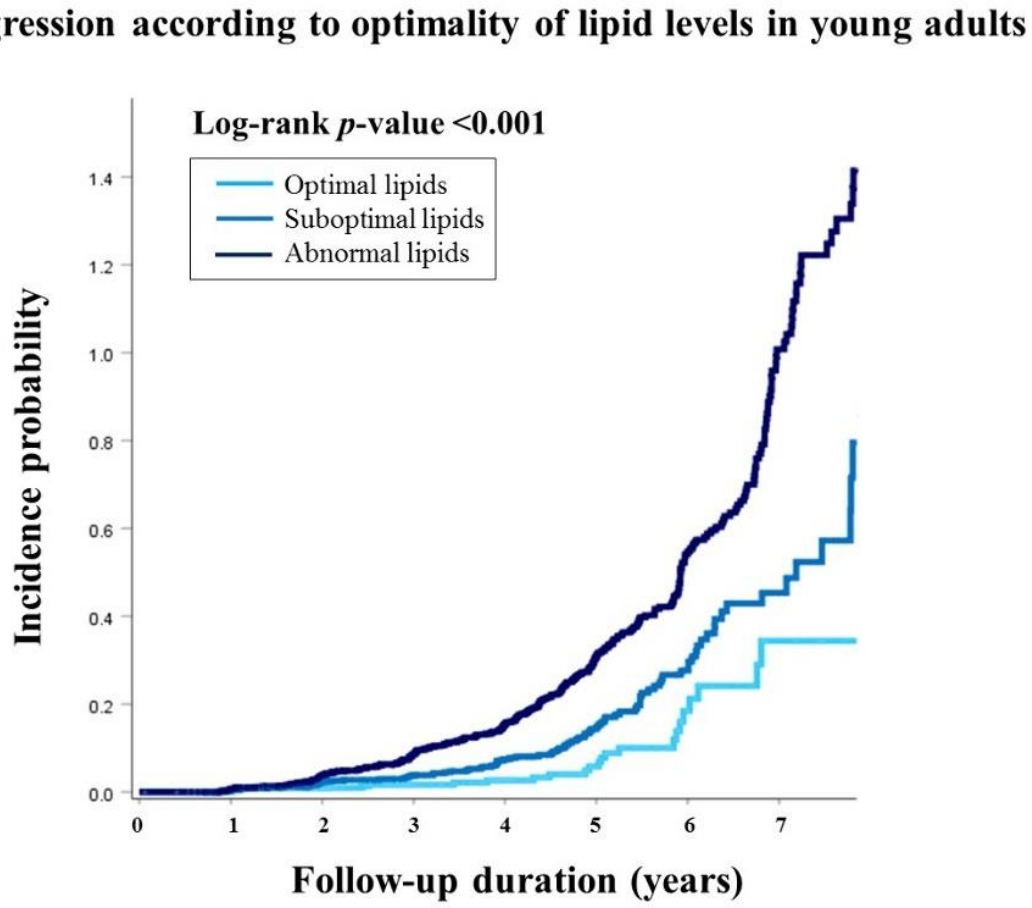
Background and Aims : Since cumulative exposure of lipids for lifetime is important to prevent and predict atherosclerotic cardiovascular disease (ASCVD), it is recommended to obtain the optimal lipid levels from young ages. We aimed to investigate the progression of coronary artery calcification (CAC) according to lipid profiles in young adults.

Methods: From the KOICA registry, we collected 2,940 statin-naïve subjects, aged 20–45 years, undergoing serial coronary artery calcium scans for the purpose of health check-ups between 2002 and 2017. CAC progression was assessed according to lipid optimality and lipid variables.

Results: In this cohort (age, 41.3 years; man 82.4%), only 477 subjects (16.2%) had the optimal lipid profile, defined as triglycerides<150 mg/dl, LDL-cholesterol<100 mg/dl, and HDL-cholesterol≥45 mg/dl. During follow-up (median 39.7 months), CAC progression was observed in 438 participants (14.8%) and more frequent in nonoptimal lipid group (16.5% vs 5.9%; p<0.001). Nonoptimal lipids during young adulthood increased the risk of CAC progression after adjusting for other risk factors (HR, 2.36; p=0.001), with stepwise risk increase according to lipid levels. In the subjects with initial calcium score of zero, those in their 20s/30s, and those without any other risk factors, nonoptimal lipid levels more than doubled the risk of CAC progression. Among lipid variables, high triglycerides appeared to have the greatest impact on CAC

progression.



Conclusions: The proportion of optimal lipid levels was lower than expected. Nonoptimal lipids were significantly associated with the risk of CAC progression in young adults, even with low-risk. Triglycerides had an independent and the strongest association with the risk of CAC progression.

SE101

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-07 Lipidomics*

MULTIVARIATE GENETIC ANALYSIS OF HUMAN PLASMA LIPIDOME IDENTIFIES TEN NOVEL LOCI

SAAG SESSION 23: THE FRONTIERS IN LIPIDOMICS

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Background and Aims : The human plasma lipidome captures information beyond routinely clinically used lipids and has disease relevance in cardiometabolic diseases and beyond. Genome-wide association studies (GWAS) of individual lipid species (univariate analysis) have identified many lipid-associated loci, however the influence of genetic variants on lipid metabolism and cardiovascular disease risk is not fully understood. Multivariate analysis of multiple correlated lipid species improves statistical power and could help identify additional lipid loci.

Methods: We performed univariate GWAS of 179 lipid species for 7177 participants from the Finnish GeneRisk cohort and multivariate analyses for 11 clusters of correlated lipid species by metaCCA software. The associations were fine-mapped with FINEMAP to identify causal variants and the causal variants were examined for associations with cardiometabolic traits in FinnGen R6, Gene Atlas and GWAS Atlas. We performed gene prioritization analysis with the tool FOCUS.

Results: Multivariate analysis across 11 clusters identified 65 loci with P-value < 5e-8, of which 58 reached the Bonferroni-corrected significance threshold (BF). We identified ten new loci whose lead variants were in or near
genes *DTL*, *STK39*, *CDS1*, *AGPAT2*, *RIC1*, *SGPL1*, *KCNJ12*, *SPHK2*, *NINL* and *AGPAT3* at BF in multivariate analysis. Of these, only *SGPL1* (Cer42:2;2) and *AGPAT2* (PC16:0;0_22:5;0) reached BF in univariate analysis*.* Fine-mapping identified missense variants as potential causal variants for novel loci *RIC1*, *SPHK2* and *AGPAT3*. FOCUS prioritized 28 genes for multivariate GWAS.

Conclusions: Multivariate genetic analysis is a powerful tool for high-dimensional data such as lipidomics and helped us identify ten novel lipid loci, of which only two were identified by univariate analysis.

SE102

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-07 Lipidomics*

MASS SPECTROMETRY IMAGING OF LIPIDS IN ADVANCED HUMAN ATHEROSCLEROTIC PLAQUE

SAAG SESSION 23: THE FRONTIERS IN LIPIDOMICS

Nuria Slijkhuis[1], Mark Towers[2], Mina Mirzaian[3], Ingeborg Nieuwenhuizen[1], Kim Van Gaalen[1], Eric Sijbrands[4], Yolanda De Rijke[3], Heleen Van Beusekom[1], Kim Van Der Heiden[1], Emmanuelle Claude[2], Gijs Van Soest[1]
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Background and Aims : Lipids play a major role in atherosclerosis and are therefore potential biomarker targets. Investigating the spatial distribution of lipids in plaques can aid in understanding the role of lipids in local pathobiology, plaque progression and subsequent biomarker discovery. In contrast to histology, mass spectrometry imaging (MSI) can visualize the spatial distribution of a wide variety of lipid species in human carotid plaques.

Methods: Plaques from 14 patients that underwent a carotid endarterectomy were collected. Cross-sectional plaque segments were gelatin embedded, cryosectioned into 10µm thick sections and thaw-mounted onto glass slides. We performed imaging experiments on a Waters SynaptXS mass spectrometer with prototype DESI source. Histology was prepared on consecutive sections.

Results: Using DESI-MSI we found a wide range of lipids from different lipid classes. We selected a subset of lipids that were present in at least half of the samples, resulting in 225 lipids. Non-negative matrix factorization (NMF) clustering was applied to this subset of lipids to extract co-occurring spectral features in the data. NMF analysis described the data in 8 spectral components that each consisted of one or two lipid classes, and were co-localized with histological plaque features. Component 2, consisting of sphingomyelins, co-localized to calcified areas of plaque as shown by Alizarin staining (Fig1). Furthermore, component 3, consisting of phosphatidylcholines, co-localized with macrophage-infiltrated plaque areas as shown by CD68

staining.

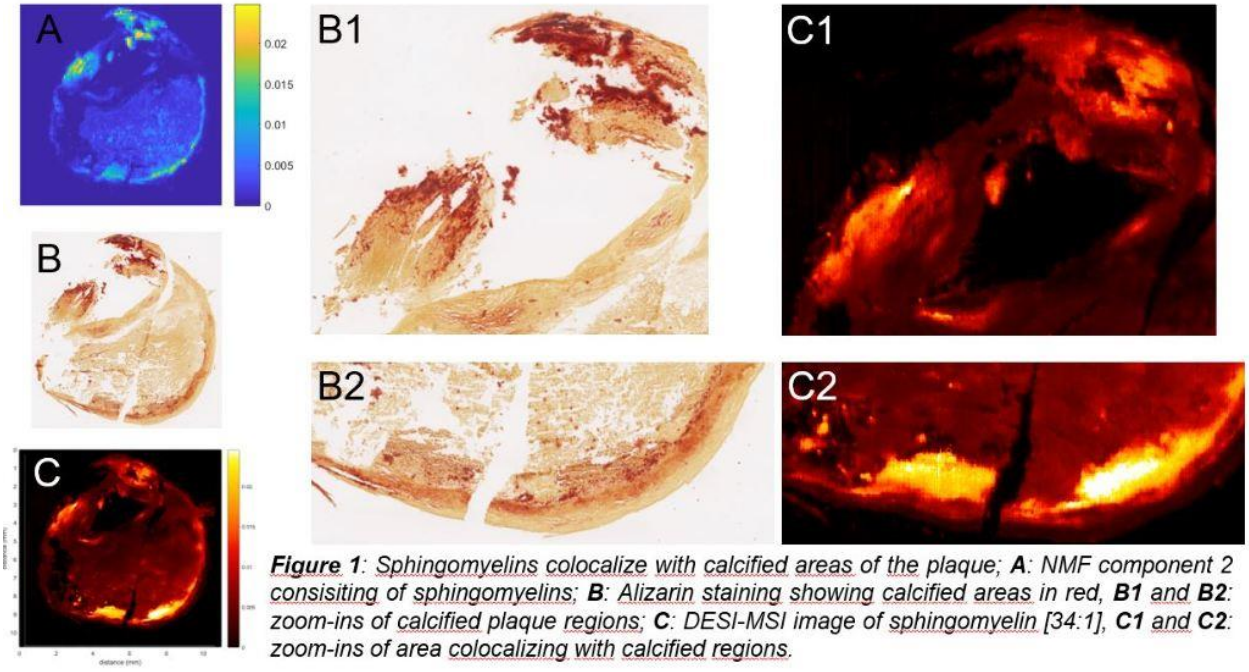

***Figure 1**: Sphingomyelins colocalize with calcified areas of the plaque; **A**: NMF component 2 consisiting of sphingomyelins; **B**: Alizarin staining showing calcified areas in red, **B1** and **B2**: zoom-ins of calcified plaque regions; **C**: DESI-MSI image of sphingomyelin [34:1], **C1** and **C2**: zoom-ins of area colocalizing with calcified regions.*

Conclusions: We uncovered highly detailed plaque lipid signatures in a large sample of human atherosclerotic plaques using DESI-MSI. NMF clustering revealed lipid patterns that co-localized with histological plaque features of interest.

SE103

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-07 Lipidomics*

REGION-SPECIFIC LIPID MARKERS OF HUMAN CAROTID ATHEROSCLEROSIS PLAQUE OUTCOME DETECTED BY MATRIX-ASSISTED LASER DESORPTION/IONIZATION MASS SPECTROMETRY IMAGING

SAAG SESSION 23: THE FRONTIERS IN LIPIDOMICS

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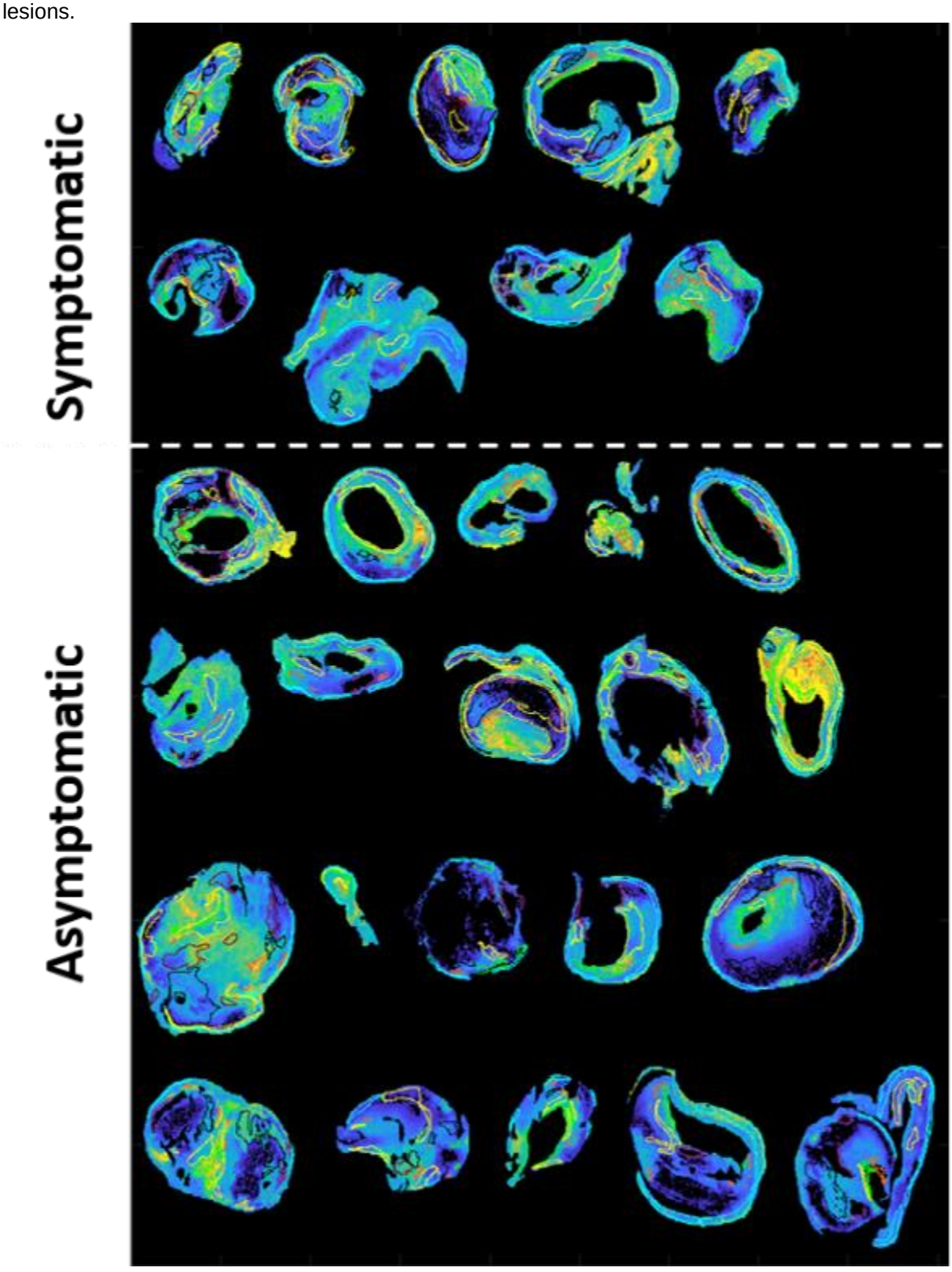
Background and Aims : Atherosclerosis is characterized by lipid-rich plaques in large and medium sized arteries. Plaque rupture can causes thrombi, occlusions of downstream vessels and adverse clinical events. Mechanism underlying plaque rupture are not completely clear, and their investigation is made difficult by the highly heterogeneous nature of atherosclerotic lesions. Matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI MSI) is a spatially resolved technique that can be used to investigate tissue composition. Here we use MALDI MSI to compare the region-specific lipid composition of symptomatic and asymptomatic human carotid atherosclerotic plaques.

Methods: Carotid atherosclerotic plaques were collected from 9 symptomatic and 20 asymptomatic patients. Plaques were analyzed by MALDI MSI of lipids using two different MALDI matrices, and adjacent sections were analyzed by histology and immunofluorescence to segment the plaque in regions. Hierarchical cluster analysis was applied to compare lipid composition of different regions. Partial least square regression was used to detect region-specific lipid markers of symptomatic and asymptomatic

lesions.



Results: The lipid profiles of macrophage-rich regions and intimal vascular smooth muscle cells exhibited the largest changes associated with plaque outcome. Macrophage-rich regions from symptomatic lesions were found to be enriched in phosphatidylcholines while the same region from asymptomatic plaques was

enriched in polyunsaturated cholesteryl esters and very long chain sphingomyelins. Intimal vascular smooth muscle cells of asymptomatic plaques were enriched in lysophosphatidylcholines.

Conclusions: MALDI MSI was used to investigate region-specific lipid composition of a large dataset of human carotid atherosclerotic plaques. Region-specific lipid markers of plaque outcome were detected in symptomatic and asymptomatic lesions.

SE104

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-07 Lipidomics*

AN UNTARGETED LIPIDOMIC ANALYSIS REVEALS DEPLETION OF SEVERAL PHOSPHOLIPID CLASSES IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA ON TREATMENT WITH EVOLOCUMAB®.

SAAG SESSION 23: THE FRONTIERS IN LIPIDOMICS

Ilenia Calcaterra[1], Alessandro Di Minno[2], Andrea Anesi[3], Viviana Cavalca[4], Maria Tripaldella[1], Elena Tremoli[4], Gabriella Iannuzzo[1], Maria Donata Di Taranto[5], Giuliana Fortunato[5], Matteo N.D. Di Minno[6]
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Background and Aims : Familial hypercholesterolemia (FH) is caused by mutations in genes involved in low-density lipoprotein cholesterol (LDL-C) metabolism. The effect of PCSK-9 inhibition on plasma lipidome has been poorly explored. We performed an untergeted-lipidomic study on FH patients receving a PCSK-9 inhibitor (Evolocumab®).

Methods: Using an ultra-high-performance liquid chromatography-electrospray ionization-quadrupole-time of flight-mass spectrometry method, the plasma lipidome of FH subjects before and at different time intervals during treatment with the PCSK-9 inhibitor Evolocumab® was explored. Lipid changes were graded between baseline, and 4- and 12-week treatment.

Results: In 25 FH subjects heterozygotes or compound heterozygotes for different LDL receptor mutations, untargeted lipidomic revealed significant reductions in 26 lipid classes belonging to phosphatidylcholine (PC), sphingomyelin (SM), ceramide (CER), cholesteryl ester (CE), triacylglycerol (TG) and phosphatidylinositol (PI). At 12-week treatment, five polyunsaturated diacyl PC, accounting for 38.6 to 49.2 % of total PC at baseline, two ether/vinyl ether forms; seven SM, five CER and glucosyl/galactosyl-ceramide (HEX-CER) were reduced, as was the unsaturation index of HEX-CER and lactosyl—CER (LAC-CER). Although, non quantitative modification were observed in phosphatidylethanolamine (PE) during treatment with Evolocumab®, shorter and more saturated fatty acyl chains were documented.

Conclusions: Depletion of several phospholipid classes occurs in plasma of FH patients during treatment with the PCSK-9 inhibitor Evolocumab®. The mechanism underlying these changes likely involves the de novo synthesis of SM and CER through the activation of the key enzyme sphingomyelin synthase by oxidized LDL and argues for a multifaceted system leading to vascular improvement in users of PCSK-9 inhibitors.

SE105

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-09 Lipid and lipoprotein metabolism*

FATTY ACID COMPOSITION OF PLASMA LIPIDS IN FAMILIAL LCAT DEFICIENCY SUPPORTS THE FORMATION OF ACAT2-DERIVED CHOLESTERYL ESTERS IN HUMANS

SAAG SESSION 23: THE FRONTIERS IN LIPIDOMICS

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Background and Aims : Mutations in the *LCAT* gene cause Familial LCAT Deficiency (FLD,OMIM#245900), a rare metabolic disorder characterized by severe HDL deficiency, hypertriglyceridemia, and an increased unesterified to total cholesterol ratio. Despite the complete lack of LCAT activity, FLD cases have circulating cholesteryl esters (CE), thus representing a tool to analyze the origin of VLDL cholesteryl esters.

Methods: Fifty carriers of LCAT deficiency were included (19 carriers of two *LCAT* mutant alleles and 31 carriers of one mutant *LCAT* allele); 39 non affected family members acted as controls. Lipoprotein composition and fatty acid distribution was evaluated in whole plasma and in isolated lipoproteins by chromatography.

Results: Plasma levels of CE were significantly reduced and highly variable among carriers of two mutant *LCAT* alleles (4-87mg/dL), and slightly reduced in heterozygous carriers. Plasma CEFA distribution showed an enrichment in saturated and monounsaturated fatty acids, and a depletion in polyunsaturated fatty acids in carriers of two *LCAT* alleles. In addition, an increased plasma oleate/linoleate ratio was also noted compared to controls (3.87±2.53 vs 0.57±0.08, P=0.002). Plasma triglyceride-fatty acid distribution was remarkably similar between carriers of LCAT deficiency and controls. CEFA profile in VLDL essentially recapitulated that of plasma. No dramatic changes were instead observed in heterozygous carriers. After fat load, chylomicrons of carriers of *LCAT* mutations showed CE containing mainly saturated fatty acids.

Conclusions: The present study, taking advantage of the availability of a large cohort of carriers of LCAT deficiency, shows that in the absence of LCAT activity CE are still present in VLDL, being derived from ACAT2.

SE106

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-07 Environmental risk factors*

EFFECTS OF TRADITIONAL CIGARETTE AND ELECTRONIC CIGARETTE ON SMOOTH MUSCLE CELL PHENOTYPIC MODULATION

SAAG SESSION 24: VASCULAR CALCIFICATION AND REMODELING

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Background and Aims : Cigarette smoke (CS) is a risk factor for cardiovascular disease. Whole traditional cigarette (TC) smoke contains over 7000 chemical components and several are deemed to be toxic. Thus, alternative next-generation tobacco products (E-cigarettes (E-cig)) are being developed as less dangerous. Vascular homeostasis is maintained by differentiated quiescent smooth muscle cells (SMCs) that, following environmental cues, may undergo transcriptional changes affecting contractile proteins and their proliferative phenotype. We studied the effects of aqueous extracts (AEs) from TC and E-cig on SMC phenotypic modulation.

Methods: Human aortic SMCs (HSMCs) were incubated for 48 hours with AEs and gene/protein expression analyzed by real-time PCR, western blot analysis, and confocal microscopy.

Results: AEs stimulated the expression of contractile markers (α-actin, calponin, and SM22) and of a network of regulatory transcription factors such as myocardin and SRF, which promote a SMC contractile state while reducing the levels of myocardin repressor Krüppel-like factor 4. E-cig showed potent and faster induction of SMC proliferation and migratory activity, while TC slowed down cell proliferation compared to control and E-cig. The incubation with TC and E-cig AEs also affected cell morphology, with the extension of filopodia, and increased F-actin levels; in particular, TC increased it by 70% and E-cig by three-fold.

Conclusions: AEs from TC and E-cig have different effects on SMC plasticity. Both extracts stimulate the expression of contractile SMC-related genes. However, E-cig potently induces SMC proliferation and migration activity, while TC reduces it. Therefore, the real long-term health effects of these next-generation cigarettes will have to be further assessed.

SE107

Topic: *ASA04 - CLINICAL VASCULAR DISEASE / ASA04-06 Aneurysms and other non-atherosclerotic arteriopathies*

THE ROLE OF RARE ENHANCER VARIANTS IN BICUSPID AORTIC VALVE PATHOLOGY

SAAG SESSION 24: VASCULAR CALCIFICATION AND REMODELING

Artemy Zhigulev[1], Karin Lång[2], Sailendra Pradhananga[1], Rapolas Spalinskas[1], Anders Franco-Cereceda[3], Hanna Björck[2], Per Eriksson[2], Pelin Sahlén[1]
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Background and Aims : The bicuspid aortic valve (BAV) is a highly heritable congenital valve defect often associated with ascending aortic aneurysm formation. Only a few coding or GWAS variants have been linked to the disease, explaining a small portion of the heritability. Given the preponderance of heritability, we hypothesized that rare regulatory variants (MAF < 0.5%) might play a crucial role in BAV pathology.

Methods: To this end, 16 tissue samples from patients either with bicuspid (8) or normal tricuspid (8) aortic valve (TAV) were obtained at the Karolinska Hospital during open-heart surgery. We generated promoter-enhancer interaction (using HiCap) and transcriptome maps of aortic endothelial cells derived from the ascending aorta. Additionally, we sequenced their entire genomes to determine sequence variants.

Results: We found a difference in the promoter-enhancer interaction landscape of BAV and TAV samples while their transcriptomes were similar. We isolated instances where the presence of a rare enhancer variant resulted in making or breaking an interaction with a promoter. We observed a 3X fold enrichment for such cases and they were enriched for binding motifs for transcription factors involved in valve formation during development. Moreover, the target genes of these rare mutations were highly enriched for processes relevant for valve formation and endothelial-to-mesenchymal transition only in BAV patients, whereas TAV patients showed no such enrichment. Resultantly, we produced a battery of genes whose mis-regulation during valve development window could be responsible for the defect.

Conclusions: These findings shed light on the genetic component of BAV pathology and provide opportunities for its non-invasive diagnosis.

SE108

Topic: *ASA04 - CLINICAL VASCULAR DISEASE / ASA04-11 Anti-inflammatory therapies*

INHIBITION OF S100A9 FUNCTION HAS IMMUNOMODULATORY AND CARDIOPROTECTIVE EFFECTS IN EXPERIMENTAL AUTOIMMUNE MYOCARDITIS.

SAAG SESSION 24: VASCULAR CALCIFICATION AND REMODELING

Gabriel Jakobsson[1], Megan Mulholland[1], Andrietta Grentzmann[1], Irena Ljungcrantz[1], Sara Rattik[1,2], Daniel Engelbertsen[1], Alexandru Schiopu[1]
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Background and Aims : Autoimmune myocarditis is characterized by non-ischemic inflammatory heart injury, leading to myocardial damage and impaired cardiac function. S100A9 is an inflammatory alarmin present in large amounts in neutrophils. The role of S100A9 as a potential treatment target in myocarditis has not been fully explored. We sought to investigate the impact of S100A9 blockade in experimental autoimmune myocarditis and assess the effects of treatment on cardiac function and immune infiltration.

Methods: BALB/C mice were immunized with αMHC peptide emulsified in Complete Freunds Adjuvant at day 0 and day 7 to induce the disease. The water-soluble small-molecule S100A9 blocker ABR-238901 was given continuously in drinking water starting on day 7. Echocardiography was performed weekly from day 21 to day 42 (n=11/group). For flow cytometry analysis, mice were sacrificed at day 21 (n=10/group).

Results: S100A9 blockade improved left ventricular ejection fraction [52.33% vs 44.87% on day 42 ($p<0.001$)]. Cardiac output was significantly improved on day 21 (12.69 vs 11.05 mL/min, $p<0.05$). We found a significant reduction of inflammatory cardiac infiltrates at day 21, characterized by reduced number of macrophages ($p<0.05$), neutrophils ($p<0.01$) and $CD4^+$ T cells ($p<0.05$). The cardiac draining lymph nodes contained fewer dendritic cells ($p<0.01$), T cells ($p<0.05$), as well as reduced numbers of inflammatory $CD4^+$ cells producing IL-17 ($p<0.05$).

Conclusions: Therapeutic S100A9 blockade inhibits inflammatory cardiac infiltration and improves cardiac function in experimental autoimmune myocarditis. Our findings highlight the important role of S100A9 in the pathogenesis of myocarditis and identify S100A9 blockade as a possible novel therapeutic avenue.

SE109

Topic: *ASA04 - CLINICAL VASCULAR DISEASE / ASA04-12 Prevention and treatment of cardiovasculardisease; miscellaneous*

ASSOCIATION OF MACROPHAGE MIGRATION INHIBITORY FACTOR AND SST2 WITH LEFT VENTRICULAR REMODELING AMONG ST-ELEVATION MYOCARDIAL INFARCTION PATIENTS

SAAG SESSION 24: VASCULAR CALCIFICATION AND REMODELING

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Background and Aims : Macrophage migration inhibitory factor (MIF) and soluble suppressor of tumorigenesis-2 (sST2) are actively involved in the regulation of inflammation in acute ST-elevation myocardial infarction (STEMI) and further development of adverse cardiac structural and functional changes, but a!ect di!erent links of this process. We hypothesized that the determination of biomarkers combination can be helpful for early risk stratification and further treatment management strategy. The aim of the study was to improve the prediction of adverse left ventricular (LV) remodeling among STEMI patients.

Methods: We enrolled 120 patients with confirmed STEMI and successful primary percutaneous coronary intervention within the first 12 hours after the onset of coronary event. LV remodeling was determined after 6 months of follow-up as an enlargement of end-diastolic and end-systolic LV volumes >10%. The levels of biomarkers were measured at admission and after revascularization.

Results: Spearman's rank correlation test showed positive relationship between MIF levels and LV end-diastolic volume (r=0.4, p=0.001), left atrial size (r=0.4; p=0.027), peak levels of troponin I (r=0.5; p=0.002), white blood cell count (r=0.33, p=0.0001), sST2 levels (r=0.33; p=0.0016). ROC analysis has indicated cut-off MIF values for predicting LV remodeling >3185 pg/ml with sensitivity 60.0 % and specificity 91.7 % (p=0.0318) and sST2 >31.21 ng/ml with sensitivity 78.6 %, specificity 58.3 % (p=0.0305). However, the results of univariate and multivariate analysis have showed that only the level of MIF was most significant predictor of LV remodeling (p=0.028).

Conclusions: The single determining of biomarker MIF before PCI could be useful in prediction of adverse cardiac remodeling after STEMI.

SE110

Topic: *ASA04 - CLINICAL VASCULAR DISEASE / ASA04-12 Prevention and treatment of cardiovasculardisease; miscellaneous*

CHARACTERIZATION OF MEDIATORS OF VASCULAR CALCIFICATION DERIVED FROM ADRENAL GLANDS

SAAG SESSION 24: VASCULAR CALCIFICATION AND REMODELING

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Background and Aims : Adrenal glands modulate cardiovascular physiology and pathophysiology via the synthesis and secretion of well-known compounds like mineralocorticoids, glucocorticoids and amine peptides. In this study, we investigated a previously unknown function of adrenal glands, that is the regulation of vascular calcification processes.

Methods: Bovine adrenal gland homogenate was separated using chromatographic fractionation. The fractions were assessed in cells, aortic rings and vitamin D3 plus nicotine renal failure rat model for effects on vascular calcification processes. Potential mediators were distinguished by mass spectrometry and comparison with pertinent databases. Moreover, the mechanism of action of CBF was studied using standard techniques such as western blotting, real time PCR and siRNA knockdown.

Results: We identified a 19 aa peptide, named “calcification blocking factor” (CBF), which shows a promising protective effect against vascular calcification. CBF is released from the parent protein ChromograninA, which is released from adrenal glands. CBF reduced the calcium content of cells and aortic rings in calcifying cultures. Pulse pressure as a marker of arterial stiffness of VDN animals treated with CBF significantly decreased. For ease of therapeutic application, smaller sections of CBF peptide assessed for their effect on Vascular Calcification.

Conclusions: In conclusion, we have identified a previously uncharacterized peptide secreted from the adrenal gland that modulates cardiovascular calcification. We show that CBF reduces calcification via PIT-1/NF-κB/BMP2/p-SMAD pathway by reducing the transdifferentiation of smooth muscle cells. Further, we identified the active site of this peptide which can be used as a therapeutic agent. These findings suggest a novel function of adrenal glands in cardiovascular calcification.

SE111

Topic: *ASA01 - PATHOGENESIS OF ATHEROSCLEROSIS / ASA01-04 Inflammation, immunity and infection in atherosclerosis*

LIGHT DEFICIENCY IN APOE-/- MICE INCREASES ATHEROMA PLAQUE VULNERABILITY BY MODULATING ARTERY TERTIARY LYMPHOID ORGANS.

SAAG SESSION 25: IMMUNITY AND ATHEROSCLEROSIS - GETTING TO THE ROOTS

Gema Hurtado Genovés, Susana Martín-Vañó, Andrea Herrero-Cervera, María Aguilar-Ballester, Ángela Vinué, Alida M. Taberner Cortés, Sergio Martínez-Hervás, Herminia González-Navarro
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Background and Aims : Previous studies indicate the participation of LIGHT(TNFSF14)-lymphotoxin beta receptor(LTβR) in atheroma progression. In apolipoproteinE-deficient mice (*Apoe-/-*), LTβR deficiency diminishes atheroma plaque, thought its absence in vascular smooth muscle cells(VSMCs) aggravates atheroma altering artery tertiary lymphoid organ (ATLO). In this research, the effect of whole body and hematopoietic stem cell (HSC) specific gene inactivation of *Light* gene in *Apoe-/-* mouse model was investigated.

Methods: *Apoe-/-Light+/+* and *Apoe-/-Light-/-* mice were placed on an atherogenic diet (HFHC, high fat high cholesterol) for two months. A subset of mice underwent bone marrow transplant (BMT) protocol at 8 weeks of age and then were fed with the HFHC diet for 9 weeks. Mice were sacrificed for analysis, consisted of whole blood and ATLO leukocyte examination by flow cytometry, histopathological characterization in aortic cross-sections and RNAseq in adventitia ATLO-free vascular tissue.

Results: Light genetic deficiency significantly augmented lesion size and necrotic core area. Leukocyte examination revealed differences in Th1-17 and Tregs between *Apoe-/-Light+/+* and *Apoe-/-Light-/-* mice (**fig.1**). Examination of *Apoe-/-Light-/-* mouse plaques revealed increased proliferation rate within atheroma and augmented CD3+ in adventitia. ATLO isolated from *Apoe-/-Light-/-* mice displayed augmented Th17 and Th1 cells. BMT from *Apoe-/-Light+/+* into *Apoe-/-Light-/-* mice significantly decreased atherosclerosis (**fig.2**), stabilized plaques and restored lymphocyte homeostasis in ATLO. RNAseq analysis of adventitia vascular tissue showed differences between *Apoe-/-Light+/+* and *Apoe-/-Light-/-* mice in pathways associated with VSMCs phenotype-switching.

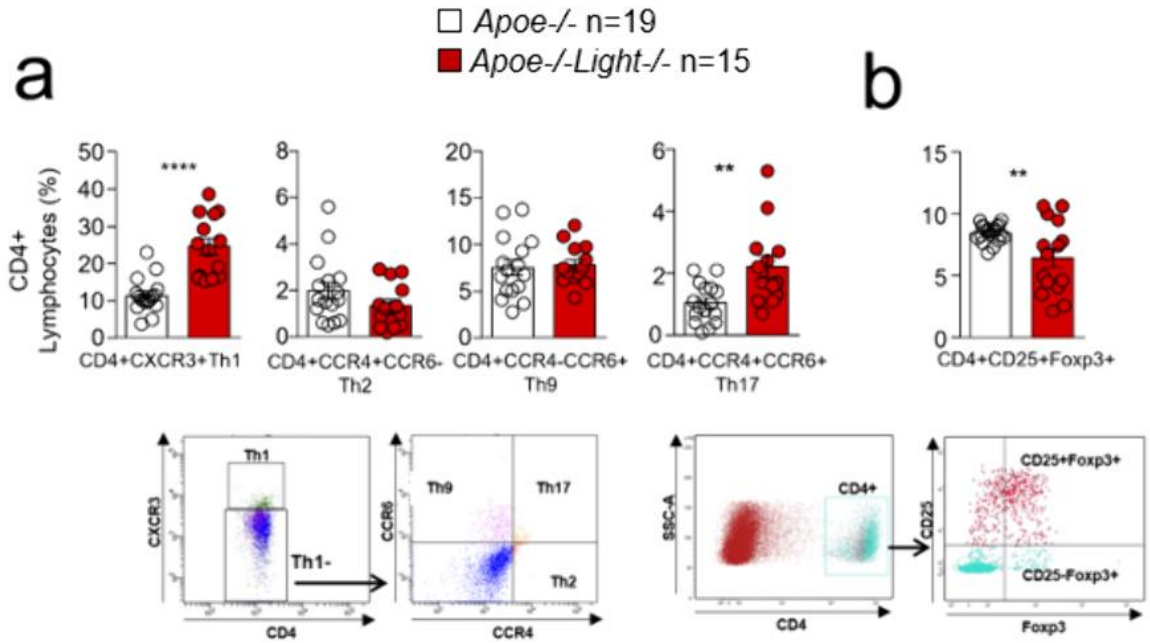

Figure 1. Effect of Light-deficiency in *Apoe-/-* mice in circulating leukocytes and inflammatory mediators. (a) Percentage of CD4+Th effector cells defined as CD4+CXCR3+Th1, CD4+CCR4+CCR6-Th2, CD4+CCR4-CCR6+Th9 and CD4+CCR4+CCR6+Th17. **(b)** Percentage of CD4+Treg cells detected as CD4+CD25+Foxp3+ leukocytes. Representative cytometry plots of the gating strategies used for the flow cytometry analysis in the different leukocyte populations are shown. Statistical analysis was performed using Student´s t-test analysis. ** $p < 0.01$; **** $p < 0.0001$.

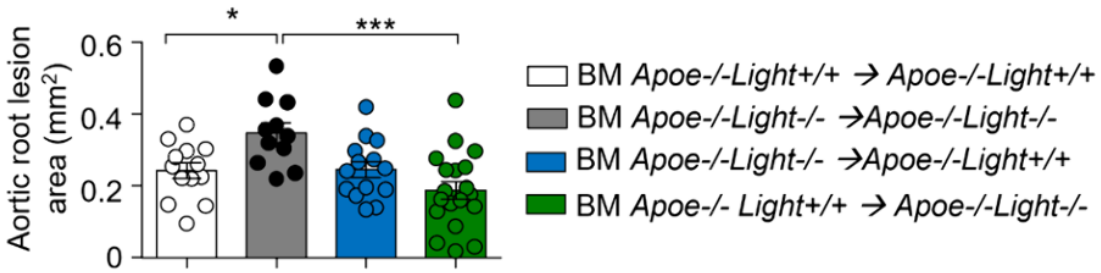

Figure 2. Atherosclerosis analysis in aortic cross-sections of paraffin-embedded heart of transplanted mice after 9 weeks of HFHC diet. Determination of aortic root atherosclerotic lesion total area (mm^2). Comparative between four groups of BMT in female mice. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. * $p < 0.05$; *** $p < 0.001$.

Conclusions: Results demonstrate augmented atherosclerosis in *Apoe-/-Light-/-* mice and atheroprotective role of Light-derived HSC. They also suggest that Light modulate atherosclerosis

through regulation of local immunity and VSMC-phenotype-switching.

Research funds: PI19/00169 from Carlos III Health Institute and FEDER funds.

SE112

Topic: *ASA01 - PATHOGENESIS OF ATHEROSCLEROSIS / ASA01-04 Inflammation, immunity and infection in atherosclerosis*

RESPONSE GENE TO COMPLEMENT-32 SERVES AS A FUNCTIONAL DYAD IN SMOOTH MUSCLE CELLS: CELL CYCLE ACTIVATOR AND MEDIATOR OF CELLULAR DIFFERENTIATION

SAAG SESSION 25: IMMUNITY AND ATHEROSCLEROSIS - GETTING TO THE ROOTS

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Background and Aims : Proliferation of endothelial cells (EC) and smooth muscle cells (SMC) and SMC phenotypic plasticity are critical processes in the development of atherosclerosis. We have previously shown that the sublytic C5b-9 effector Response Gene to Complement-32 (RGC-32) regulates EC proliferation, migration, and cytoskeletal reorganization. Our goal was to explore the effect of RGC-32 on cell cycle activation and TGF-β induced differentiation of SMC and the extracellular matrix (ECM) production.

Methods: For overexpression of RGC-32 in human aortic SMC (ASMC), we chose pVP22 or pVP22-RGC-32 plasmid transfection using Effectene. ASMC proliferation was assessed through [^{3}H]thymidine and *BrdU* incorporation assays. The effect of RGC-32 on ECM production and SMC differentiation was studied by silencing RGC-32 expression in ASMCs using transfection of RGC-32 siRNA in the presence of Lipofectamine 3000. The expression of collagens I, myocardin, SM22 and α-smooth muscle actin (α-SMA) were determined by real-time PCR.

Results: Overexpression of RGC-32 resulted in ASMC-induced cell cycle activation and proliferation; ERK1 proved to be indispensable for sublytic C5b-9/RGC-32-mediated activation of CDC2 and the cell cycle. Cell cycle activation in ASMC by sublytic C5b-9 required the participation of RGC-32 phosphorylated at threonine 91. In cultured ASMC α-SMA, collagen I, myocardin and SM22 were significantly induced at 18 h of stimulation with TGF-β. Silencing of RGC-32 in ASMC was followed by a significant reduction in TGF-β induced expression of myocardin, SM22, α-SMA and collagen I.

Conclusions: RGC-32 plays a dual role in ASMC, participating in both sublytic C5b-9-induced cell cycle activation and TGF-β-induced ECM production, while modulating SMC differentiation.

SE113

Topic: *ASA01 - PATHOGENESIS OF ATHEROSCLEROSIS / ASA01-04 Inflammation, immunity and infection in atherosclerosis*

CIRCULATING NATURAL KILLER CELLS IN PATIENTS WITH SYMPTOMATIC ATHEROSCLEROSIS ACQUIRE THE CD9+CD49A PRO-INFLAMMATORY PHENOTYPE AND SUPPORT M1-LIKE MACROPHAGE POLARIZATION.

SAAG SESSION 25: IMMUNITY AND ATHEROSCLEROSIS - GETTING TO THE ROOTS

Martina Cucchiara[1], Matteo Gallazzi[1], Sergio Losa[2], Valentina Carlini[3], Tiziana Mazzullo[4], Massimiliano Martelli[2], Douglas M. Noonan[1], Antonino Bruno[5], Adriana Albini[6]
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Background and Aims : Inflammation represents a hallmark of atherosclerosis (ATS), with monocytes/macrophages and T cells as the most investigated immune cells. Natural killer (NK) cells have been proposed as immunoregulatory cells in ATS, but the mechanisms still require investigations. We phenotypically and functionally characterized peripheral blood NK cells in patients with symptomatic atherosclerosis (sATS) and investigated their functional interactions with endothelial cells and monocyte/macrophages.

Methods: NK cell subset distribution in peripheral blood of sATS patients was investigated, by multicolor flow cytometry, for surface antigens expression. Protein arrays were performed to characterize the secretome on FACS-sorted peripheral blood NK cells (pbNKs). Conditioned media (CM) from FACS-sorted pbNKs of sATS patients and healthy control (HC) were used to determinate their ability to induce pro-inflammatory/pro-angiogenic phenotype in endothelial cells, recruit endothelial cells and monocytes and polarize macrophages.

Results: We found that pbNKs from sATS patients acquire the pro-inflammatory $CD56^{bright}CD9^{+}CD49a^{+}$phenotype. Secretome analysis showed that pbNKs from sATS have increased secretion of CXCL8, a chemokine involved in endothelial cell recruitment and pro-inflammatory activation, TGFb, a cytokine involved in macrophage polarization, bFGF (pro-angiogenic factor), GRO and RANTES (factors involved in monocyte recruitment). CMs from pbNK cells of sATS patients recruit endothelial cells and monocytes and elicit a M1-like macrophages polarization.

Conclusions: Our works provided the rational to propose the profiling of pbNK cell polarization in sATS patients as possible circulating biomarker to trace and/or stratify sATS patients, as consequence on their NK cell polarization state, using a minimally invasive, liquid biopsy-based methods.

SE114

Topic: *ASA01 - PATHOGENESIS OF ATHEROSCLEROSIS / ASA01-04 Inflammation, immunity and infection in atherosclerosis*

OSTEOPOROSIS AND VASCULAR CALCIFICATION – AN IMMUNOLOGICAL LINK

SAAG SESSION 25: IMMUNITY AND ATHEROSCLEROSIS - GETTING TO THE ROOTS

Wera Pustlauk[1], Ioanna M. Dimitriou[1], Toralf Roch[1], Ulrik Stervbo[2], Moritz Anft[2], Arturo Blázquez Navarro[1], Timm H. Westhoff[2], Sven Geißler[1], Nina Babel[1]
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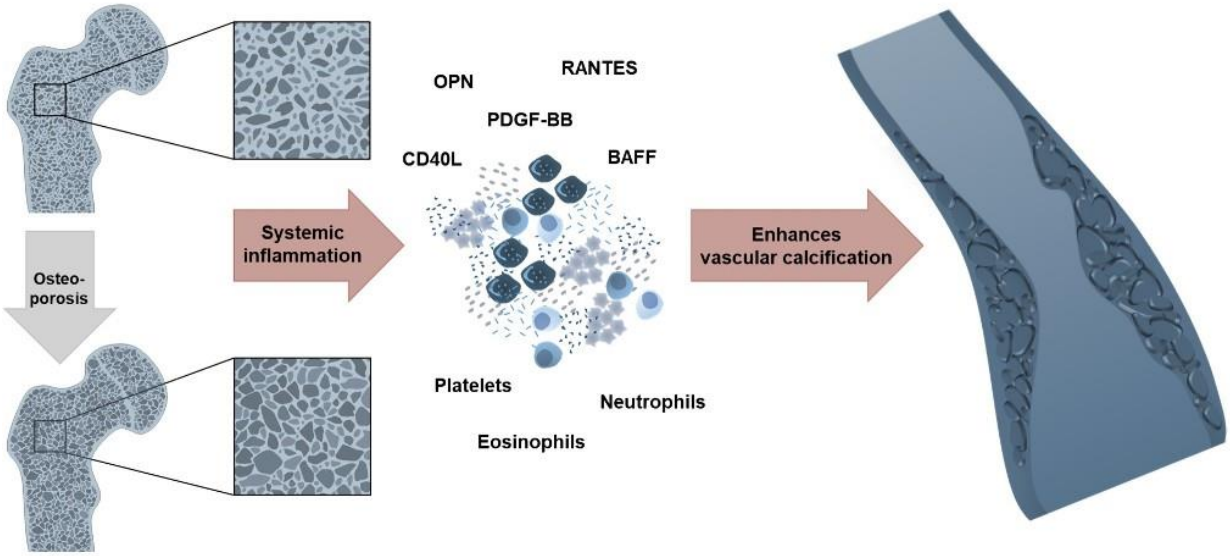
Background and Aims : Osteoporosis and low bone mineral density were identified as independent risk factors for enhanced vascular calcification and cardiovascular diseases, associated with increasing mortality. Common risk factors for both disorders, such as age, lack of physical activity, and menopause are insufficient to explain a mutual pathogenic mechanism. However, broad evidence emphasises the important role of systemic inflammation in both diseases, indicating a pathologically immunological regulation.

Methods: To address this question, the capacity of male and female sera pools from osteoporotic patients as well as non-osteoporotic controls to impact osteogenic differentiation was assessed using an *in vitro* model for calcification of human vascular smooth muscle cells (SMC). Furthermore, sera were screened for soluble mediators using different multiplex immunoassays. The impact on SMC calcification of the mediators identified was investigated. In addition, from all patients circulating immune cell subsets were quantified by flow cytometry.

Results: Stimulation with osteoporotic sera resulted in an earlier onset as well as enhanced calcification of SMC irrespective of the sex. BAFF, RANTES, CD40L, PDGF-BB, and OPN were found to be significantly increased in osteoporotic patients compared to non-osteoporotic controls. Thereof, PDGF-BB significantly increased SMC calcification *in vitro*. Analysis of immune cell subsets indicated a distinct increase of neutrophils, eosinophils and platelets in osteoporotic patients, while no differences in lymphocyte, monocyte, and natural killer cell subsets were detected.

Conclusions: These findings support a pathologically immunological link between an impaired bone metabolism in patients with osteoporosis and enhanced vascular

calcification.



SE115

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-01 Lp(a)*

STATIN THERAPY AND LIPOPROTEIN(A) LEVELS: A SYSTEMATIC REVIEW AND META-ANALYSIS

SAAG SESSION 26: THE SIGNIFICANCE OF LP(A)

Lotte M. De Boer[1], Anna O.J. Oorthuys[2], Albert Wiegman[2], Miranda W. Langendam[3], Jeffrey Kroon[4], René Spijker[3], Aeilko H. Zwinderman[3], Barbara A. Hutten[3]
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Background and Aims : Lipoprotein(a) (Lp(a)) is a causal and independent risk factor for cardiovascular disease (CVD). People with elevated Lp(a) are often prescribed statins as they also often show elevated low-density lipoprotein cholesterol (LDL-C) levels. While statins are well-established in lowering LDL-C, their effect on Lp(a) remains unclear. We evaluated the effect of statins compared to placebo on Lp(a) and the effects of different types- and intensities of statin-therapy on Lp(a).

Methods: We conducted a systematic review and meta-analysis of randomized-trials with a statin- and placebo-arm. Medline and EMBASE were searched until August 2019. Quality-assessment of studies was done using Cochrane risk-of-bias tool (RoB 2). Mean difference of absolute- and percentage changes of Lp(a) in the statin- versus the placebo-arms were pooled using a random-effects meta-analysis. We compared effects of different types- and intensities of statin-therapy using subgroup- and network meta-analyses. Certainty of the evidence was determined using GRADE (Grading of Recommendations Assessment, Development and Evaluation).

Results: Overall, 39 studies (24,448 participants) were included. Mean differences (95% CI) of absolute- and percentage changes in the statin- versus the placebo-arms were 1.1 mg/dl (0.5-1.6, $p<0.0001$) and 0.1% (-3.6%-4.0%, $p=0.95$), respectively (moderate-certainty evidence). None of the types of statins changed Lp(a) significantly compared to placebo (very low- to high-certainty evidence), as well as intensities of statin-therapy (low- to moderate-certainty evidence).

Conclusions: Statin-therapy does not lead to clinically important differences in Lp(a) compared to placebo in patients at risk for CVD. Our findings suggest that in these patients, statin-therapy will not change Lp(a)-associated CVD risk.

SE116

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-01 Lp(a)*

ASSOCIATION OF LIPOPROTEIN(A) WITH CORONARY ARTERY DISEASE IN PERIPHERAL ARTERY DISEASE PATIENTS

SAAG SESSION 26: THE SIGNIFICANCE OF LP(A)

Narek A. Tmoyan[1], Marat V. Ezhov[1], Olga I. Afanasieva[2], Elena A. Klesareva[2], Marina I. Afanasieva[2], Uliana V. Chubykina[1], Sergey N. Pokrovsky[2]
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Background and Aims : To evaluate the relationship of lipoprotein(a) [Lp(a)] level with coronary artery disease (CAD) in patients with peripheral artery disease (PAD).

Methods: The study included 285 patients with PAD. The patients were divided into two groups depending on presence of CAD. The group with CAD consisted of 208 patients and the group without CAD contained 77 patients. The levels of lipids, Lp(a), C-reactive protein (CRP) were measured in serum.

Results: The groups with and without CAD were comparable by age and sex. In group with CAD the frequency of hypertension, type 2 diabetes, smoking was higher than in the group without CAD. The level of Lp(a) was significantly higher in patients with CAD than in those without CAD: 36 [15; 80] vs. 30 [10; 49] mg/dL, p=0.01. Prevalence of Lp(a) ≥50 mg/dL [hyperlipoproteinemia(a)] was significantly higher in patients with both PAD and CAD comparing to patients with PAD but without CAD: 78 (38%) vs. 18 (23%), p=0.03. The odds ratio of CAD in PAD patients in the presence of Lp(a) level ≥50 mg/dL was 2.0 (95% confidence interval, 1.1-3.6, p=0.03). There were no differences between groups in lipids and CRP level. In logistic regression analysis adjusted for hypertension, type 2 diabetes and smoking, Lp(a) was an independent predictor of CAD in PAD patients.

Conclusions: In PAD patients Lp(a) concentration and prevalence of hyperlipoproteinemia(a) were higher in patients with CAD than without CAD. Lp(a) level is an independent predictor of CAD in PAD patients.

SE117

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-01 Lp(a)*

THE ASSOCIATION OF LIPOPROTEIN(A) WITH MACE INCLUDING CORONARY REVASCULARISATIONS IN PATIENTS WITH ATHEROSCLEROTIC CARDIOVASCULAR DISEASE: DATA FROM UK BIOBANK

SAAG SESSION 26: THE SIGNIFICANCE OF LP(A)

Paul I. Welsh[1], Rosemary Brown[1], Ana Filipa Fonseca[2], Gabriella Farries[3], Taha Itani[2], Anas Al Zabiby[4], Shruti Narasimham[3], Jonathan Little[5], Naveed Sattar[1]
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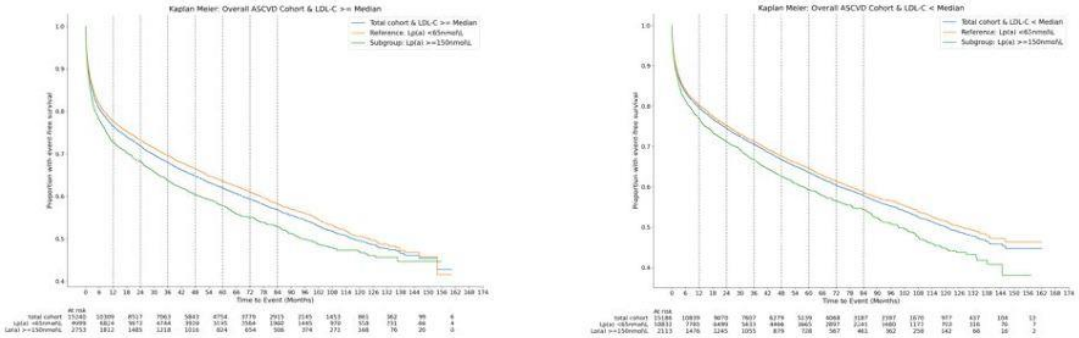
Background and Aims : To investigate the association of elevated Lp(a) with Major Adverse Cardiovascular Events (MACE) outcomes in a population with previous ASCVD.

Methods: This was an observational retrospective study including 30,510 participants from UK Biobank with a prior ASCVD event (CHD, cerebrovascular or peripheral arterial disease). The study modelled associations of serum Lp(a) with time to first composite MACE (n=11,163 events) defined as myocardial infarction (n=5,724), ischaemic stroke (n=919), CV-death (n=1,533) or coronary revascularisation (n=2,987). Coronary revascularisation was also reported as a disaggregated outcome. These associations were examined using both unadjusted (Kaplan Meier) and adjusted for classical risk factors using Cox proportional hazards models.

Results: Median follow-up time was 4.7 years and median Lp(a) was 23.6 nmol/L (interquartile interval 8.3-98.7 nmol/L). Lp(a) of ≥150nmol/L versus <65nmol/L was associated with increased MACE, irrespective of LDL-C subgroup (Fig 1). In adjusted models, 100nmol/L increase in Lp(a) was associated with 8.0% (95%CI 5.8-10.4%) and 18.6 % (95%CI 14-23%) higher risks of MACE and coronary revascularisation, respectively. Risk associations of Lp(a) with composite MACE were similar in men and women (7.4% and 9.5%, respectively), by age group (for <65 years 6.9% and for ≥65 years 8.8%), and by LDL-cholesterol below or above the cohort median (8.1% and 7.9%, respectively).

Figure 1. Kaplan-Meier curve of time without MACE separately in participants with LDL-C <median (135.6 mg/dL) or ≥ median in an ASCVD population



Conclusions: Elevated Lp(a) in patients with ASCVD was associated with increased risk of MACE, and particularly with the need for coronary revascularisation. Adopting Lp(a) measurements among ASCVD

patients in clinical practice may aid understanding of an individual's risk of developing MACE or requiring coronary revascularisation.

SE118

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-09 Lipid and lipoprotein metabolism*

TRIGLYCERIDE METABOLISM MODIFIES LIPOPROTEIN(A) PLASMA CONCENTRATION

SAAG SESSION 26: THE SIGNIFICANCE OF LP(A)

Itziar Lamiquiz Moneo[1,2], Maria Ramos-Cáceres[2], Ana Cenarro[3], Pilar Calmarza[2], Victoria Marco-Benedí[4], Ana Maria Bea[5], Rocio Mateo-Gallego[2], José Puzo[2], José Ordovás[6], Fernando Civeira[2], Martin Laclaustra[7]
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Background and Aims : Lipoprotein(a) (Lp(a)) is a significant cardiovascular risk factor. This study aimed to analyze the relationship between triglycerides (TG) and Lp(a) concentrations, cross-sectionally and longitudinally, and the influence of the number and composition of TG-rich lipoproteins, and the *APOE* genotype.

Methods: Data from Aragon Workers Health Study (AWHS) (n=5467), NHANES III (n=3860), and Lipid Clinic from the Hospital Universitario Miguel Servet (HUMS) (n=2079) were used to analyze the cross-sectional TG-Lp(a) relationship. The relationship TG-Lp(a) intra-subject variation was studied in AWHS participants and HUMS patients with repeated measurements. TG-rich lipoproteins were quantified by NMR in a subsample from AWHS. Apolipoproteins B and E were quantified by Luminex in VLDL isolated by ultracentrifugation, from HUMS serum samples. *APOE* genotyping was carried out by the Sanger method in AWHS and HUMS participants. Regression models adjusted for age and sex were used to study the association.

Results: The three studies showed an inverse relationship TG-Lp(a), especially for TG ≥300 mg/dL. Increased VLDL number, size and TG content were associated with significantly lower Lp(a) concentrations. There was an inverse association between the apo E concentration in VLDL and Lp(a). Conversely, no significant association was observed for apo B. Subjects carrying apoE2/E2 genotype had significantly lower Lp(a)
levels.

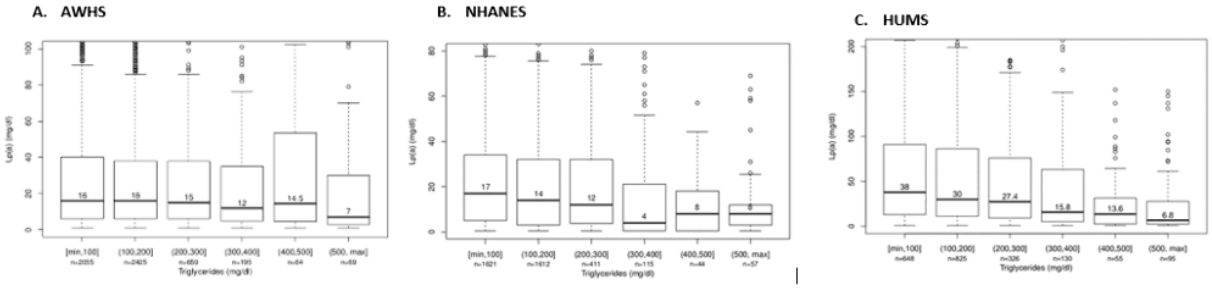

Table 1. Effect of total VLDL particle concentration, triglyceride content of VLDL and VLDL size on Lp(a) concentration.

A. Separate regression each VLDL variable

VLDL variable	**Lp(a) change per standard deviation**
VLDL particle concentration.	x0.793 (95%CI 0.682,0.922)
Triglycerides in VLDL	x0.783 (95%CI 0.677,0.906)
VLDL size	x0.770 (95%CI 0.664,0.892)

B. VLDL variables mutually adjusted (all three rows included in a single regression model)

VLDL variable	**Lp(a) change per standard deviation**
VLDL particle concentration.	x0.913 (95%CI0.575,1.449)
Triglycerides in VLDL	x1.069 (95%CI0.537,2.127)
VLDL size	x0.778 (95%CI0.512,1.181)
p (likelihood ratio test)	0.007

Table 2. Lp(a) concentration according to *APOE* genotype in AWHS and HUMS

	***APOE* genotype**					
	E3E3	**E2E2**	**E2E3**	**E2E4**	**E3E4**	**E4E4**
AWHS						
n	3535	21	526	59	824	43
G-mean Lp(a), mg/dl	13.6	4.1	13.1	11.5	14.4	13.8
Model (x-times)	1.000 (ref.)	0.300 (95% CI 0.170 , 0.530)	0.963 (95% CI 0.852 , 1.087)	0.837 (95% CI 0.595 , 1.178)	1.053 (95% CI 0.953 , 1.165)	1.007 (95% CI 0.676 , 1.501)
p	-	<0.001	0.538	0.308	0.311	0.972
HUMS						
n	1014	16	124	24	332	34
G-mean Lp(a), mg/dl	27.9	11.1	21.9	21.4	26.3	14.9
Model (x-times)	1.000 (ref.)	0.426 (95% CI 0.208 , 0.873)	0.807 (95% CI 0.615 , 1.058)	0.777 (95% CI 0.432 , 1.398)	0.933 (95% CI 0.779 , 1.117)	0.538 (95% CI 0.328 , 0.884)
p	-	0.020	0.121	0.399	0.450	0.014

G-mean denotes: geometric mean (unadjusted). Model adjusted for age and sex.

Conclusions: Our results show an inverse relationship Lp(a)-TG. Subjects with larger VLDL size have lower Lp(a), and lower values of Lp(a) were present in patients with apoE-rich VLDL and apoE2/E2 subjects. Our results would suggest that bigger VLDLs and VLDLs enriched in apoE are not suitable substrates for the formation of Lp(a).

SE119

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-12 Adipose tissue biology and pathology*

ROLE OF HISTONE DEACETYLASE 3 IN IMMUNOPHENOTYPE OF ADIPOSE TISSUE

SAAG SESSION 27: THE SECRETS OF ADIPOSE TISSUE

Lara Coppi[1], Carolina Peri[1], Fabrizia Bonacina[2], Raffaella Longo[1], Rui Silva[1], Giuseppe Danilo Norata[2], Alberico L. Catapano[3], Maurizio Crestani[1]
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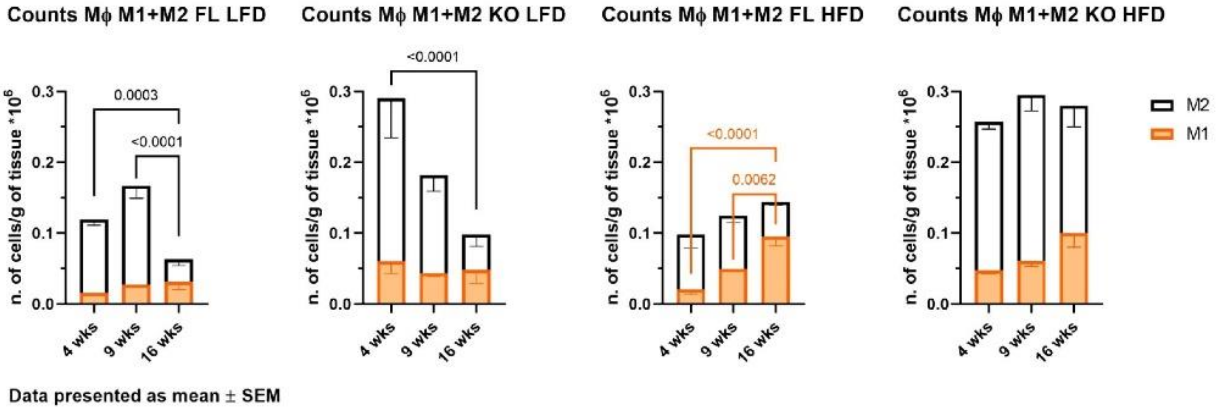
Background and Aims : Obesity is associated to comorbidities like cardiovascular disease, type 2 diabetes, and infectious diseases. We showed that histone deacetylase 3 (HDAC3) regulates white adipose tissue (WAT) physiology and its genetic inactivation leads to metabolic rewiring of WAT towards browning. Since inflammation of WAT affects adipocyte functionality, we aimed to explore the immunophenotype of WAT from Hdac3 KO (H3fatKO) mice to test whether the observed phenotype was associated to different immune cell profile.

Methods: Immunophenotype of visceral WAT (epiWAT), blood, bone marrow and spleen of H3fatKO and floxed control (FL) C57Bl6/J male mice (n=5-9), fed high- (HFD) or low-fat diet (LFD) for 4, 9 and 16 weeks, was characterized by FACS. Statistical analysis was conducted with 2-way ANOVA.

Results: EpiWAT mass of H3fatKO vs. FL mice was reduced. Number of macrophages in epiWAT of H3fatKO vs. FL mice increased after 4 weeks of both diets, whereas at 9 and 16 weeks of diet increased only upon HFD. The increased macrophage number was related to M2-phenotype. Moreover, despite the initial difference in KO vs. FL at LFD, the number of M2 macrophages decreased with aging in both groups. As expected, in FL HFD M1 macrophages increased upon HFD treatment, whereas this was not observed with KO HFD.



Conclusions: HDAC3 is a key factor for WAT phenotype and its inactivation triggers events supporting WAT browning. Based on our results, we hypothesize that yet unidentified specific factors released by Hdac3 KO adipocytes are involved in establishing and maintaining the immunophenotype observed in H3fatKO mice.

SE120

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-12 Adipose tissue biology and pathology*

NFE2L1 PROTECTS WHITE ADIPOCYTES FROM CHOLESTEROL-INDUCED INFLAMMATION

SAAG SESSION 27: THE SECRETS OF ADIPOSE TISSUE

Carolin Muley, Janina Caesar, Alexander Bartelt
Institute For Cardiovascular Prevention (ipek), Ludwig-Maximilians-University, Munich, Germany

Background and Aims : Adipocytes are crucial regulators of cardiovascular health. During obesity, adipocytes become dysfunctional, which is associated with inflammation and insulin resistance, major risk factors for atherosclerosis. Here we determine the role of the proteostatic transcription factor Nfe2l1 in the regulation of cholesterol homeostasis, adipocyte inflammation and atherosclerosis.

Methods: We assessed ER stress and inflammatory markers in white adipose tissue (WAT) of adipocyte-specific Nfe2l1 knockout (KO) mice using RNAseq, qPCR and Western blot. Immune cell distribution in WAT was analyzed using multicolor flow-cytometry. Mechanistic studies were performed in 3T3-L1 adipocytes as well as in primary white adipocytes using RNAi. ER stress was induced with cholesterol and/or epoxomicin.

Results: Mice lacking adipocyte-Nfe2l1 displayed adipocyte hypertrophy, diminished browning and severe inflammation characterized by an upregulation of T cell response in adipose tissue. On high-fat diet (HFD, 60% kcal fat), KO mice were more glucose intolerant and insulin-resistant than wild-type controls, despite similar weight gain and adiposity. On Western diet (WD, 42% kcal fat with 0.2% added cholesterol), KO mice displayed lipodystrophy, insulin resistance, and hepatic steatosis compared to wild-types. In 3T3-L1 adipocytes, cholesterol treatment in combination with proteasomal inhibition impaired the activation of Nfe2l1 and exacerbated ER stress and inflammation. The combined toxicity of cholesterol and proteasomal dysfunction was dependent on Atf3, an ER stress-induced transcription factor.

Conclusions: Our results demonstrate that Nfe2l1 limits Atf3-mediated ER stress and inflammation in response to excessive cholesterol, through improved proteasomal protein quality control. These findings highlight a novel proteostasis-cholesterol relationship in adipocytes.

SE121

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-12 Adipose tissue biology and pathology*

PROTECTIVE ROLE FOR C3A-DES ARG IN ATHEROGENESIS AMONG LOW RISK PATIENTS.

SAAG SESSION 27: THE SECRETS OF ADIPOSE TISSUE

Sofya O. Eliashevich[1], Batogab B. Shoibonov[2], Mikhail B. Khudyakov[3], Valida A. Dadaeva[1], Oleg V. Senko[4], Anna V. Kuznetsova[5], Oxana Drapkina[6]
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Background and Aims : Genetic, dietary and immune factors contribute to the pathogenesis of atherosclerosis in humans. Complement activation is an integral part of the innate immune defence but also influences directly triglyceride synthesis. The study aim was to evaluate a potential association between central obesity, C3a-desArg and atherosclerosis in individuals with low SCORE-2 risk.

Methods: A pilot study, including patients at low risk according to SCORE-2, was designed. Anthropometry and bioimpedance analysis were performed in every subjects alongside with laboratory tests for cholesterol profile, multiply modified LDL (mmLDL) and C3a-desArg. Central obesity (CO) was detected according to the IDF criteria. Individuals were consecutively screened for carotid atherosclerosis using ultrasound method. ANOVA test and Pearson’s chi-square test were used to assess the impact of different metabolic parameters on atherosclerosis prevalence.

Results: 127 adult subjects were screened (mean age: 43(3) years; BMI 27(5) kg/m2; and 47% male). 80 (63%) participants with central obesity. Prevalence of atherosclerosis was 32% (41).The level of mmLDL was significantly higher in the CO group than that in the control, regardless of gender: 20.8 U (8.5) vs 13.6 U (7), respectively ($p<0.01$). cIMT average were correlated with WC, body fat mass, TG, TC, LDL, and mmLDL ($r = 0.4; 0.3; 0.3; 0.4; 0.4; 0.5$, respectively, $p = 0.001$) and C3a-desArg ($r = - 0.4$, $p = 0.001$). According to multivariate analysis: at a C3a-desArg>10.7 ng/ml, atherosclerosis was not detected.

Conclusions: C3a-desArg plays an unexpectedly beneficial role in the atherosclerosis. C3a-desArg is critical for triglyceride synthesis and the maintenance of lipid homeostasis,resulting in increased adiposity and atheroprotection.

SE122

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-04 Adipose tissue homeostasis*

TUMOR NECROSIS FACTOR (TNF) RECEPTOR-ASSOCIATED FACTOR 5 DEFICIENCY IN DIET-INDUCED OBESITY INDUCES A PRO-INFLAMMATORY RESPONSE IN ADIPOCYTES AND AGGRAVATES METABOLIC COMPLICATIONS.

SAAG SESSION 27: THE SECRETS OF ADIPOSE TISSUE

Mark Colin Gissler[1], Nathaly Anto-Michel[2], Xiaowei Li[1], Timoteo Marchini[1], Tijani Abogunloko[1], Timothy Mwinyella[1], Andreas Zirlik[2], Christoph Bode[1], Florian Willecke[3], Dennis Wolf[1]
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Background and Aims : Accumulation of inflammatory leukocytes is a prerequisite of adipose tissue inflammation during cardiometabolic disease. We previously reported that a genetic deficiency of TRAF5 accelerates atherosclerosis in mice by increasing inflammatory cell recruitment. Here, we tested the hypothesis that an impairment of TRAF5 signaling modulates adipose tissue inflammation and its metabolic complications in a model of diet-induced obesity (DIO) in mice.

Methods: *Traf5*$^{-/-}$ and *Wt* mice were fed a HFD for 18 weeks and subsequently assessed for their metabolic and inflammatory phenotypes. Selective hematopoietic*Traf5*-deficiency was generated by bone marrow transplantation. To assess a potential significance for human disease, we studied *TRAF5*-expression in adipocytes and blood samples of obese and non-obese patients as well as *TRAF5*-expression in adipose tissue of patients before and one year after undergoing bariatric surgery.

Results: *Traf5*$^{-/-}$mice on HFD gained significantly more weight compared to *Wt* mice and showed aggravated adipose tissue inflammation and metabolic complications. Surprisingly, selective hematopoietic *Traf5*-deficiency did not resemble the obese and inflammatory phenotype of global *Traf5*$^{-/-}$mice. Conversely,expression of inflammatory cytokines was significantly upregulated in *Traf5*-deficient adipocytes but not in *Traf5*-deficient leukocytes from visceral adipose tissue. In obese mice, *Traf5* was most downregulated in adipocytes, suggesting an adipocyte-dependent effect. In human disease, *Traf5* expression was lower in blood and adipocytes from obese patients and mice and recovered in adipose tissue of obese patients one year after bariatric surgery.

Conclusions: We show that a genetic deficiency of TRAF5 in mice aggravates diet-induced obesity and its metabolic derangements by a proinflammatory response in adipocytes. Our data indicate that TRAF5 may promote anti-inflammatory and obesity-preventing signaling events in adipose tissue.

SE123

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-04 Adipose tissue homeostasis*

FIBROBLAST GROWTH FACTOR 21 POTENTLY REDUCES ATHEROSCLEROSIS AND NASH DEVELOPMENT

SAAG SESSION 27: THE SECRETS OF ADIPOSE TISSUE

Cong Liu[1,2], Milena Schönke[1,2], Borah Spoorenberg[1,2], Joost Lambooij[3,4], Van Der Zande Hendrik[3], Enchen Zhou[1,2], Niek Dekker[5], Anne-Christine Andréasson[6], Andrew Park[7], Martin Uhrbom[6], Ingela Ahlstedt[6], Yasuhiro Ikeda[7], Kristina Wallenius[6], Xiao-Rong Peng[6], Bruno Guigas[3], Mariëtte Boon[1,2], Yanan Wang[1,2], Patrick Rensen[1,2]
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Background and Aims : Fibroblast growth factor 21 (FGF21), a key regulator of energy metabolism, is currently evaluated in humans for treatment of obesity. However, its effect on lipoprotein metabolism in relation to cardiometabolic diseases including NASH and atherosclerotic cardiovascular disease remains elusive.

Methods: By using APOE*3-Leiden.CETP mice, a well-established mouse model mimicking human-like cardiometabolic diseases, we investigated the role of FGF21 in atherosclerosis and NASH via administration of recombinant FGF21 and an AAV8 vector encoding murine-optimized FGF21, respectively.

Results: FGF21 largely lowered plasma cholesterol within lipoprotein remnants. Mechanistically, FGF21 promoted brown adipose tissue (BAT) activation and white adipose tissue (WAT) browning, thereby enhancing the selective uptake of fatty acids from triglyceride-rich lipoproteins into BAT and into WAT, consequently accelerating the clearance of the cholesterol-enriched remnants by the liver. Ultimately, FGF21 largely reduced atherosclerotic lesion area and severity. FGF21 also improved adipose tissue function, accompanied by alleviated insulin resistance. Moreover, FGF21 abolished hepatic steatosis, and largely alleviated hepatic inflammation as evidenced by decreased crown-like structures, Kupffer cell activation, infiltrated monocytes and lipid/scar-associated macrophages, correlating with less liver fibrosis as demonstrated by reduced collagen accumulation.

Conclusions: FGF21 largely increases fatty acid oxidation in thermogenic tissues and in the liver, thereby reducing improving lipid metabolism, and attenuating the development of atherosclerosis and all features of NASH. Our data provide a strong experimental basis for the clinical development of FGF21 to treat atherosclerotic cardiovascular disease and NASH.

SE124

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-02 Epidemiology of dyslipidemias*

DO ATHEROSCLEROTIC EVENTS CHANGE LIPID LOWERING THERAPY USE IN CLINICAL PRACTICE? - THE ANSWER WITH REAL-WORLD DATA

SAAG SESSION 28: LATEST EVIDENCE FROM EPIDEMIOLOGY

Daniel Seabra[1], Francisco Araújo[2,3], Marta Afonso-Silva[4], Diana Grangeia[5], Tiago Taveira-Gomes[6,7,8,9,10], Cristina Gavina[1,3,11,12]
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Background and Aims : Acute atherosclerotic cardiovascular events (ASCV) significantly increase risk of its recurrence and should prompt lipid lowering therapy (LLT) intensification towards more ambitious targets. This study aims to analyze LLT changes after an ASCV and respective LDL-C control.

Methods: Retrospective population-based study from a region of Northern Portugal. Population was composed of patients with ≥1 General Practice appointment in the three years prior to the index date. We created incident cohorts for Myocardial Infarction, Peripheral Artery Disease, Ischemic stroke, Recurrent ASCV (2+ events at most 2 years apart). We performed descriptive analysis of the cohorts at baseline (pre-event) and reported LDL-C control and LLT switches at discharge time (post-event) and at 1-year follow up.

Results: Moderate intensity statins monotherapy is the most used LLT before and after ASCV (Table 1). After ASCV hospitalization, LLT is upscaled in 19.1% of MI patients, in 9.9% of PAD, in 9.8% of ischemic stroke (IS) and in 11.0% of recurrent events patients. LDL-C mean absolute value one-year after the ASCV is 100mg/dl, 105mg/dl, 107mg/dl and 102mg/dl for patients with MI, PAD, IS, and recurrent events, respectively. LDL-C target values are achieved in <5% of the patients (Table2).

Table 1.

	Myocardial Infarction	*Peripheral Artery Disease*	*Ischemic stroke*	*Recurrent event <2y*
n	1817	762	5251	2807
LLT medication 1y pre-event (%)				
Low intensity statin	6.3	6.6	6.1	7.5
Moderate intensity statin	58.1	50.9	42.9	67.1
High intensity statin	3.3	4.9	1.9	4.8
High intensity statin+ezetimibe	0.3	0.8	0.3	0.4
Moderate intensity statin+ezetimibe	1.4	2.0	1.1	2.2
Low intensity statin+ezetimibe	1.0	1.6	0.9	1.25
Ezetimibe	1.7	2.1	1.1	2.3
Fibrates	5.2	6.8	3.7	5.2
PCSK9 inhibitors	0.0	0.0	0.0	0.0
Other	0.1	0.4	0.0	0.1
LLT medication 1y post-event (%)				
Low intensity statin	9.2	7.0	8.4	7.0
Moderate intensity statin	82.2	63.4	60.7	70.6
High intensity statin	18.1	10.8	7.6	12.3
High intensity statin+ezetimibe	2.6	1.3	1.1	1.9
Moderate intensity statin+ezetimibe	7.1	5.0	3.2	5.2
Low intensity statin+ezetimibe	2.8	2.4	1.8	2.2
Ezetimibe	7.9	4.9	3.7	5.8
Fibrates	8.7	7.6	6.1	6.1
PCSK9 inhibitors	0.0	0.0	0.0	0.0
Other	0.6	0.7	0.1	0.3

Table 2.

	Myocardial Infarction	*Peripheral Artery Disease*	*Ischemic stroke*	*Recurrent event <2y*
n	1817	762	5251	2807
Age, years (median (IQR))	66.0 (20.0)	71.0 (18.0)	71.0 (20.0)	70.0 (21.0)
Males (%)	64.2	62.9	43.4	53.3
LLT switches post-event (%)				
LLT Step up	19.1	9.9	9.8	11.0
LLT Step down	18.7	10.1	9.3	12.3
≥ 50% LDL-C reduction 1y post-event (%)	25.9	32.5	22.1	17.4
LDL-C 1y post-event (mg/dl)	100.0	105.0	107.0	102.0
Control LDL-C ESC 2019 pre-event (%)	2.8	4.1	2.1	3.4
Control LDL-C ESC 2016 pre-event (%)	8.6	11.3	6.7	10.2
Control LDL-C ESC 2019 3M post-event (%)	3.7	4.7	3.0	4.1
Control LDL-C 2016 3M post-event (%)	11.8	13.8	9.3	12.3
Control LDL-C ESC 2019 1y post-event (%)	4.9	4.7	3.3	4.4
Control LDL-C ESC 2016 1y post-event (%)	15.9	13.1	10.3	14.4

Conclusions: These real-world data show that LLT is not adequately adjusted for goals after an acute cardiovascular event, which may explain the low rate of patients with LDL-C at the therapeutic target recommended in the Guidelines. There is a need to optimize LLT in clinical practice in order to reduce ASCV and mortality.

SE125

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-16 Other*

CAROTID AND FEMORAL ARTERY PLAQUES AS PREDICTORS OF CARDIOVASCULAR AND ALL-CAUSE MORTALITY IN THE MIDDLE-AGED GENERAL POPULATION

SAAG SESSION 28: LATEST EVIDENCE FROM EPIDEMIOLOGY

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Background and Aims : To analyze the association of different markers of carotid and femoral artery plaques with all-cause and cardiovascular mortality in the middle-aged general population.

Methods: The sample of our study consisted of participants from the population-based cohort of the ESSE-RF, led in the Ivanovo region (1,102 people aged 40-67; median age 54 (48-60) years). The frequency of statin treatment was 11.2%. Carotid and femoral arteries were analyzed for plaque number (total number of plaques) and maximum stenosis using Samsung Medison MySono U6. The endpoints were cardiovascular death and all-cause death. Multivariate Cox regression adjusted for age and sex was used for statistical analysis.

Results: Carotid plaques were found in 73.1% of the men and 53.7% of the women, femoral plaques – in 50.6% and 25.5%, respectively. The follow-up period was 5.8±0.2 years. There were 29 endpoints. The presence of one carotid plaque increased the risk of cardiovascular and all-cause death by 1.4 times (HR=1.44, 95%CI 1.08–1.91, p=0.012 and HR=1.41, 95%CI 1.14–1.75, p=0.001, respectively), as well as one femoral plaque did (HR=1.37, 95%CI 1.07–1.74, p=0.011 and HR=1.38, 95%CI 1.15–1.65, p=0.001, respectively). Maximum carotid stenosis equal to 20% enhanced the risk of the endpoints by 2 times (HR(per 10%)=1.56, 95%CI 1.21–2.02, p=0.001 and HR=1.42, 95%CI 1.16–1.74, p=0.001, respectively), as maximum femoral stenosis did (HR=1.32, 95%CI 0.98–1.78, p=0.064 and HR=1.36, 95%CI 1.09–1.70, p=0.007, respectively).

Conclusions: Plaques in carotid and femoral arteries are approximately equal independent predictors of all-cause and cardiovascular mortality in the middle-aged general population.

SE126

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-02 Epidemiology of dyslipidemias*

POST-PRANDIAL ANALYSIS OF FLUCTUATIONS IN THE PLATELET COUNT AND PLATELET ACTIVITY IN PATIENTS WITH THE FAMILIAL CHYLOMICRONEMIA SYNDROME

SAAG SESSION 29: GENOTYPES AND PHENOTYPES IN DYSLIPIDEMIA

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Background and Aims : Background: Thrombocytopenia has been reported in patients with the familial chylomicronemia syndrome (FCS) due to complete lipoprotein lipase deficiency (LPLD), treated or not with volanesorsen, a second generation APOC3 anti-sense oligonucleotide. Chylomicrons are the lipoproteins delivering fat after a meal and therefore, FCS is a post-prandial disease. Platelet count and activity have not been studied post-prandially in FCS. **Objective:** To evaluate post-prandial fluctuations in the platelet count and platelet activity in relation to other hematologic parameters in FCS vs controls.

Methods: The platelet count (PLC), platelet activity and other hematologic variables on the complete blood count (CBC) were measured hourly, up-to 5 hours after a meal in 6 LPLD patients (FCS), 6 heterozygotes for LPLD causing mutations and 7 normolipidemic controls (wild-type LPL). Platelet activity was measured with occlusion time on a collagen matrix coupled with epinephrine.

Results: Compared to the other groups, the PLC significantly decreased 1-hour after a meal compared to baseline in FCS (p=0.03) whereas it tended to increase in normolipidemic controls (p=0.016). Platelet activity remained constant for all subjects, in all groups. In FCS, the post-prandial PLC correlated with post-prandial triglyceride level and lymphocyte count (p=0.037 and p=0.005, respectively).

Conclusions: The platelet count rapidly decreases post-prandially in FCS. Post-prandial fluctuations in the platelet count in FCS is not associated with changes in platelet activity but correlates with triglyceride level and the lymphocyte count.

SE127

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-05 Inherited dyslipidemias*

LOMITAPIDE EFFECTIVELY REDUCES TRIGLYCERIDE (TG) LEVELS IN FAMILIAL CHYLOMICRONEMIA SYNDROME (FCS)

SAAG SESSION 29: GENOTYPES AND PHENOTYPES IN DYSLIPIDEMIA

Angelo Baldassare Cefalù[1], Laura D'Erasmo[2], Antonina Giammanco[1], Anna Montali[2], Gabriella Iannuzzo[3], Alberto Zambon[4], Francesco Forte[3], Davide Noto[1], Patrizia Suppressa[5], Stefano Giannini[6], Marcello Arca[7], Maurizio Averna[8]
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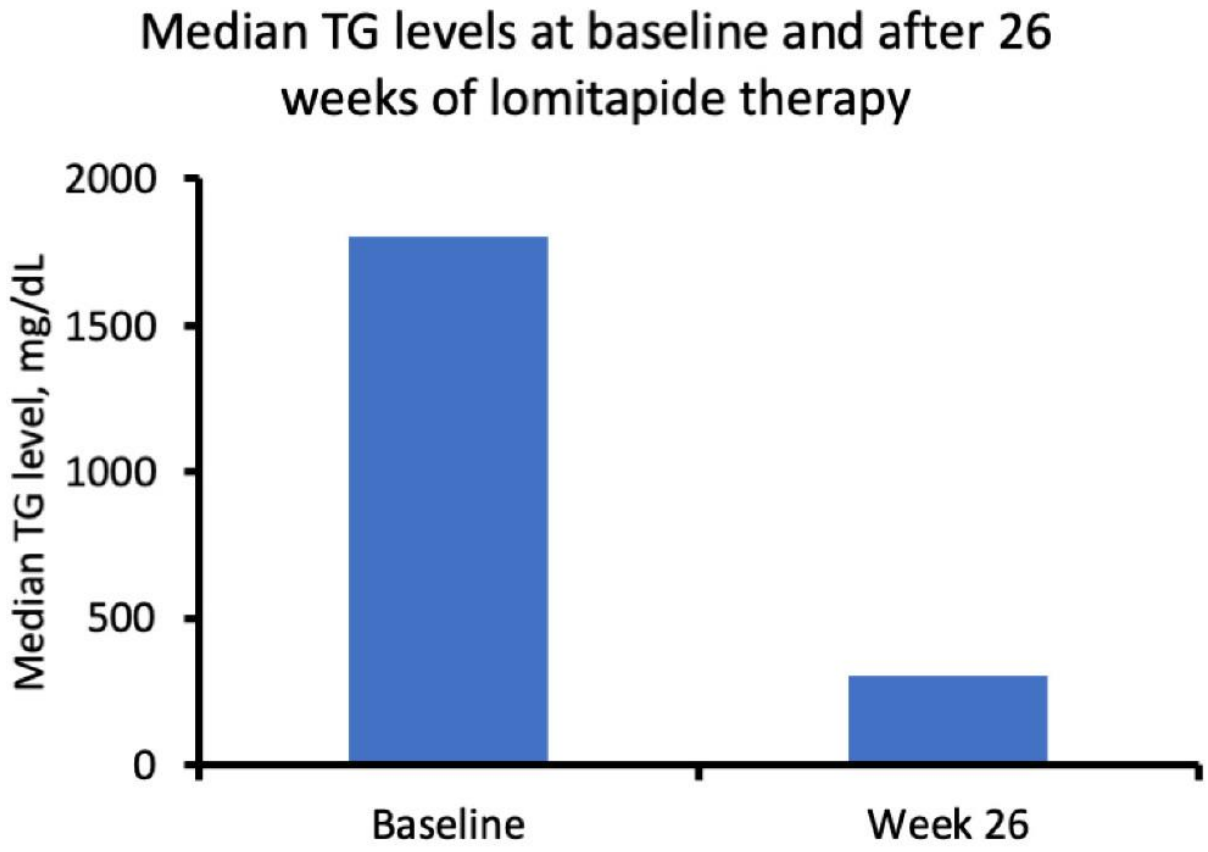
Background and Aims : FCS is a rare autosomal recessive disorder of impaired lipoprotein lipase (LPL) function, elevated TG levels, abdominal pain and pancreatitis. Current treatment (extremely low-fat diet <10% fat/day to reduce TG levels ≤750mgldL) is ineffective. Lomitapide is a microsomal triglyceride transfer protein (MTP) inhibitor that reduces circulating levels of TGs.

Methods: The open-label, single-arm 'LOCHNES' study of lomitapide in FCS, enrolled adult patients ≥18 years with genetically confirmed FCS, elevated fasting TG≥750mg/dL and a history of pancreatitis. Patients were administered escalating doses of lomitapide to maximum tolerated dose for 26 weeks. The primary endpoint was the percent change in TGs from baseline to Week 26.

Results: Eighteen patients were enrolled (mean±SD: age 46.6±16.7y; body mass index 23.7±4.1kg/m^2). Median baseline TG levels were 1804mg/dL (810–4151mg/dL). At Week 26, mean lomitapide dose was 32.8±17.8mg/day, and median fasting TGs reduced to 305mg/dL (70–1818mg/dL; 70.5% reduction; Figure); 13 patients achieved TGs ≤750mg/dL. Adverse events were mild-to-moderate and mainly related to gastrointestinal tolerability (n=10 and ALT/AST enzyme elevations ≥3x upper limit of normal (n=4), with no discontinuations. Median FIB-4 score increased 38.4% from baseline to 1.03 (p=0.054). Liver imaging (n=9) revealed increases in hepatic fat in 8 patients, but only three patients had an increase >20%. No patient experienced an episode of acute pancreatitis or severe abdominal pain during lomitapide

treatment.



Conclusions: Lomitapide is effective in reducing triglycerides in FCS and preventing recurrence of acute pancreatitis. Larger studies are warranted.

SE128

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-05 Inherited dyslipidemias*

CHYLOMICRON RETENTION DISEASE: A CASE REPORT IN GREECE

SAAG SESSION 29: GENOTYPES AND PHENOTYPES IN DYSLIPIDEMIA

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Background and Aims : Chylomicron retention disease (CRD) is a rare autosomal recessive disorder, in which intestinal fat malabsorption is the main cause of diverse severe manifestations. The specific molecular defect was identified in 2003 and consists of mutations in the SAR1B gene encoding for intracellular SAR1B GTPase protein which is involved in chylomicron transport from the endoplasmic reticulum (ER) to the Golgi apparatus.

Methods: 11 month's old girl admitted in the Pediatric Clinic of University Hospital of Irakleion-Crete PAGNI with growth delay, increased liver fuction tests, and occasioanally mucusy stools. Blood samples were collected from the proband and both parents. DNA Isolation, PCR, Cardiogena Panel-NGS (Illumina MiSeq) and bioinformatics analysis (Sophia Genetics DDM) were performed.

Results: The patient’s lipid profile was Chol 56mg/dl, HDL 18mg/dl, LDL 21,4mg/dl Trig 83mg/dl, apoA 67 mg/dl, apoB 33 mg/dl, Lp(a) 2.7 mg/dl. Parents’ lipid levels are normal. Genotyping showed homozygosity for the splice site mutation NM_016103.4(SAR1B):c.349-1G>C. This mutation activates a nearby cryptic splice site, production of an unspliced mRNA, disrupting the protein. The mutation is confirmed by Sanger.

Conclusions: Genetic testing confirmed chylomicron retention disease and the SAR1B:c.349-1G>C mutation was the first described in Greece. Hypocholesterolemic disorders should be suspected in infants with growth delay as early diagnosis is very challenging and not always suspected due to non-specific symptoms.

SE129

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-05 Inherited dyslipidemias*

GPR146 GENE VARIANTS ARE ASSOCIATED WITH REDUCED PLASMA LIPIDS AND CARDIOVASCULAR HEALTH: A NOVEL ROLE FOR GPR146 IN HYPOLIPIDEMIA

SAAG SESSION 29: GENOTYPES AND PHENOTYPES IN DYSLIPIDEMIA

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Background and Aims : In 2013, a common genetic variant in the *GPR146*-gene locus has been associated with increased cholesterol levels. A subsequent study in mice has shown that ablation of GPR146 causes hypolipidemia and protects against atherosclerosis independent of the LDL-receptor.

Methods: We made use of common (rs1997243 and rs2362529) and rare genetic variants (*GPR146*-p.Pro62Leu) in the *GPR146*-gene locus, as genetic research instruments in general population cohorts (UK-Biobank, Copenhagen City Heart study and Lifelines) and in cohorts of subjects with undefined extreme hypocholesterolemia, to study the role of GPR146 on cholesterol metabolism.

Results: We show that carriers of rs1997243, associated with increased gene expression of *GPR146*, present with increased LDL-C, apo-B, HDL-C and apoAI. In contrast, carriers of rs2362529, which is instead associated with a decreased GPR146 gene expression, present with the exact opposite phenotype with a reduction in risk of coronary artery disease. We further show that carriers of a rare variant (GPR146-p.Pro62Leu) present with markedly lower LDL-C and HDL-C compared to non-carriers. In a family with hypocholesterolemia, we finally show that the p.Pro62Leu variant cosegregates with low LDL-C.

Conclusions: The key novel finding in this study is that carriers of new genetic variants in *GPR146* are characterized by low levels of plasma lipids with a concordant reduction in risk of CAD. We also show that heterozygosity for a variant, predicted to be damaging, is associated with familial hypobetalipoproteinemia. The study supports a novel role for GPR146 in human hypolipidemia and the development of GPR146 antagonists to reduce LDL-C and decrease the risk of atherosclerosis.

SE130

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-05 Inherited dyslipidemias*

LABORATORY DIAGNOSIS OF SEVERE HYPERTRIGLYCERIDAEMIA. CASES FROM THE DYSLIPIDAEMIA REGRISTY OF THE SPANISH ATHEROSCLEROSIS SOCIETY

SAAG SESSION 29: GENOTYPES AND PHENOTYPES IN DYSLIPIDEMIA

María José Ariza[1], José Rioja[1], Ovidio Muñiz-Grijalvo[2], Teresa Arrobas Velilla[3], Emilio Ortega[4,5], Daniel Zambón[4,5], Mónica Domenech[4,5], Luis A. Álvarez-Sala[6], Javier Delgado-Lista[7], Aurora González-Estrada[2], Verónica Adriana Seidel[8], Francisco J. Fuentes[7], Ana Camacho[9], Manuel J. Romero[9], Miguel Ángel Sánchez-Chaparro[1,10], Pedro Valdivielso Felices[1,10]
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Background and Aims : Severe hypertriglyceridaemia (sHTG) increases the risk of cardiovascular disease and acute pancreatitis episodes. Patients with sHTG fit mainly into two clinical entities: Familial or Multifactorial Chylomicronemia Syndromes (FCS and MCS, respectively). FCS and MCS exhibit clinical differences but also separate genetic and biochemical characteristics that can be assessed in the laboratory. The aim of this work has been to implement a laboratory workflow to help diagnose sHTG patients with either FCS or MCS.

Methods: Patients with two fasting triglycerides >1000mg/dL determinations were sequenced with a capture probe panel of 24 triglycerides-related genes using massive parallel sequencing (n=200). Two-step sequential ultracentrifugation was performed (n= 159) to diagnose Type I hyperlipoproteinemia (HLP I) and post heparin lipoprotein lipase activity was measured to discard or confirm its deficiency (n=60).

Results: Most patients had MCS as they: (i) did not exhibit HLPI and/or (ii) their genetic profile was not compatible with FCS and (iii) were not deficient in LPL activity. FCS cases were identified as they had: (i) HLPI, and/or (ii) biallelic pathogenic variants in *LPL* (n=5), GPIHBP1 (n=3), or *LMF1* (n=2) genes and/or (iii) LPL activity deficiency. We identified 4 FCS patients with HLPI, biallelic pathogenic variants in *APOA5* but a rescued LPL activity. An additional study of Apo-AV functionality was designed to confirm the FCS diagnosis in these cases.

Conclusions: Laboratory studies, in patients with severe hypertriglyceridaemia, provide with information of clinical utility to distinguish between Familial and Multifactorial Chylomicronemia Syndromes.

SE131

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-01 Epidemiology of cardiovascular diseases and risk factors*

ELEVATED REMNANT CHOLESTEROL APPROPRIATELY RECLASSIFIES INDIVIDUALS WHO DEVELOP MYOCARDIAL INFARCTION

SAAG SESSION 30: SCORES, OMICS, AND IMAGING IN RISK STRATIFICATION

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Background and Aims : Elevated remnant cholesterol levels cause atherosclerotic cardiovascular disease. We tested the hypothesis that elevated remnant cholesterol will lead to appropriate reclassification of individuals who later develop atherosclerotic cardiovascular disease.

Methods: We followed 41,928 Danish individuals for more than 10 years from the Copenhagen General Population Study without a history of diabetes, coronary artery disease, or statin use. Using predefined cut points for elevated remnant cholesterol, we calculated net reclassification indices (NRI) from below to above 5%, 7.5%, or 10% separately and combined for absolute 10-year occurrence of myocardial infarction and major adverse cardiovascular events (MACE) defined as a composite of death from coronary artery disease, myocardial infarction and coronary revascularization.

Results: For individuals with remnant cholesterol levels ≥95th percentile (≥1.6 mmol/L, 61 mg/dL), 23% ($p<0.001$) of myocardial infarction events and 21% ($p<0.001$) of MACE were reclassified correctly from below to above 5% for 10-year occurrence when adding remnant cholesterol levels to models based on conventional risk factors, while no events were reclassified incorrectly for either endpoint. Consequently, addition of remnant cholesterol levels yielded NRI of respectively 10% (95% confidence interval 1–20%) for myocardial infarction and 5% (-3–13%) for MACE. Of note, when combining reclassification from below to above 5%, 7.5%, and 10% risk of events, 42% ($p<0.001$) of individuals with myocardial infarction and 41% ($p<0.001$) with MACE were reclassified appropriately, leading to NRI of respectively 20% (9–31%) and 11% (2–21%).

Conclusions: Elevated remnant cholesterol levels considerably improved myocardial infarction and MACE risk prediction.

SE132

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-08 Novel risk factors and biomarkers*

HIGH PLASMA LIPOPROTEIN LIPASE IS ASSOCIATED WITH A LOWER RISK FOR FUTURE MAJOR ADVERSE CARDIOVASCULAR EVENTS IN PATIENTS FOLLOWING CAROTID ENDARTERECTOMY.

SAAG SESSION 30: SCORES, OMICS, AND IMAGING IN RISK STRATIFICATION

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Background and Aims : Since carotid plaque intraplaque hemorrhage is associated with plaque progression leading to cardiovascular events, we hypothesized that blood proteins associated with carotid plaque IPH are also likely to be associated with MACE after CEA.

Methods: In 688 patients undergoing CEA of the Athero-Express biobank, we measured 276 proteins using three OLINK® proteomics immunoassays in preoperative blood samples. We analyzed the association of proteins with IPH using logistic regression analyses. In addition, we analyzed the association of candidate proteins with the three-year postoperative risk of MACE (including stroke, myocardial infarction, and cardiovascular death).

Results: A total of 130 patients (18,9%) developed MACE during the three-year follow-up. We found six proteins to be significantly associated with IPH, from which Lipoprotein Lipase was associated with the postoperative risk of MACE undergoing CEA. We analyzed the association of LPL with MACE within two different time periods, since the proportional hazard assumption for LPL was violated. High LPL was associated with an increased risk for 30-day MACE (adjusted HR per SD:1.60(1.10-2.30), *p*=0.014), while high LPL was associated with a lower risk for MACE within 30-days up and till 3-year (adjusted HR per SD:0.80(0.65-0.99), *p*=0.036).

Conclusions: Of the six proteins that were associated with IPH, only LPL was associated with the risk for MACE. High LPL was independently associated with an increased risk of MACE within the first 30-days after CEA, while high LPL was associated with a lower risk of MACE in the period 30 days up and till 3-years.

SE133

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-08 Novel risk factors and biomarkers*

UNRAVELING THE TRANSCRIPTIONAL DYNAMICS OF NASH PATHOGENESIS AFFECTING ATHEROSCLEROSIS DEVELOPMENT

SAAG SESSION 30: SCORES, OMICS, AND IMAGING IN RISK STRATIFICATION

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Background and Aims : The prevalence of non-alcoholic steatohepatitis (NASH) is rapidly increasing and associated with cardiovascular disease (CVD), the major cause of mortality in NASH patients. Although sharing common risk factors, the mechanisms by which NASH may directly contribute to the development to CVD remain poorly understood. The aim of this study was to gain insight into the dynamics of key molecular processes of NASH that drive atherosclerosis development.

Methods: A time-course study was performed in Ldlr-/-.Leiden mice fed a high-fat diet to induce NASH/atherosclerosis. The effects on NASH and atherosclerosis were assessed and transcriptome analysis was performed. Dynamical GENIE3 (dynGENIE3) was used for gene regulatory network inference using the differentially expressed genes from time series. Liver regulators were identified for each aorta target gene by extracting dynamics in the liver that precede dynamics in the aorta and were linked to atherosclerosis development.

Results: Ldlr-/-.Leiden mice developed obesity, hyperlipidemia, insulin resistance with steatosis and hepatic inflammation preceding atherosclerosis development. Transcriptome analysis revealed a time-dependent increase of pathways related to NASH and fibrosis followed by an increase in proatherogenic processes the aorta. DynGENIE3 identified specific liver regulators related to lipid metabolism (SC5D and TM7SF2), inflammation (IL1A and TNF) and fibrosis (PDGF) linked to a set of aorta target genes related to vascular inflammation (TNFA) and atherosclerosis signaling (VCAM1 and CCL2).

Conclusions: Time-resolved analyses of NASH and atherosclerosis in Ldlr-/-.Leiden reveal pathogenic liver processes precede atherosclerosis development. Hepatic key regulators and gene modules driving the atherogenic pathways and regulators in the aorta were identified.

SE134

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-08 Novel risk factors and biomarkers*

DOES A 6-MONTH CHANGE IN CIRCULATING BIOMARKERS IMPROVE THE PROGNOSTIC POWER OF BASELINE VALUES FOR PREDICTING CARDIAC MRI PATHOLOGIES IN PATIENTS WITH STEMI?

SAAG SESSION 30: SCORES, OMICS, AND IMAGING IN RISK STRATIFICATION

Franziska Holtkamp[1], Dimitri Gruen[1], Anna Frey[2], Valerie Jahns[2], Roland Jahns[2], Tobias Gassenmaier[2], Christian W. Hamm[1], Stephan Frantz[2], Till Keller[1], Roland Klingenberg[1]
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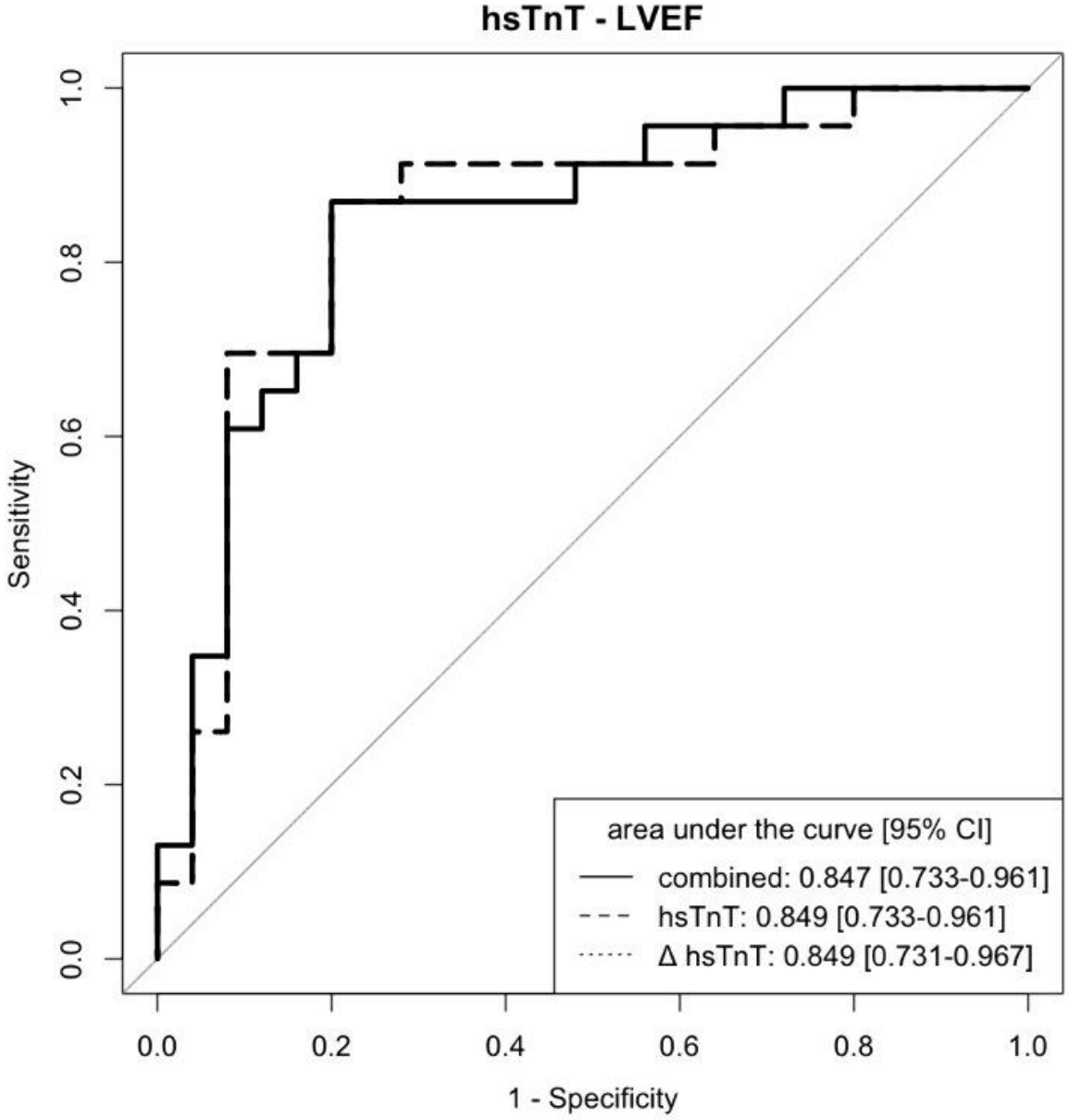
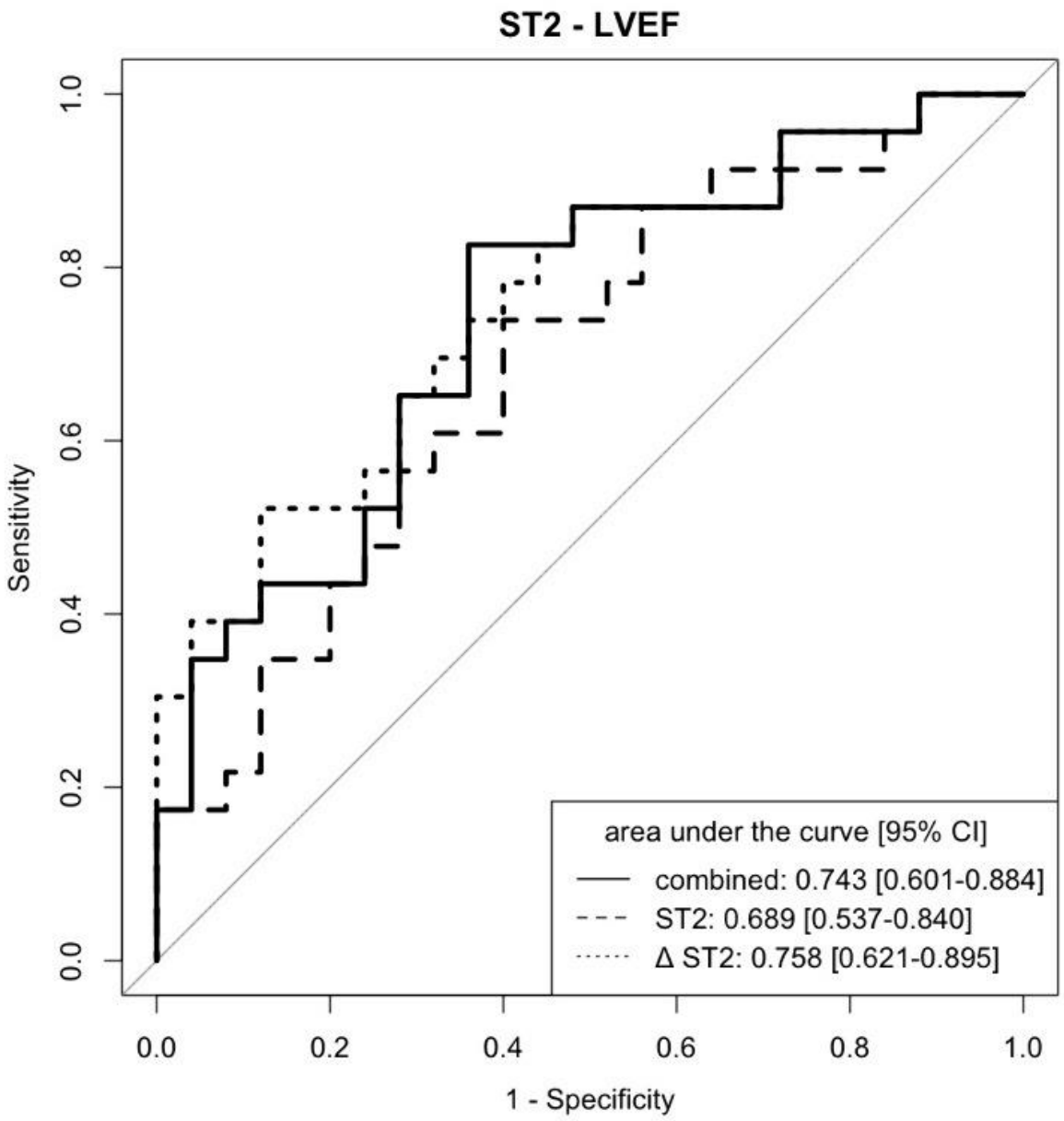
Background and Aims : To compare the predictive power of circulating biomarkers at baseline and their change over time for the development of structural changes typical of heart failure after STEMI.

Methods: Serial cardiac 3 Tesla MRI scans were performed to determine LVEF, LVESV, LVEDV, infarct mass, and relative infarct mass and were correlated with serial measurements of CCN1, ST2, hs-TnT, and NT-proBNP. The prognostic significance of these biomarkers was assessed through multiple logistic regression analysis by examining their performance in predicting dichotomized cardiac MRI values 12 months after STEMI based on their median. For each biomarker three models were created, using baseline (BL), Δ-value (BL-6 months), and both values together as predictors. All models were adjusted for age and MDRD-based eGFR.

Results: The logistic regression analysis, focused on LVEF, identified hsTnT and ST2 as significant and strong predictors of dichotomized cardiac MRI-parameters after 12 months (AUC >0.7). BL-measurement (hsTnT: AUC 0.849 [CI:0.733-0.961], ST2: AUC 0.689 [CI:0.537-0.840]) as well as the Δ-value BL-6M (hsTnT: AUC 0.849 [CI:0.731-0.967], ST2: AUC 0.758 [CI:0.621-0.895]) showed a high prognostic value without a statistically significant difference for the comparison of BL-model vs. Δ-value (BL-6M)-model for hsTnT (p=1) and ST2 (p=0.177). The combined model including baseline and Δ-value as predictors was not able to improve the ability to predict LVEF (hsTnT: AUC 0.847 [CI:0.733-0.961], p=0.918; ST2: AUC 0.743 [CI:0.601-0.884],
p=0.448).


hsTnT - LVEF
Sensitivity
1 - Specificity
0.0
0.2
0.4
0.6
0.8
1.0
area under the curve [95% CI]
combined: 0.847 [0.733-0.961]
hsTnT: 0.849 [0.733-0.961]
Δ hsTnT: 0.849 [0.731-0.967]



Conclusions: ST2 and hsTnT were identified as biomarkers that can predict LVEF 12 months after STEMI. Combining baseline biomarkers with their Δ-changes BL-6M yielded no advantage in prognostic value.

SE135

Topic: *ASA04 - CLINICAL VASCULAR DISEASE / ASA04-02 Endothelial dysfunction; Clinical assessment*

EARLY LIFE DETERMINANTS OF VASCULAR STRUCTURE IN FOETUSES, INFANTS, CHILDREN AND ADOLESCENTS: A SYSTEMATIC REVIEW AND META-ANALYSIS

SAAG SESSION 30: SCORES, OMICS, AND IMAGING IN RISK STRATIFICATION

Benjamin J. Varley[1], Reeja Nasir[2], Michael R. Skilton[2,3,4], Maria E. Craig[1,5,6], Megan L. Gow[1,5,6]
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Background and Aims : The first 1,000 days of life shape lifelong health, including cardiovascular disease (CVD) risk. Aortic intima-media thickness (aIMT), a measure of subclinical atherosclerosis, is an early indicator of CVD, amenable to change from as early as *in-utero*. We investigated the association between early life risk factors/exposures during the first 1,000 days and aIMT in youth.

Methods: MEDLINE, EMBASE, Scopus, CINAHL and AMED were searched from inception to July 2021. Eligible criteria: observational controlled studies in youth aged <20 years with risk factors/exposures during the first 1,000 days and aIMT measurement. Outcome data were pooled using random-effects meta-analysis. Meta-regression and moderator analysis were used to investigate potential confounders.

Results: 8,657 articles were identified and 34 included. Age ranged 22.9 weeks gestation *in-utero* to 10.9 years. In meta-analysis (n= 1,220 cases, n=1,977 controls), greater aIMT was associated with small for gestational age (SGA): 14 studies, mean difference (MD) 0.082mm, [95% CI 0.051, 0.112], p<.001, I^2=97%; intrauterine growth restriction (IUGR), 6 studies, 0.198mm [0.088, 0.309], p<.001, I^2=97.3%; preeclampsia: 2 studies, 0.038mm [0.024, 0.051], p<.001, I^2=38%, and large for gestational age (LGA): 3 studies, 0.089mm [0.043, 0.0136], p<.001, I^2=93%. In meta regression, older age (p<.001), maternal smoking (p=.040) and SGA (p<.001) were associated with greater difference in aIMT in pre-term participants vs controls.

Conclusions: Greater aIMT in youth with SGA, IUGR, preeclampsia or LGA is putatively consistent with increased risk for CVD later in life. Further research is required to determine whether intervention and preventative strategies reduce future CVD risk in this population.

SE136

Topic: *ASA04 - CLINICAL VASCULAR DISEASE / ASA04-03 NASH and other ectopic lipid diseases*

PROC3 DERIVED SCORES FOR LIVER FIBROSIS PREDICT CARDIOVASCULAR EVENTS IN NAFLD PATIENTS

SAAG SESSION 30: SCORES, OMICS, AND IMAGING IN RISK STRATIFICATION

Francesco Baratta[1], Daniele Pastori[2], Giulia Tozzi[3], Alessandra Colanotni[2], Nicholas Cocomello[2], Stefano Cecchi[3], Mattia Coronati[2], Francesco Angelico[2], Maria Del Ben[1]
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Background and Aims : Serum levels of PRO-C3 have been shown to have a good correlation with biopsy-proven liver fibrosis in patients with nonalcoholic fatty liver disease (NAFLD). PRO-C3 has been also included in some risk scores, namely ADAPT, FIB-C3 and ABC3D. Aim of the study was to investigate the association between serum PRO-C3 levels, scores including PRO-C3, and cardiovascular events (CVEs) incidence in the PLINIO study cohort.

Methods: Were included 663 PLINIO patients with US evidence of liver steatosis who complete at least 6 months of follow-up. PRO-C3 was dosed using a commercial elisa-kit. ADAPT, FIBC3 and ABC3D were calculated. Prospective data on CVEs incidence were collected.

Results: Median follow up length was 47.8 months yielding 2786.5 person-years of observation. During the follow-up, 41 patients (1.5% year) experienced CVEs. Patients who experienced CVEs were more frequently male (p=0.010), had higher prevalence of diabetes (p=0.006), metabolic syndrome (p=0.002) and prior CVEs (p<0.001). There was no difference in median PRO-C3 according to CVEs. Instead, we found a higher prevalence of impaired ADAPT (p=0.038), FIBC3 (p=0.015) and ABC3D (p<0.001). CVEs were associated with ABC3D>3 (HR: 2.29, p<0.05) in the whole population and with FIBC3 (HR: 1.40, p<0.05) and ABCD3 (HR: 1.40, p<0.05) in patients in primary prevention. The AUROC for the prediction

of CVEs was 0.65 [0.56-0.73] for both FIBC3 and ADAPT scores and 0.63 [0.54-0.73] for ABC3D.

Multivariate cox regression analysis in the whole population (Panel A) and in patients in primary prevention (Panel B)

Panel A.	CVEs aHR (95% C.I. for aHR)
ADAPT[2]	1.24 [0.93-1.64]
ADAPT>6.3287[2]	1,23 [0.42-3.63]
FIBC3[3]	1.22 [0.97-1.55]
FIBC3>0.4[3]	1.40 [0.66-2.98]
ABC3D[3]	1.24 [0.98-1.57]
ABC3D>3[3]	2.29 [1.17-4.47]*
Panel B.	**CVEs aHR (95% C.I. for aHR)**
ADAPT[2]	1.34 [0.91-1.99]
ADAPT>6.3287[2]	1,98 [0.46-8.54]
FIBC3[3]	1.40 [1.06-1.85]*
FIBC3>0.4[3]	1.22 [0.44-3.39]
ABC3D[3]	1.40 [1.06-1.84]*
ABC3D>3[3]	1.69 [0.69-4.18]

[1]Adjusted for age, sex, prior CVEs, smoking habits, BMI, diabetes, platelets; [2]Adjusted for sex, prior CVEs, smoking habits and BMI; [3]Adjusted for sex, prior CVEs, smoking habits; *p<0.05. CVEs: cardiovascular and cerebrovascular events; composite: CVEs + new onset atrial fibrillation: CHD: coronary heart disease; HR: Hazard ratio; aHR: adjusted HR; C.I: confidence interval. PRO-C3: N-terminal propeptide of type III collagen; ADAPT: age, of diabetes, PRO-C3, and platelet algorithm; FIBC3: FIBC3 diagnostic panel; ABC3D: age, BMI, platelet count, PROC3, diabetes derived score.

Conclusions: PROC3 levels didn't predict CVEs, differently from PROC3 derived scores. These scores both detect advanced liver fibrosis and predict CVEs, identifying a subgroup of NAFLD patients needing a multidisciplinary management.

SE137

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-09 Lipid and lipoprotein metabolism*

IDENTIFICATION OF A GAIN-OF-FUNCTION LIPC VARIANT AS A NOVEL CAUSE OF FAMILIAL COMBINED HYPOCHOLESTEROLEMIA

SAAG SESSION 31: CURRENT FINDINGS IN FAMILIAL DYSLIPIDEMIAS

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Background and Aims : Atherosclerotic cardiovascular disease is the main cause of mortality worldwide and is strongly influenced by plasma low-density lipoprotein cholesterol (LDL-C) levels. So far, only few genes with a strong causal relationship to plasma LDL-C levels have been identified. Here, we aimed to elucidate the genetic origin of an unexplained combined hypocholesterolemia inherited in four generations of a French family.

Methods: Using new generation sequencing, we identified a novel dominant rare variant in the *LIPC* gene, which encodes for hepatic lipase (HL), and that co-segregates with the phenotype.

Results: Nuclear magnetic resonance and lipidomics analysis revealed that family members carrying the LIPC-E97G variant had extremely low levels of LDL-C, high-density lipoprotein cholesterol (HDL-C), LDL particle numbers and phospholipids. The lysoPL/PL ratio was increased in plasma of LIPC-E97G carriers, suggestive of an increased lipolytic activity on phospholipids. *In vitro* and *in vivo* studies confirmed that the LIPC-E97G variant specifically increases the phospholipase activity of HL without altering TG lipase activity. Finally, APOE3-CETP Leiden mice overexpressing LIPC-E97G recapitulated the combined hypocholesterolemic phenotype of the family and demonstrated that the increased phospholipase activity promotes catabolism of triglyceride-rich lipoproteins by peripheral tissues, but not the liver.

Conclusions: In summary, we identified *LIPC* as the second gene causally involved in familial combined hypocholesterolemia. Our mechanistic data highlight the critical role of HL phospholipase activity in LDL-C homeostasis and suggest a new LDL clearance mechanism.

SE138

Topic: *ASA02 - LIPIDS AND LIPOPROTEINS / ASA02-13 Other*

GENETIC TESTING FOR FAMILIAL HYPERCHOLESTEROLEMIA USING LOW-COST HIGH-THROUGHPUT MICROARRAY-BASED GENOTYPING

SAAG SESSION 31: CURRENT FINDINGS IN FAMILIAL DYSLIPIDEMIAS

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Background and Aims : Familial hypercholesterolemia (FH) is an autosomal dominant disorder characterized by elevated low-density lipoprotein cholesterol (LDL-C) levels and premature cardiovascular disease (CVD). Current methods for genetically confirming FH are expensive and time-consuming. In this study we set out to validate a low cost, high throughput genotyping array, dubbed GOALL-GSA_v1, developed to detect known FH-causing single-nucleotide variants (SNVs), small indels and copy number variations (CNV) (~€30 per sample) in a cohort of genetically diagnosed FH patients.

Methods: The Illumina Global Screening Array (GSA) was customized to include probes for 899 proven FH causing variants in the *LDLR, APOB,* and *PCSK9* genes. The validation cohort consisted of randomly selected 1343 FH patients diagnosed through next-generation sequencing (NGS) or sanger sequencing methods. Variant detection was performed by using standard Illumina array processing by the Human Genotyping Facility (HuGe-F, Erasmus MC) and optimized with the Zcall algorithm for rare variant calling and PennCNV method for CNV calling.

Results: The customized GSA correctly diagnosed SNVs and small indels in 1199 of 1260 patients (sensitivity of 95%) with an 4.8% false-negative rate. In 32 patients, the array identified variants that were previously unobserved by NGS or sanger sequencing (2.5%). CNV analysis is awaited. Exclusion of patients with variants currently not present on the array, resulted in an 1.4% false-negative rate.

Conclusions: The customized GSA is a promising tool for genetically diagnosing FH at low costs and has the potential to greatly increase accessibility to genetic testing for FH. Continuous customization of GSA will further improve the diagnostic yield for FH.

SE139

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-05 Inherited dyslipidemias*

LDLR VARIANT CLASSIFICATION WITH CLINGEN FAMILIAL HYPERCHOLESTEROLEMIA VARIANT CURATION EXPERT PANEL SPECIFICATIONS

SAAG SESSION 31: CURRENT FINDINGS IN FAMILIAL DYSLIPIDEMIAS

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Background and Aims : Recently the ClinGen Familial Hypercholesterolemia variant curation expert panel (FH VCEP) thoroughly adapted the American College of Medical Genetics and Genomics standards for interpretation of sequence variants to the FH and *LDLR* context.

Methods: A total of 352 *LDLR* variants have already been classified following FH VCEP specifications, 82 by FH VCEP consensus curation and 270 by internal laboratory efforts, the latter with only publicly published or internal case data. ClinVar interpretations were extracted on 6/12/2021.

Results: *LDLR* variants spanned the whole gene and were 63% missense, 14% null (nonsense, frameshift and large deletions), 12% splicing, 6% in the 5'UTR, and 1-2% each in frame, synonymous and intronic. Variant classifications were 11 Benign (B), 15 Likely benign (LB), 99 Likely pathogenic (LP), 115 Pathogenic (P), 1 conflicting and 111 variants of uncertain significance (VUS). Classifications matched ClinVar interpretations for 212/345 variants. Concordance in classification increased with increasing ClinVar star status. Of the 125 unclassified variants in ClinVar (conflicting and VUS), 55 (44%) achieved a definite classification (P/LP or B/LB). Of the remaining 111 VUS, 16 would become LP with just 1 or 2 more index cases identified, 21 with just functional studies, and 34 with a combination of both.

Conclusions: A crucial step to accurate diagnosis for FH patients worldwide, is to correctly determine the potential disease-causing variants' pathogenicity. Our results show that, although the FH VCEP *LDLR*-specific guidelines improved ClinVar interpretations, case data and functional studies are still fundamental data in variant classification and sharing of this evidence should be encouraged.

SE140

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-05 Inherited dyslipidemias*

UNCOMMON PRESENTATION OF CHOLESTERYL ESTER STORAGE DISEASE (CESD): DESCRIPTION OF A CASE AND GENETIC CHARACTERIZATION BY NEXT GENERATION SEQUENCING

SAAG SESSION 31: CURRENT FINDINGS IN FAMILIAL DYSLIPIDEMIAS

Chiara Scrimali[1], Federica Brucato[1], Marina Caserta[2], Mariagrazia Gueli Alletti[1], Cinzia Castana[2], Rossella Spina[1], Valeria Ingrassia[1], Grazia Ida Altieri[1], Francesca Fayer[1], Davide Noto[1], Gabriella Misiano[1], Antonina Giammanco[1], Carlo M. Barbagallo[1], Antonina Ganci[1], Maria Piccione[3], Angelo Baldassare Cefalù[1], Maurizio Averna[4]
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Background and Aims : The LIPA gene encodes the lysosomal acidic lipase (LAL), an enzyme which hydrolyzes cholesterol esters (CE) and triglycerides (TG). Cholesteryl Ester Storage Disease (CESD) is a rare recessive disease caused by mutations in LIPA gene which result in residual LAL activity. Complete LAL deficiency is associated with a more severe form of disease known as Wolman's disease. Hyperlipidemia and liver steatosis are common clinical features of CESD.

Methods: The proband, a 2 years old child, was evaluated for microcephaly. Routine laboratory data showed total high cholesterol levels (243 mg/dL) and triglycerides (272 mg/dL). Next generation sequencing was carried out on an Ion GeneStudio S5 Plus System using the Ion 540 Chip. We designed a custom panel to analyze candidate genes related to LDL, HDL e triglycerides metabolism.

Results: No pathogenic mutations were identified in the major candidate genes for familial hypercholesterolemia and hypertrygliceridemia. However, the proband was found to be carrier of two mutations in LIPA gene (c.883C>T -p.His295Tyr- and c.929G>A - p.Trp310Ter). This result prompted to the assay of LAL activity by DBS Analysis. LAL activity was < 5% of the normal range. The His295Tyr variant is a pathogenic missense mutation associated with CESD, while the Trp310Ter variant has been previously identified in homozygosity in two newborns of Sicilian origin with Wolman's disease. The family cascade screening revealed the presence of His295Tyr mutation in the proband's father and the Trp310Ter in the proband's mother.

Conclusions: We report a case of CESD with uncommon clinical presentation features compound heterozygous for two mutations in LIPA gene.

SE141

Topic: *ASA03 - DYSLIPIDEMIA AND RISK FACTORS / ASA03-13 Genomics, GWAS and population genetics; Mendelian randomization*

LDL CHOLESTEROL POLYGENIC SCORE DOES NOT REFLECT POLYGENIC FAMILIAL HYPERCHOLESTEROLEMIA IN ARABS

SAAG SESSION 31: CURRENT FINDINGS IN FAMILIAL DYSLIPIDEMIAS

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Background and Aims : Familial hypercholesterolemia (FH) is a monogenic disease but can also present as a polygenic disease due to small-effect LDL-C-raising alleles of 12 SNPs from The Global Lipid Genetic Consortium GEWAS study. Here to determine polygenic score in FH patients, assess the utility of 6-SNP polygenic score or 12-SNPs polygenic score in identification of polygenic FH.

Methods: We studied total of 93 FH patients and control subjects and genotyped all 12 LDL-C determining SNPs using real time PCR. The polygenic score was determined by the mean effect of each variant.

Results: We identified strong correlation (r= 0.90) between the 12-SNPs and the 6-SNPs polygenic score. The polygenic score of FH with pathogenic LDLR mutation group (FH/M+) (0.98+/-0.13) was not significantly different to the control group (0.97 +/- 0.23). similarly, no difference was observed in the FH patients with no mutation (FH/M-) group (1.02 +/- 0.11) compared to the control group. Also, no differences observed in the polygenic score between FH/M- group and FH/M+ groups.

Conclusions: The use of 6 polygenic score provided cost-effective alternative than the 12 SNP score in determination of polygenic score. Also, the reported polygenic LDL-C score failed to differentiate between polygenic familial hypercholesterolemia and monogenic forms in the Omani Arab population. Future studies are required to establish LDL-C polygenic score on Arabic population.