Suomen ateroskleroosiyhdistys ry, SATY ry
12th Symposium and Annual meeting
15-16th of March, 2018
Porthania III, Yliopistonkatu 3, Helsinki

PROGRAM

Thursday March 15, 2018

13.00 Opening words: Vesa Olkkonen

SESSION I.

Chairpersons Pirkko Pussinen and Minna Kaikkonen-Määttä

13.05-13.50 Juha Sinisalo, Heart and Lung Center, Helsinki University Hospital and Helsinki University: *Acute diagnostics and care of MI patients at HUCS*

13.50-14.10 Matti Jauhiainen, Minerva Foundation Institute for Medical Research: *USF1 silencing triggers beneficial anti-atherogenic effects in mouse peritoneal and human THP-1 macrophages*

14.10-14.30 Anna Alexanova, Faculty of Medicine and Life Sciences, University of Tampere: *Modeling lipid metabolism of coronary artery disease patients with induced pluripotent stem cell derived hepatocytes*

14.30-14.50 Mostafa Kiamehr, Faculty of Medicine and Life Sciences, University of Tampere: *hiPSC-derived hepatocytes closely mimic the lipid profile of primary hepatocytes - Future personalised cell model for studying lipid metabolism of the liver*

14.50-15.00 Presentation by Raisio Group: Prof. Ingmar Wester

15.00-15.30 Coffee

15.30-15.50 Jaakko Leskelä, Oral and Maxillofacial Diseases, University of Helsinki and Helsinki University Hospital: *Genome-wide association study of endotoxemia*

15.50-16.10 Milla Pietiäinen, Oral and Maxillofacial Diseases, University of Helsinki: *Aggregatibacter actinomycetemcomitans serotypes associate with periodontal and coronary artery disease status*

16.10-16.30 Jonna Weisell, School of Pharmacy, University of Eastern Finland, Kuopio: *Effect of Vitamin K_2 on Development of Aortic Valve Calcification in Hypercholesterolemic mice*

16.30-16.50 Laura Lahdentausta, Oral and Maxillofacial Diseases, University of Helsinki: *Associations of cardiometabolic medications with cardiovascular diseases and related serum biomarkers*

16.50-17.10 Maykel López Rodríguez, Institute of Clinical Medicine, Internal Medicine, University of Eastern Finland, Kuopio: *Regulation of lactate levels in human by a common functional variant at the glucokinase regulatory protein (GCKR) gene*

17.10-17.30 Saara Laitinen, Finnish Red Cross, Blood Service: *Research and Development activities in FRC Blood Service: Biobanking for future*

17.30-17.40 Presentation by Amgen AB

18.00-19.00 Annual assembly of the society

19.00- Dinner at Unicafe Porthania

Friday March 16, 2018

SESSION II.

Chairpersons Katariina Öörni and Tuire Salonurmi

9.00-9.45 Hanna Savolainen-Peltonen, University of Helsinki and Helsinki University Hospital, Obstetrics and Gynecology, HUS: *Menopause, hormone replacement therapy, and CVD*

9.45-10.05 Maija Ruuth, Wihuri Research Institute, Helsinki: *Susceptibility of LDL particles to aggregate is reduced by PCSK9 inhibitor and by healthy diet*

10.05-10.25 Veera Karkamo, Finnish Food Safety Authority Evira, Veterinary Pathology, Helsinki: *Atherosclerosis in two Korat cats*

10.25-10.45 Minna Holopainen, Finnish Red Cross Blood Service and Department of Biosciences, University of Helsinki: *Mesenchymal stromal cells and their extracellular vesicles enhance the anti-inflammatory phenotype of regulatory macrophages by down-regulating the production of IL-23 and IL-22*

10.45-11.20 Coffee

11.20-11.40 Anu Toropainen, A. I. Virtanen Institute, University of Eastern Finland, Kuopio: *Role of CAD-associated genetic variation in the regulation of cell-type specific enhancer activity*

11.40-12.00 Eija Nissilä, Department of Bacteriology and Immunology, Haartman Institute, and Research Programs Unit, Immunobiology, University of Helsinki: *Complement Factor H downregulates complement mediated inflammation in human THP-1 macrophages and monocytes*

12.00-12.20 Suvi Kuosmanen, A.I. Virtanen Institute for Molecular Sciences, University of Eastern Finland: *MicroRNAs Mediate the Aging-Associated Decline of NRF2 in Endothelial Cells*

12.20-12.40 Su Nguyen, Wihuri Research Institute, Helsinki: *VEGF-B gene therapy decreases macrophage uptake of modified LDL, foam cell formation, and atherosclerosis*

12.40-13.00 Hanna Ruhanen, Minerva Foundation Instutute for Medical Research and University of Helsinki, Molecular and Integrative Biosciences: *ANGPTL3 depletion alters lipid profile and metabolism in human hepatocytes*

13.00 Closing of the symposium: Vesa Olkkonen

ABSTRACTS

USF1 silencing triggers beneficial anti-atherogenic effects in mouse peritoneal and human THP-1 macrophages

Maija Ruuth[1], Jarkko Soronen[2], Essi Kaiharju[3], Krista Merikanto[3], Julia Perttilä[2], Jari Metso[2], Miriam Lee-Rueckert[1], Marja-Riitta Taskinen[4], Petri T. Kovanen[1], Katariina Öörni[1], Vesa M. Olkkonen[2], Pirkka-Pekka Laurila[3,5], Matti Jauhiainen[2,3]

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Aim - The upstream stimulatory factor 1 (USF1) was originally associated with familial combined hyperlipidemia. We recently showed that inactivation of USF1 in mice protects against obesity, insulin resistance, and cardiovascular disease. Even when fed a high-fat diet, USF1-deficient mice stayed lean and maintained a beneficial serum lipid profile with low triglycerides and elevated high-density lipoprotein (HDL) cholesterol (*Laurila et al. Science Transl Med 2016*). Here the aim was to study the impact of USF1 silencing on HDL functionality in macrophages, focusing on cholesterol efflux, cholesterol uptake and inflammation.

Methods - *USF1* was silenced in THP-1 macrophages using lentiviral preparations containing either the control shRNA expression vector (MISSION® pLKO.1-puro Non-Target shRNA) or the USF1 silencing shRNA expression vector in. Cholesterol efflux and acetyl-LDL uptake were measured in THP-1 macrophages. Cytokines were analyzed with ELISA methods. Gene expression microarray was performed in peritoneal macrophages from USF1-deficient mouse.

Results - Usf1 deficiency improved the function of HDL. HDL particles derived from Usf1-deficient mice removed cholesterol from macrophages more efficiently than HDL of wild-type mice, which was attributed to a higher phospholipid/APOA1 mass ratio in Usf1 knock-out mice HDL particles. We achieved 79 % silencing of *USF1* in THP-1 macrophages using shRNA, and observed significant increase in cholesterol efflux capacity from these cells that was associated with increased *ABCA1* mRNA levels. We further conducted genome-wide expression array analysis of peritoneal macrophages isolated from Usf1+/+ and Usf1-/- mice. These results demonstrated that in the Usf1-/- macrophages the expression of Lipa was upregulated by 63%, Acat1 by 23%, and Nceh1 by 41%. In human THP-1 macrophages similar increases in mRNA expression upon USF1 silencing were observed for both LIPA and NCEH1. Furthermore, secretion of pro-inflammatory cytokines MCP-1 and IL-1β was significantly reduced in USF1-silenced THP-1 macrophages when compared to control cells, demonstrating attenuated inflammatory burden in USF1 deficient macrophages. Lack of USF1 protected against LPS-induced macrophage cholesterol uptake and was associated with a reduced *SR-A1* mRNA level. Usf1 deficiency in mouse induced upregulation of macrophage genes involved in fatty acid β-oxidation and mitochondrial oxidative phosphorylation thus resembling the transcriptome of the brown adipose tissue of USF1-deficient mice.

Conclusions - Our findings identify USF1 as a novel factor regulating HDL functionality, demonstrating that USF1 deficiency improves the functional quality of HDL particles, enhances cholesterol efflux, attenuates cholesterol accumulation, and reduces macrophage inflammation. Improved cholesterol flux through macrophages and alleviated inflammation are associated with the anti-atherogenic function of USF1 deficiency.

MODELING LIPID METABOLISM OF CORONARY ARTERY DISEASE PATIENTS WITH INDUCED PLURIPOTENT STEM CELL DERIVED HEPATOCYTES

Alexanova A[1], Viiri L[1], Kiamehr M[1], Aalto-Setälä K[1,2],

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Coronary artery disease (CAD) is the most common cause of death in Finland. Atherosclerosis that is causing CAD develops over a period of decades and leads to accumulation of fatty substances, plaques, in the arterial wall. The plaques obstruct arteries gradually and some of the plaques are fragile and can cause myocardial infarction and sudden death if they rupture. However, some of the patients never experience rupturing of the plaque, but identifying the patients with vulnerable plaques, and thus those with increased risk for myocardial infarction, is challenging. It is widely known that lipids are involved in atherogenesis, and presence of some lipids in blood has been linked to increased risk of myocardial infarction. Because liver has an important role in lipid production, modeling its lipid metabolism can provide novel and valuable information on mechanisms leading to CAD and myocardial infarction. Production of induced pluripotent stem (iPS) cells from skin fibroblasts and differentiating iPS cells into hepatocytes provides a source of patient specific hepatocytes without invasive liver biopsy.

The aim of this project is to utilize stem cell technology to produce hepatocytes from CAD patients and apply them to model inherited traits of lipid metabolism. The objective is to use cell lines derived from patients with either acute or stable CAD, as well as healthy controls. This hepatocyte model will be studied comprehensively by applying "omics" technologies: lipidomics, proteomics and miRNA microarrays. For this project we have differentiated hepatocytes from 27 iPS cell lines of 15 patients in total. The preliminary results indicate that there are differences between the patient groups at least in miRNA levels.

Finding the mechanisms of lipid metabolism that predispose towards CAD can lead to identification of new biomarkers, which would improve diagnostics of CAD and finding the patients predisposed to acute coronary events.

hiPSC-derived hepatocytes closely mimic the lipid profile of primary hepatocytes - Future personalised cell model for studying lipid metabolism of the liver

Mostafa Kiamehr[1*], Anna Alexanova[1], Leena E. Viiri[1], Laura Heiskanen[2], Terhi Vihervaara[2], Dimple Kauhanen[2], Kim Ekroos[5], Reijo Laaksonen[1,2], Reijo Käkelä[3] & Katriina Aalto-Setälä[1,4]

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Hepatocyte-like cells (HLCs) differentiated from human induced pluripotent stem cells (hiPSCs) offer an alternative platform to primary human hepatocytes (PHHs) for studying lipid metabolism of the liver. However, despite their great potential, HLCs' lipid profile has not yet been characterised. Here, we comprehensively studied the lipid profile and fatty acid (FA) metabolism in HLCs and compared those to the current standard hepatocyte models: HepG2 cells, PHHs, as well as human liver tissue. We differentiated HLCs by five commonly used methods from three cell lines and thoroughly characterised them by gene and protein expression. HLCs generated by each method were assessed for their functionality and the ability to metabolise long- and very long-chain saturated and unsaturated FAs. In the next stage, the lipid profile of HLCs was investigated by both mass spectrometry and gas chromatography, and was compared to PHHs and HepG2 cells. Our results showed that HLCs closely mimic the functionality, lipid profile and FA metabolism of PHHs. HLCs also revealed to be superior to HepG2 cells in terms of their FA metabolism and lysophospholipid content. Furthermore, HLCs were able to utilise medium-derived FAs and modify simple lipids into more complex ones according to their needs. Additionally, we propose that increasing PUFA supply of the culture medium may positively affect the lipid profile and functionality of HLCs. In conclusion, our data showed that HLCs provide a functional and relevant model to investigate human lipid homeostasis at both molecular and cellular levels.

Keywords: Human induced pluripotent stem cell, hiPSC; hepatocyte-like cell, HLC; primary human hepatocyte, PHH; HepG2 cell; fatty acid, FA; lipidomics; mass spectrometry; gas capohromatography.

Genome-wide association study of endotoxemia

Toppila I[1, 2, 3]*, Leskelä J[4]*, Härma AM[1, 2, 3]*, Kopra KAE[4], Palviainen T[8], Salminen A[4], Sandholm N[1, 2, 3], Pietiäinen M[4], Salomaa V[6], Kaprio J[7, 8], Groop PH[1, 2, 3], Perola M[5], Lehto M[1, 2, 3], Pussinen PJ[4]

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Lipopolysaccharide (LPS) is a virulence factor of gram-negative bacteria. The translocation of LPS into circulation results in endotoxemia, which is associated with both acute and chronic inflammatory conditions. We performed genome-wide association study (GWAS) of endotoxemia measured by Limulus Amebocyte lysate (LAL)-assay, in 11,296 individuals with Finnish ancestry.

We identified five loci with 740 SNPs reaching a genome-wide significant association with the measured endotoxemia. Four out of five of the hits were located next to genes affecting the contact activation cascade (rs5030082 near *KNG1*, $p=2.95\times10^{-19}$; rs71640036 near *KLKB1* and *F11;* $p=5.41\times10^{-78}$; rs1801020 near *F12*, $p=6.62\times10^{-65}$; and rs2081361 near *SERPING1*, $p=4.37\times10^{-9}$). The fifth loci was located next to gene *LIPC* (rs10152355, $p=2.51\times10^{-9}$) affecting lipoprotein metabolism. The SNP associations were independent of each other. Clinical parameters explained 27.8-50.8% of the LPS variability and adding the five lead SNPs in the model increased the explanatory rate by 1.5-9.2%. Adding the genotype information of the four SNPs affecting contact activation cascade in regression models for BMI and incident CVD event increased the estimates and strengthened their association with endotoxemia.

Our results suggest that the biological activity of LPS in the circulation, i.e. endotoxemia, has a relatively small but highly significant genetic component. Endotoxemia seems to be associated with genetic variation in contact activation pathway and lipoprotein metabolism, which all play a role in host defense and LPS neutralization. Whether the observed associations are due to interference within the LAL-assay or a direct biological effect, remains to be solved.

Aggregatibacter actinomycetemcomitans serotypes associate with periodontal and coronary artery disease status

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Background: Periodontitis is a local infection and inflammation of tooth-supporting tissues leading to attachment loss of affected teeth. It contributes also to the increased risk for coronary artery disease (CAD). *Aggregatibacter actinomycetemcomitans* is one of the few periodontal pathogens, whose presence in the oral cavity has been linked to CAD. Seven serotypes ("a"—"g") of *A. actinomycetemcomitans* have been identified up to date based on the structural characteristics of O-antigen of lipopolysaccharide (LPS). Several studies support the idea that distinct strains and serotypes of *A. actinomycetemcomitans* have differences in their potential to cause periodontitis, but the association of different *A. actinomycetemcomitans* serotypes with the increased risk for systemic diseases, such as CVD, is rarely studied. We investigated the association of *A. actinomycetemcomitans* serotypes in saliva with periodontal and CAD status.

Methods: The study population included 497 patients of the Parogene study who underwent both coronary angiography and extensive clinical oral examination. We designed quantitative polymerase chain reaction (qPCR) assays to distinguish different *A. actinomycetemcomitans* serotypes from saliva samples. The patients were selected for serotyping if their salivary or subgingival sample were found *A. actinomycetemcomitans* positive in our previous studies.

Results: Serotype was identified from 16.5% of the *A. actinomycetemcomitans* positive samples. Serotype "c" was the most frequent (35.7%) followed by serotypes "b" (28.6%), "a" (26.2%), "e" (7.1%) and "d" (2.4%). None of the patients had serotype "f". The subjects with any detectable serotype had less teeth and more bleeding of gums than those with no serotype. Serotypes "b" and "c" associated with deepened periodontal pockets.

The bacterium quantities and serum antibody levels against *A. actinomycetemcomitans* were highest in patients carrying serotype "c". Serotypes "b" and "c" were most frequently found (59.3%) from CAD patients (p=0.040) and they associated with the risk of stable CAD with an odds ratio of 2.67 (1.06-7.44). Also, the severity of CAD, i.e. the number of stenosed arteries, associated with serotypes "b" and "c" (p=0.018).

Conclusions: *A. actinomycetemcomitans* serotypes "b" and "c" associate both with periodontal status and with an increased risk of having CAD. Detectable serotypes associate with the quantity and the serology of the bacterium emphasizing both local and systemic effect of the *A. actinomycetemcomitans* serotypes. Our findings emphasize the importance of recognizing the great variance in virulence among the same pathogenic species.

Effect of Vitamin K_2 on Development of Aortic Valve Calcification in Hypercholesterolemic mice

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Background: Calcific aortic valve disease (CAVD) is a progressive disease that ranges from mild valve thickening to calcification that leads to aortic valve obstruction. Disease process initiates from endothelial damage of the aortic valve followed by accumulation of oxidized LDL and inflammatory cells into the valve, and extra cellular matrix remodeling. As disease progresses, chondrocytes, cartilage, and endochondral calcification may also be present in the calcified valves. Currently, no pharmacologic treatment is available for CAVD. Vitamin K dependent Matrix Gla protein (MGP) and Gla-rich protein (GRP) are known to inhibit vascular calcification in mice and recently, in the small interventional proof of concept study in patients with asymptomatic or mildly symptomatic aortic valve calcification, vitamin K attenuated the progression of CAVD. Thus, vitamin K could be potential for CAVD treatment.
Purpose: Study the effect of vitamin K_2 on valvular calcification in the hypercholesterolemic mouse model of CAVD.
Methods: $LDLr^{-/-}ApoB^{100/100}$ mice were divided in two groups. Mice received western diet (WD) for 5 months to induce CAVD (n=10), study group with added 0.2 mg/g vitamin K_2 (n=10). At the end of the study, echocardiography was performed. Valvular samples were stained with Haematoxylin-eosin (HE) and MAC-3 for macrophages, to analyse aortic valve and atheroma plaque area and morphology.
Results: Preliminary results of histopathological analysis did not indicate differences in the atheroma plaque area, cusp area or in the stenosis between the control and vitamin K_2 group. There was no difference in total amount of macrophages in the atheroma or aortic valve area among the groups. Additionally, in the echocardiography, stroke volume was increased significantly in mice treated with vitamin K_2 when compared to control group (44.7% vs. 36.2%, $p<0.05$). Body weight gain was equal in both study groups.
Conclusions: Although vitamin K_2 have no effect on aortic stenosis or valvular area in the hypercholesterolemic mouse, further analysis is needed to investigate its effect on valvular and atheroma plaque morphology.

Associations of cardiometabolic medications with cardiovascular diseases and related serum biomarkers

Laura Lahdentausta, Susanna Paju, Kåre Buhlin, Päivi Mäntylä, Taina Tervahartiala, Markku S Nieminen, Juha Sinisalo, Timo Sorsa, Pirkko J Pussinen

Background: We investigated the effects of cardiometabolic medications on cardiovascular disease -related serum biomarkers, matrix metalloproteinase (MMP)-8, MMP-9, tissue inhibitor of metalloproteinase (TIMP)-1 and myeloperoxidase (MPO).

Methods: Serum samples were collected during hospitalization and coronary angiography in the Parogene study (N=481), a random cohort of patients who underwent coronary angiography and oral examination. Serum MMP-8, MMP-9, TIMP-1, and MPO concentrations were measured with immunoassays. Medications used before and prescribed during hospitalization were registered. The confounders in the logistic and Cox regression models were age, gender, coronary artery disease status, and diabetes. In multinomial logistic regression no CAD group was set as reference category. Patients were followed-up on average for 6.8 years (median 2469 days, IQR 119 days) by using registries.

Results: The number of cardiometabolic medications used before hospitalization associated with stable CAD and ACS, ORs (95% CI) being 1.30 (1.14-1.47, p<0.001), and ACS 0.72 (0.63-0.82, p<0.001).

Serum MMP-8, MMP-9, TIMP-1, and MPO associated significantly with ACS in multivariate multinomial models, 6.36 (3.52-11.47, p<0.001), 2.07 (1.08-3.97, p=0.029), 0.037 (0.005-0.28, p=0.001), and 5.80 (2.24-15.0, p<0.001), respectively. The further adjustment with number of medications used before hospitalization did not affect these associations. Serum TIMP-1 provided an OR 0.073 (0.01-0.55, p=0.011) for stable CAD and further adjustment with number of medications used before hospitalization improved the odds to 0.043 (0.005-0.39, p=0.005). Serum MMP-8, MMP-9, or MPO did not associate with stable CAD.

Serum MMP-8 (28.1 vs. 60.7 ng/ml, p<0.001), MMP-9 (148.7 vs. 188.3 ng/ml, p=0.02), and MPO (294.1 vs. 361.7 ng/ml, p<0.001) concentrations were significantly lower in statin users compared to no-users.

The number of cardiometabolic medications prescribed during hospitalization associated with cardiovascular death with an HR 1.42 (1.07-1.89, p=0.015). The statins protected from cardiovascular death in the follow-up with HR 0.30 (0.12-0.73, p=0.008).

Serum MMP-8 and TIMP-1 associated with death (due to any reason) with HRs 1.85 (1.01-3.38, p=0.05) and 100.9 (13.4-763.0, p<0.001), respectively. The corresponding HRs for cardiovascular death were 2.18 (1.02-4.67, p=0.05) and 204.8 (17.5-2394.2, p<0.001). Further adjustment with statins used during hospitalization increased the HR of serum MMP-8 to 2.34 (1.11-4.95, p=0.026).

Conclusions: Serum biomarkers associated with CAD and CAD outcome. Serum biomarkers, especially MMP-8 and TIMP-1, have prognostic value and adjustment with statins strengthened the association of MMP-8 with CVD death. Statins may have a direct effect on these biomarkers.

Regulation of lactate levels in human by a common functional variant at the glucokinase regulatory protein (*GCKR*) gene.

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The glucokinase regulatory protein (GKRP) regulates hepatic glucose metabolism by inhibiting glucokinase (GCK) in the nucleus of the hepatocyte. The functional variant rs780094 at the glucokinase regulatory protein gene (*GCKR*) associates with a large group of metabolic traits and the risk for Type 2 Diabetes (T2D), non-alcoholic fatty liver disease (NAFLD), insulin resistance and cardiovascular diseases (CAD). rs780094 lies in an intragenic enhancer that regulates *GCKR* expression in a haplotype specific way. We hypothesized that a differential expression of *GCKR* may explain the genetic associations at this locus. Given the direct relationship between hepatic metabolism of lactate and glucose, we investigated the association for rs780094 with lactate in an oral glucose tolerance test (OGTT) in two subsets of participants in the cross sectional Metabolic Syndrome in Men (METSIM) study in Kuopio, Finland. Additionally, we investigated the effect of increasing GKRP in the production of lactate and GCK availability in HepG2 cells and human primary hepatocytes (HPH) to model the physiological effect of the rs780094-enhancer. Our results showed that rs780094 exhibited a glucose dependent association with lactate levels in humans. The rs780094-CC genotype associates with lower lactate levels at fasting but higher increase in lactate levels upon glucose stimulation. Additionally, the found that increasing expression of *GCKR* induces higher lactate production in the liver cellular models. In conclusion, our results provide a mechanistic explanation for the genetic associations at *GCKR*, including the risk for T2D, NAFLD and CAD, which involve differential *GCKR* expression between rs780094 genotypes in the fasting state.

Susceptibility of LDL particles to aggregate is reduced by PCSK9 inhibitor and by healthy diet

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Aims
We have previously shown that inter-individual differences in the intrinsic propensity of LDL particles to aggregate upon modification associate with coronary death and that aggregation-prone LDL particles are enriched in sphingolipids. Here we examined whether in humans PCSK9 inhibition or dietary changes, both of which can influence LDL lipidome, would alter the susceptibility of LDL to aggregate.

Methods
Plasma samples were derived from EQUATOR study, a randomized placebo-controlled phase II trial with a fully human monoclonal anti-PCSK9 antibody RG7652. LDL was isolated before and after treatment with RG7652 (n=25) or placebo (n=15) for 29 days. LDL was isolated also from healthy subjects participating in the SYSDIET study, in which they were randomly assigned to a Healthy Nordic diet (n=33) or a Control diet (n=24) for 18-24 weeks. Aggregation of LDL was triggered by lipolysis and followed by dynamic light scattering. LDL lipid composition was analysed by mass spectrometry.

Results
Lipidomic analysis of LDL particles revealed that PCSK9 inhibitor decreased the proportion of sphingolipids and increased the proportion of phosphatidylcholines in LDL particles and rendered them less aggregation-susceptible. Increased consumption of vitamin E and decreased consumption of sucrose in the Healthy Nordic diet were associated with similar changes in LDL lipidome and the aggregation susceptibility of LDL. Placebo-treatment or control diet had only minor effects on LDL lipid composition and they did not alter the aggregation-susceptibility of LDL particles.

Conclusions
PCSK9 inhibition and healthy diet reduce LDL-sphingomyelin and LDL aggregation susceptibility, which may partially explain the anti-atherosclerotic effects of these interventions.

Atherosclerosis in two Korat cats

Veera Karkamo[1], Karoliina Hagner[2], Anna Knuuttila[2], Niina Airas[2], Ilona Kareinen[2,]

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Introduction

In most animal species, atherosclerosis is of little clinical relevance. Above all, it is interesting because of possibility for developing animal models of the human disease. Atherosclerosis in animals is typically morphologically different from human disease as lipids in the arteriolar walls is present not in intima, but mainly in media and adventitia.

The Korat cat breed originates from Thailand and has remained isolated from other cat breeds since the 1960's. The Finnish Korat population originates from only 27 cats and the population size is approximately 800 registered cats, which indicates a marked inbreeding ratio.

Materials and Methods

Two related adult Korat cats, a male and a female, suffered from progressive cardiovascular symptoms. The male cat died after a short period of progressive lethargy and the female cat was euthanazied after a sudden onset of paraplegia. The cats underwent clinical, ultrasound and radiographic examinations. A post-mortem investigation was performed for both.

Results

Clinical, ultrasound and radiographic findings included cardiovascular changes characterized by pleural effusion, cardiac hypertrophy, hypercholesterolemia and vascular structural changes (thickening of arteriolar walls, luminal narrowing). On clinical examination the male cat showed a progressive weakness characterized by decreased appetite, lethargia, hyperproteinemia due to hyperglobulinemia, dehydration, leukocytosis, uremia, hypothermia and shock. The cat died despite intensive care. The female cat had a sudden onset of hind leg paraparesis and paraplegia with signs of pain. It was euthanazied because of nonresponsiveness to analgesics and a poor prognosis.

In both cats post-mortem examination revealed severe vascular changes in arteries. There were generalized thickening, hardening, loss of elasticity, and luminal narrowing of large and medium-sized arterial walls with multifocal white plaques on the inner surface in both cats. In addition the male cat had moderate cardiac hypertrophy, multifocal thrombosis in vessels, marked chronic multifocal myocardial fibrosis, mild chronic pulmonary and hepatic congestion. Also a moderate acute multifocal ulcerative gastritis with hemorrhage and a moderate chronic focal ulcerative colitis were seen. The female cat had mild to moderate multifocal myocardial necrosis and fibrosis, marked diffuse chronic pulmonary edema and mild multifocal chronic pyelonephritis with locally extensive papillary necrosis and mineralization.

On histopathology, the arteriolar changes showed severe diffuse intimal thickening with accumulation of cholesterol, lipid macrophages ("foam cells"), and mineral within the tunica intima with smooth muscle cell proliferation. In addition there were mild to moderate multifocal lymphoplasmacytic inflammatory infiltrate, hemorrhage and thrombosis in vessels.

Conclusions

These cats were diagnosed with a clinically devastating severe progressive atherosclerosis with arteriolar intimal thickening not typical for animals, but comparable with human disease. The findings support the diagnosis of an inherited atherosclerosis in these cases. Further investigations in this breed of cats are ongoing to clarify the possibility for the development of a spontaneous animal model for the human disease.

Mesenchymal stromal cells and their extracellular vesicles enhance the anti-inflammatory phenotype of regulatory macrophages by down-regulating the production of IL-23 and IL-22

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Regulatory macrophages (Mregs) orchestrate active dampening of the inflammation, resolution, by secreting anti-inflammatory and proresolving agents including interleukin (IL)-10 and lipid mediators. Mesenchymal stromal cells (MSCs), a cell population that also modulates inflammation, are multipotent adult stem cells that are used in experimental clinical trials in the treatment of various immunological disorders. The mechanisms of the immunomodulation of MSCs are known to a certain extent, however, the overall picture remains unclear. One known mechanism is the secretion of extracellular vesicles (EVs) that mediate cellular communication. We investigated the effects of both human bone marrow-derived MSCs and MSC-derived EVs (MSC-EVs) on mature human Mregs. The cytokines and lipid mediators were determined from cell culture media of Mregs cultivated with MSCs or MSC-EVs. Additionally, the alterations in the expression of cell surface markers and the phagocytic ability of Mregs were investigated. Our novel findings indicate that both MSC co-culture and MSC-EVs down-regulated the production of IL-23 and IL-22 enhancing the anti-inflammatory phenotype of Mregs and amplifying proresolving properties. The levels of prostaglandin E_2 (PGE_2) were substantially upregulated in MSC co-culture media, which may endorse proresolving lipid mediator class switching. Additionally, our results demonstrate for the first time that MSC-EVs mediate the Mreg phenotype change via PGE_2. These data suggest that both human MSC and MSC-EVs may potentiate tolerance-promoting proresolving phenotype of human Mregs, which is of interest when developing novel and more potent cell therapies.

Role of CAD-associated genetic variation in the regulation of cell-type specific enhancer activity

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Coronary artery disease (CAD) is one of the leading causes of death in Western societies and the number of patients is growing as a result of the increasing prevalence of obesity, type 2 diabetes and metabolic syndrome. To improve CAD prevention, diagnosis and treatment, we need to gain better understanding of the genetic and environmental factors that promote the progression of the disease. Hundreds of single nucleotide polymorphisms (SNPs) associated with CAD risk have been identified by means of genome-wide association studies (GWASs). A growing body of evidence suggests that most of the SNPs associated with risk of common diseases including CAD are located in the non-coding regions of the genome rather than in the exons of genes. These SNPs are frequently located in cell-type-specific enhancer regions, which play a key role in gene expression regulation through recruitment of transcription factors (TFs). The enhancer variants modulate transcriptional output, offering a mechanistic basis to explain their association with risk for many common diseases. The SNPs are thought to mainly function by altering TF binding to these regulatory regions. However, the functional processes through which enhancer variants mediate their effect on the phenotype are not entirely understood.

Here, we aim to bring the functional characterization of genetic variants associated with CAD to date by identifying and interpreting the role of enhancer variants across five disease relevant cell types (macrophages, endothelial cells, smooth muscle cells, adipocytes and hepatocytes). By identifying the target genes in physical interaction with the candidate enhancers and establishing causal relationships between the enhancer activity and gene expression, as well as deciphering the interaction networks of the affected genes we hope to obtain a more complete picture of the gene regulatory events driving the disease progression. The enhancer candidates are further characterized by reporter assays, a novel high-throughput quantitative enhancer assay called STARR-Seq and CRISPR/Cas9 genomic engineering. Ultimately, correlating these findings with patient data for cardiovascular risk factors, genotype, gene expression and tissue biomarkers has the potential to improve risk prediction, biomarker identification and treatment selection in the clinics.

Complement Factor H downregulates complement mediated inflammation in human THP-1 macrophages and monocytes

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The complement system plays a central role in humoral innate immune response. It attacks immediately microbes and foreign particles invading the human body. Complement can be activated through three pathways, the classical, alternative (AP), and lectin pathways. AP is constantly active in plasma and can easily be activated on cell surfaces and thereby trigger local inflammation once the efficient regulators are missing. The molecular mechanism how host cells are protected from the AP attack is based on recognition of complement C3b deposits on host cells by Factor H (FH), the main AP regulator in plasma. FH is an elongated molecule composed of 20 domains. Domains 1-4 are responsible for FH regulatory activity while domains 19-20 and 6-7 mediate the interaction with cell surface sialic acids, glycosaminoglycans and C-reactive protein. We have previously shown that domains 5-7 of FH bind to both lipid-free apoE as well as apoE located on the surface of high density lipoprotein (HDL) particles thereby reducing complement activation in plasma.

Interestingly, a common polymorphism Y402H in FH domain 7 is associated with age-related macular degeneration (AMD), a disease that is significantly associated with prevalence of atherosclerosis. Atherosclerosis is characterized by subendothelial cholesterol accumulation in concert with chronic inflammatory response in the walls of arterial blood vessels where the complement system plays an important role. However, the molecular mechanisms by which complement regulation participate in suppressing atherosclerotic lesion progression is not fully understood.

The aim of this study was to investigate the role of FH in reducing complement mediated inflammation at early stages of atherosclerosis.

We used primary peripheral blood monocytes as well as PMA and acetyl-LDL stimulated THP-1 monocytes as model cells. Binding of FH and apoE on these cells as well as secretion of apoE by these cells were analysed by flow cytometry and ELISA. We show that FH binds to peripheral blood monocyte CD11b receptor via domains 11-15. When peripheral blood monocytes were with increasing concentrations of FH, binding of apoE to these cells increased while anti-heparin antibody clearly attenuated this interaction. ApoE binding was also increased to THP-1 cells in the presence of FH while the cell culture supernatant indicated reduction in secreted apoE. Preincubation of THP-1 cells with FH resulted in reduced C3b deposition and C3a formation when the cells were exposed to serum. These data indicate that apoE interacts with monocytic cells predominantly via a dual interaction involving FH and cell surface heparan sulfate, and that FH does not only downregulate complement activation on atherosclerotic lesions but may also participate in promoting the suggested beneficial effects of apoE in suppressing atherosclerotic lesion progression.

MicroRNAs Mediate the Aging-Associated Decline of NRF2 in Endothelial Cells

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Oxidative stress predisposes to several aging-associated diseases, such as cardiovascular diseases and cancer. Simultaneously with the increase in the production of reactive oxygen species, the adaptive stress responses decline. The decline is majorly due to a decrease in antioxidant production. Nuclear factor E2-Related Factor 2 (NRF2) is a transcription factor considered as the key regulator of oxidative and electrophilic stress responses, but it has also been shown to play a regulatory role in cellular metabolism. NRF2 expression declines in aging, but the mechanisms remain unclear. In this study, we show that downregulation of NRF2 in aging decreases endothelial glycolytic activity and stress tolerance both of which are restored after reinstating NRF2. In addition, microRNAs (miRNAs) that are abundant in old endothelial cells are shown to decrease NRF2 expression by direct targeting of NRF2 mRNA. The effect can be reversed by miRNA inhibition. We conclude that aging-associated miRNAs are involved in the decline of NRF2 expression and thus contribute to the repression of adaptive responses during aging.

VEGF-B gene therapy decreases macrophage uptake of modified LDL, foam cell formation, and atherosclerosis

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Background and Aim

Vascular endothelial growth factor-B (VEGF-B) has been shown to increase coronary and adipose angiogenesis by displacing endogenous VEGF from VEGF receptor-1 (VEGFR1) for binding to VEGFR2, which transduces signals for vascular growth. Here, we have evaluated the effects of VEGF-B overexpression on atherosclerosis.

Methods and Results

To study the effect of VEGF-B on the development of atherosclerosis, we increased systemic VEGF-B concentration by using adeno-associated viral vector 9 (AAV9) into *apoE*$^{-/-}$ mice that were subsequently fed high-fat diet. VEGF-B reduced atherosclerotic lesion formation in the aorta without changes in plasma lipid levels or lipoprotein distribution profiles. VEGF-B reduced the monocyte/macrophage content in atherosclerotic lesions, and the uptake of acetylated LDL and foam cell formation in cultured macrophages. We found that VEGF-B suppressed the expression of scavenger receptors (*SR-AI, CD36 or LOX-1*) without altering the expression of receptors involved in cholesterol efflux (*ABCA1, ABCG1 or SR-BI*), HDL level, or HDL quality. VEGF-B did not affect the M1/M2 polarization of macrophages or aortic vascular relaxation.

Conclusions

We demonstrate that increased systemic VEGF-B decreases monocyte/macrophage accumulation in atheromas and prevents macrophage uptake of modified LDL and foam cell formation, thereby protecting against atherosclerosis development.

ANGPTL3 DEPLETION ALTERS LIPID PROFILE AND METABOLISM OF HUMAN HEPATOCYTES

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Aim: Angiopoietin-like 3 (ANGPTL3) is a known inhibitor of lipoprotein lipase and endothelial lipase. Human carriers of loss-of-function variants of ANGPTL3 are protected from cardiovascular disease. However, the intracellular function of ANGPTL3 in hepatocytes is still unknown. Therefore we studied the effect of stable ANGPTL3 knock-down on the lipid profile, metabolism and transcriptome of immortalized human hepatocytes (IHH).

Methods: We analyzed the lipidome of control and ANGPTL3 knock-down IHH cells using electrospray ionization triple quadrupole mass spectrometry and gas chromatography. We also performed a *de novo* lipogenesis assay using [^{3}H] acetic acid and differential transcriptome analysis (RNA sequencing) of control and knock-down cells.

Results: We observed a significant decrease in relative levels of monounsaturated fatty acids and an increase in the levels of polyunsaturated fatty acids (PUFAs) in the total lipids of ANGPTL3 knock-down cells compared to the controls. A similar change was detected in the major membrane phospholipid classes and cholesterol esters. In addition, the total level of cholesterol esters was significantly reduced in the ANGPTL3 knock-down cells, and this change was further confirmed in the *de novo* lipogenesis assay revealing reduced synthesis of cholesterol esters. The RNA sequencing demonstrated gene expression changes in several pathways related to hepatic lipid metabolism, fibrosis and inflammation. Of note, SOAT1 (encoding ACAT1) was significantly down-regulated, and also several mRNAs in the eicosanoid synthesis pathways were altered.

Conclusions: Our findings suggest that the functions of hepatocellular ANGPTL3 involve cholesterol ester synthesis, PUFA metabolism, and VLDL secretion. More insight into these functions is required when hepatic ANGPTL3 is targeted by antisense oligonucleotide therapy.