Front page image: Visualization of lipid droplets in a U2OS osteosarcoma cell overexpressing low-density lipoprotein receptor (LDLR). Lipid droplets are shown in green, cell nucleus in blue and the cytoplasm in red (only intense areas) (courtesy of Simon Pfisterer/Membrane biology).
CONTENTS

4 SUMMARY OF RESEARCH AND ACTIVITIES DURING THE YEAR 2016
5 ADMINISTRATION

Research groups

6 CARDIOVASCULAR RESEARCH
Ilkka Tikkanen, M.D., Dr.Med.Sci., Docent, Head

8 CELLULAR NEUROSCIENCE
Pirta Hotulainen, Ph.D., Docent, Head

10 CELLULAR PHYSIOLOGY
Kid Törnquist, Ph.D., Professor, Head

12 ENDOCRINOLOGY
Hannele Yki-Järvinen, M.D., Dr.Med.Sci., F.R.C.P, Professor, Head

14 LIPID SIGNALING AND HOMEOSTASIS
Vesa Olkkonen, Ph.D., Professor, Head

16 MEMBRANE BIOLOGY
Elina Ikonen, M.D., Dr.Med.Sci., Professor, Head

18 METABOLISM
Heikki Koistinen, M.D., Dr.Med.Sci., Docent, Head

20 NEURONAL SIGNALING
Dan Lindholm, M.D., Dr.Med.Sci., Professor, Head

22 TARGETED GENE-EXPRESSION ANALYSES
Jakob Stenman, M.D., Dr.Med.Sci., Docent, Head

23 TELOMERE RESEARCH
Frej Fyhrquist, M.D., Dr.Med.Sci., Dhc, Professor Emeritus

24 RALPH GRÄSBECK IN MEMORIAM

25 PUBLICATIONS 2016
Minerva Foundation Institute for Medical Research is a privately owned research institute located at Biomedicum, Academic Medical Center Helsinki, Finland. The Institute, the history of which dates back to 1959, combines basic biomedical research with clinical investigation relevant for common diseases.

The overarching aims of the Minerva Foundation Institute are to generate 1) a new knowledge base, 2) innovations for the development of future diagnostic approaches, and 3) preventive measures and treatments for common diseases such as diabetes and cardiovascular diseases, as well as neurodegenerative and neuropsychiatric disorders. The study objectives, rooted in fundamental molecular mechanisms of disease, are addressed at Minerva Foundation Institute through scientific approaches ranging from the use of cultured cells and genetically manipulated animal models to investigation of human patients. The research undertaken in the groups of the Institute during the year 2016 is outlined in this report.

The resources of Minerva Foundation are directed at maintaining and further developing a research infrastructure that serves, in the most effective way, the work in the research groups. The groups are responsible for acquiring external funds to cover the costs of special reagents, stipendium support of doctoral students, and salaries of personnel. In 2016, external funds raised by the research groups covered 51% of the total Institute budget.

Research at the Institute thrived in 2016. The number and the quality of publications by the Minerva scientists showed an increasing trend: A total of 42 articles were published in international peer-reviewed journals, with a median impact factor of 4.92. In addition, one doctoral thesis was finalized and defended during the year.

To promote the exchange of information and seed new collaborations, the Institute organized, in collaboration with the Finnish Brain Research Society, the symposium 'Healthy and Pathological Wiring of the Developing Brain', held at Biomedicum Helsinki on October 25, 2016. The symposium focused on the establishment of brain connectivity and how it is compromised in neurological disorders. The meeting with six international and four domestic lecturers was a success, gathering more than 100 participants. The integration and spirit of the Institute were promoted by a recreational event organized in June. During the year, special emphasis was given to the public image of the Institute; press releases were prepared on research highlights and the Institute web pages were further developed.

**EVENTS AT MINERVA 2016**

**Seminars and Symposia**

**MINERVA SEMINAR, BIOMEDICUM HELSINKI:**
Mika Ala-Korpela, Computational Medicine, Faculty of Medicine, University of Oulu and Biocenter Oulu: *Quantitative serum metabolomics in large-scale systems epidemiology*. August 12, 2016.

Daoguang Yan, Department of Cell Biology, Jinan University, Guangzhou, China: *ORPs and the metabolic patterns in different types of cancer cells*. November 11, 2016.

**MEDIX PRIZE OF THE MINERVA FOUNDATION AWARD CEREMONY, BIOMEDICUM HELSINKI, SEPTEMBER 19, 2016:**
Pekka Katajisto, Institute of Biotechnology, University of Helsinki: *Age-selective segregation of organelles by stem cells*.

The winning article 2016:

**MINERVA FOUNDATION SYMPOSIUM & NEUROSCIENCE FINLAND 2016, BIOMEDICUM HELSINKI, OCTOBER 25, 2016**

Healthy and Pathological Wiring of the Developing Brain

Franck Polleux, Columbia University, New York, New York, USA: *SRGAP2A and its human-specific paralog SRGAP2C coordinates the development of excitatory and inhibitory synapses*.

Pirta Hotulainen, Minerva Foundation Institute for Medical Research, Finland: *Dendritic spine morphogenesis*.

Sari Lauri, University of Helsinki, Finland: *Activity-dependent plasticity mechanisms constructing glutamatergic synapses*.

Sampsa Vahatalo, University of Helsinki, Finland: *Early development of functional networks in the human*. Neuroscientist of the Year: *Asla Pitkänen*, University of Eastern Finland, Finland: *Post-traumatic epilepsy: from mechanisms to therapy*. 

THE MINERVA FOUNDATION

The main purpose of the Foundation is to promote research in medicine and biosciences by maintaining the Minerva Foundation Institute for Medical Research. This scientific review covers the period January 1 – December 31, 2016. During this period, the board of trustees included the following persons:

Professor Caj Haglund, chair
Professor Johan Eriksson
Docent Patrik Finne
Professor Per-Henrik Groop
M.Sc. (Econ. & Bus. Adm.) Thomas Ramsay
M.Sc. Ann-Christine Sundell
Docent Carina Wallgren-Pettersson
M.Sc. (Econ. & Bus. Adm.) Carl-Magnus Westermarck

Scientific Committee
Professor Per-Henrik Groop, chair
Professor Tom Böhling
Professor Johan Eriksson
Docent Patrik Finne
Docent Carina Wallgren-Pettersson

THE MINERVA FOUNDATION INSTITUTE FOR MEDICAL RESEARCH

Board of Directors
Professor Vesa Olkkonen, chair
Docent Pirta Hotulainen
Professor Elina Ikonen
Docent Heikki Koistinen
Professor Dan Lindholm
Docent Jakob Stenman
Docent Ilkka Tikkanen
Professor Kid Törnquist
Professor Hannele Yki-Järvinen
Carita Estander-Kortman
Cia Olsson, secretary

Neuroscience PhD thesis of the Year: **Enrico Glerean**, Department of Neuroscience and Biomedical Engineering, Aalto University, Finland: *Dynamic similarity of brain activity in humans: from single areas to functional networks.*

**Scott Soderling**, Duke University Medical Center, North Carolina, USA: *Synaptic pathologies underlying developmental brain disorders.*

**Stephan Ripke**, Charité, Berlin, Germany: *Common variants genetics: what they tell us about biology and treatment of psychiatric illness.*


**Klas Blomgren**, Karolinska Institutet, Sweden: *Injury and repair in the developing brain.*

**Outi Hovatta**, Karolinska Institutet, Sweden: *Stem cell treatments.*

**Sanna Janhunen**, Orion Pharma, Finland: *Cognition animal models in schizophrenia and new treatment mechanisms.*

**Dan Lindholm**, Minerva Foundation Institute for Medical Research, Finland: *Peroxisome proliferator-activated receptor (PPAR) agonists have beneficial actions in brain disorders.*

**Doctoral Dissertation**

**Johanna Mäkelä**: *Neuroprotective effects of PGC-1α activators in dopaminergic neurons.* University of Helsinki, May 20, 2016. (Neuronal signaling)
Main research activities

Our research group studies mechanisms and repair of end organ damage in cardiovascular and renal diseases. Specifically, we are investigating the local expression and effects of components of the renin-angiotensin-aldosterone system and other vasoactive factors, as well as apoptosis in hypertension, heart failure, and progression of renal damage. In addition, the cardiovascular and renal protective properties of new cardiovascular drugs are evaluated in our group. During recent years, the group’s research has focused on exploring the regenerative and reparative mechanisms of cardiac injury after myocardial infarction and heart failure development to identify new, potential targets for novel cardiovascular medicines.

We have shown that heme oxygenase-1 (HO-1) and carbon monoxide (CO) have potential roles in cardiac recovery and repair after myocardial injury. Our findings demonstrated that treatment with a specific CO-donor, CORM-3, improves both structural and functional cardiac recovery after myocardial infarction (MI) in rats. These effects were associated with altered expression of myocardial miRNA molecules, notably miR-206, involved in cardiac remodeling and repair. Thus, modulation of the HO-1-CO pathway may prove to be a novel tool to facilitate cardiac recovery after myocardial injury and protect against development of heart failure after MI.

During 2016, we continued our collaboration with Docent Hannele Laivuori, University of Helsinki, and her research group to explore the association of HO-1 gene (HMOX1) polymorphisms and
risk of preeclampsia. Our recent findings demonstrated that both the maternal and fetal long GTn repeat in the promoter of HMOX1 are associated with preeclampsia. The long fetal GTn repeat may increase the mother's risk of severe and early-onset preeclampsia. We also established a collaboration with Prof. Per-Henrik Groop and the FinnDiane Study Group, University of Helsinki, Helsinki University Hospital (HUCH), and Folkhälsan Institute of Genetics, to study the possible involvement of HO-1 and HO-1 gene polymorphisms in the development of cardiovascular and renal complications in type 1 diabetes.

A study on the role of HO-1 in cardiac arrest patients in collaboration with Prof. Ville Pettilä, HUCH, and the FINNRESUSCI Study Group showed that higher plasma HO-1 levels are associated with a longer time to return spontaneous circulation as well as poor long-term outcome.

Molecular mechanisms of cardiac regeneration and repair, notably the role and effects of selected miRNAs, antagonists and transcription factors, have been studied in several collaborative projects with Profs. Eero Mervaala and Heikki Ruskoaho and their research groups at the University of Helsinki, and with Prof. Risto Kerkelä and coworkers at the University of Oulu. In these studies, the cardiac cryoinfarction model in adult zebrafish and a drug-induced cardiac hypertrophy/failure model in embryonic zebrafish were utilized. In addition, rat neonatal and zebrafish cardiomyocyte cell culture models were used to examine molecular and cellular pathways related to cardiac regeneration and repair. In collaboration with Dr. Surjya Dash and Prof. Sanna Lehtonen, University of Helsinki, we were able to show that septin7b, the zebrafish ortholog of human septin 7, is essential for the subcellular organization of cardiomyocytes and cardiac function in zebrafish.

Lastly, we have been working in several collaborative clinical studies that continued in 2016. Specifically, with Docent Veli-Pekka Harjola, HUCH, and Dr. Yvan Devaux, Luxembourg Institute of Health, Luxembourg, we analyzed changes in potential miRNAs associated with the outcome of cardiogenic shock patients (CardShock project). High plasma levels of miR-21, miR-122a, miR-320 and miR-423 predicted mortality in these patients. The effects of drug treatment on the progression of disease in aortic stenosis patients, notably on factors and signal cascades involved in formation of fibrosis, have been investigated in collaboration with Prof. Markku Kupari, HUCH (ROCKAS study). In collaboration with Docent Ville Pettitilä, HUCH, potential new biomarkers of acute kidney injury (FINNAKI study) have been examined. Finally, group leader Ilkka Tikkanen has participated in the international collaboration to elucidate the blood pressure lowering and vascular effects of the sodium glucose co-transporter 2 (SGLT2) inhibitor empagliflozin, a novel glucose lowering drug with cardiovascular protective properties, in the treatment of type 2 diabetes.

**Publications 2016**


**Group members**

Pirta Hotulainen, Ph.D., Docent, Head
Enni Bertling, Ph.D.
Rimante Minkeviciene, Ph.D.
Merja Joensuu, Ph.D. (2016–2018 at University of Queensland, Australia)
Amr Abou Elezz, M.Sc.
Iryna Hlushchenko, M.Sc.

**External funding**

The Academy of Finland
The Instrumentarium Foundation
The Liv och Hälsa Foundation
University of Helsinki Brain and Mind graduate school (Abou Elezz)

**Main research activities**

Disorders of the central nervous system (CNS) are some of the most prevalent, devastating and yet poorly treated illnesses. The development of new therapies for CNS disorders could significantly improve the patients’ quality of life, as well as reduce the future burden on health-care systems. However, few truly innovative CNS drugs have reached the market in recent years. Defective regulation of the neuronal cytoskeleton underlies many neurological diseases, making the neuronal cytoskeleton a perfect target for drug innovation.

A neuron typically extends a long thin axon to transmit information to target cells and several shorter dendrites that receive input from other cells through specialized connections known as synapses. The axon initial segment (AIS) is the site of action potential initiation. At same time, the AIS serves as a barrier between dendrites and axons, by sorting vesicles and proteins to the axon or somatodendritic compartment. The majority of excitatory synapses in the central nervous system exist on small bulbous structures on dendrites known as dendritic spines. The dendrites of a single neuron can contain hundreds to thousands of spines. During learning, new spines will appear. At the same time, other spines and synapses will be removed.

Thus, dendritic spines can be considered “memory-units” or “stuff that memories are made of”. Through adding or removing or strengthening or weakening these units, the brain modulates its function. By re-organizing synaptic pathways, new skills are stored in the brain. Precise control of the dendritic spine

Figure. Confocal-microscopy image of primary hippocampal neuron expressing tyrosine phosphorylation mimicking GFP-actin (green). Red shows filamentous actin stained by phalloidin (courtesy of Enni Bertling).
morphology and density as well as the length and location of AISs are critical for normal brain function. Accordingly, both aberrant spine morphology and non-functional AISs are linked to many neurological diseases. The actin cytoskeleton is a structural element underlying proper morphology of dendritic spines as well as proper structure of the AIS.

Goal: We are aiming to develop a comprehensive model of the actin cytoskeleton regulation in dendritic spines and the axon initial segment during neuronal development as well as in neurological diseases. So far we have elucidated the molecular mechanisms underlying dendritic spine initiation (Saarikangas et al., 2015), dendritic filopodia elongation (Hotulainen et al., 2009), spine head growth (Hotulainen et al., 2009) and spine head maintenance (Koskinen et al., 2014).

During 2016, these studies were extended by the discovery of a novel actin regulatory mechanism in mammalian cells – actin tyrosine phosphorylation. In our “paper of the year” (Bertling et al., 2016), we showed that actin is phosphorylated during neuronal development, when dendritic spines undergo rapid changes, as well as during synaptic plasticity, when dendritic spines need to quickly change their shape and size in order to “save” neuronal activity-induced changes to synaptic pathways. The discovery of mammalian actin phosphorylation is important as this will help us to understand the molecular basis of learning, how cells can change and store structural changes rapidly, in only a few minutes.

The regulation of changes in the actin cytoskeleton during synaptic plasticity where discussed in detail in the review article “Dendritic spine actin dynamics in neuronal maturation and synaptic plasticity” (Hlushchenko, et al., 2016).

In our current projects, we are revealing the roles of the actin regulating proteins Rif and gelsolin in neurons. In addition, we are studying novel mechanisms to regulate the neuronal actin cytoskeleton (pH-dependent actin regulation). Furthermore, we elucidate the effects of genetic mutations linked to schizophrenia and autism spectrum disorder on dendritic spine density and morphology. Moreover, we are clarifying the special actin regulation underlying the structure of the axon initial segment. In addition, in 2016, we started a new project where we aim to improve culturing of neurons derived from human induced pluripotent cells (iPSC) so that they resemble mature neurons, e.g. exhibit dendritic spines. As dendritic spine morphology and/or density has been altered in many neurological diseases, these mature looking neurons with dendritic spines could be used for screening novel CNS drugs, using dendritic spines as a readout.

Dendritic spine density and morphology are altered in various neurological diseases. The actin cytoskeleton is a structural component regulating dendritic spine density and morphology. Manipulation of the dendritic spine actin cytoskeleton provides a means to change dendritic spine morphology and density. Thus, manipulating the actin cytoskeleton could be used to rescue the altered dendritic spine density and morphology in neurological diseases.

See also lab home page: www.helsinki.fi/neurosci/hotulainenlab/

Publications 2016


Group members
Kid Törnquist, Ph.D., Professor, Head
In addition, the following researchers were working at Åbo Akademi University:
Kati Kemppainen, Ph.D.
Muhammad Yasir Asghar, Phil.lic.
Melissa Magnusson, M.Sc.
Ilari Pulli, M.Sc.
Taru Lassila, B.Sc.
Emilia Holm, B.Sc.

External funding
The Liv och Hälsa Foundation
The Sigrid Juselius Foundation
Svenska Kulturfonden
Magnus Ehrnrooth Foundation

Main research activities
Our research group investigates calcium and lipid signaling in cancer cells. Specifically, the group studies three main areas of calcium and lipid signaling in thyroid and other human tumor-derived cancer cells: 1) the importance of the canonical transient receptor potential (TRP) C-family of ion channels, as well as the actions of the sphingomyelin metabolite sphingosine 1-phosphate (S1P), 2) the interactions between TRPC-channels and S1P, and 3) the receptors important for the regulation of cell migration in different cell types.

Investigations performed in the group have shown that the S1P-receptor functional agonist FTY-720 (Fingolimod), an immunosuppressive drug currently used for treatment of multiple sclerosis, effectively attenuated the proliferation and both the basal and S1P-evoked invasion of several thyroid cancer cell lines. The mechanism of action involved downregulation of both the S1P receptor and the VEGF receptor 2 (i.e. the receptors needed for S1P-evoked invasion), as well as several protein kinase C isoforms. Furthermore, the S1P-evoked secretion of matrix-metalloproteinases 2 and 9, two important matrix-metalloproteinases for

Figure. Cytoplasmic and mitochondrial calcium measurements. A. Validation of the experimental set-up is usually done by employing conventional fluorescence microscopy. Shown here are patient fibroblast cells that were loaded with the fluorescent calcium indicator Fura-2. The red color indicates a high intracellular calcium concentration. B. After validation, high-throughput mitochondrial and cytoplasmic calcium measurements can be conducted by employing a Hidex Sense plate reader. The image shows a visual representation of raw data (i.e. the increase in intracellular calcium concentration) from fibroblast cells that were transfected with the mitochondrially targeted luminescent calcium indicator, aequorin.
the invasion of thyroid cancer cells, were downregulated. FTY720 also attenuated thyroid cancer cell proliferation in the chick embryo chorioallantoic membrane assay. Since FTY720 has shown promising results in attenuating proliferation and invasion of several cancer cell types, our results suggest that FTY720 could be beneficial in the treatment of thyroid cancers.

Our previous investigations have shown that another lipid, sphingosylphosphorylcholine (SPC), potently attenuates proliferation of several different types of cancer cells. In a microarray screen, we observed that the mRNA for large tumor suppressor 2 (Lats2) was potently upregulated. Lats2 is part of the Hippo signaling pathway, and we hypothesized that the SPC-evoked inhibition of proliferation could be mediated by this pathway. Knockdown of S1P receptor 2, a receptor known to inhibit proliferation, prevented the SPC-induced up-regulation of Lats2 and attenuated the anti-proliferative effect of SPC. In line with this we observed that knockdown of Lats2 enhanced cell proliferation. However, knockdown or over expression of kinase-dead Lats2 or intermediates in the Hippo signaling pathway did not attenuate the SPC-evoked inhibition of proliferation. We also observed a transient phosphorylation of the YAP transcriptional regulator, followed by a substantial inhibition of YAP target genes. However, the potent SPC-induced effects seen in Lats2 and YAP did not mediate the anti-proliferative effect of SPC.

Another angle of our work involves ongoing investigations of calcium signaling. Several proteins participate in regulating cellular calcium homeostasis, and we have been interested in the importance of the transient receptor potential (TRP) family of ion channels, especially TRPC channels. These channels are present in normal, as well as cancerous cells, including human thyroid cancer cells, and potently enhance proliferation, migration and invasion of cancer cells. Current studies are now aimed at investigating the importance of Orai channels and the stromal interacting molecules (STIM), in particular STIM-1, on thyroid physiology and how signaling through these channels differs from that observed for TRPC channels. Orai1 and STIM-1 are of importance in regulating store-operated calcium entry, the calcium entry process activated when intracellular calcium stores are depleted due to agonist-induced signaling. Preliminary results suggest that similarities exist with TRPC-evoked signaling, but there are also some clear differences. Future work is aimed to shed light on these differences.

Ongoing work in our group also aims to understand compartmentalized calcium signaling in different types of cells. Since compartmentalized calcium signaling results in ion hot spots in cells it may affect specialized signaling pathways or organelles. Together with our collaborators, we have developed novel tools to enhance these ongoing studies. Our current investigations aim at understanding how sphingolipids and related proteins modulate organelar calcium homeostasis. In these studies a new, high-throughput screening method using the Hitex plate reader has been developed and employed.

Kati Kemppainen defended her Ph.D. thesis in June. The thesis, entitled "Novel cancer-related regulatory targets for the signaling sphingolipids sphingosine-1-phosphate and sphingosylphosphorylcholine," was accepted at Åbo Akademi University (Turku) and was graded "With honors".

Publications 2016


**Main research activities**

We have shown in a population-based study that non-alcoholic steatohepatitis (NASH) is as common in Finland as elsewhere.

The key aim of our work centers on determining the molecular mechanisms responsible for hepatic insulin resistance in human non-alcoholic fatty liver disease (NAFLD). In one approach, we used a common human genetic model of NAFLD (‘PNPLA3 NAFLD’) and the model of ‘Metabolic NAFLD’, to define how the molecular signature of the human liver differs between the two conditions, of which only the latter is insulin resistant. We collected 125 liver biopsies over four years. The human liver triacylglyceride (TAG) composition differed markedely between the two types of NAFLD. The liver was enriched with polyunsaturated TAGs in ‘PNPLA3 NAFLD’ and with saturated and monounsaturated TAGs in ‘Metabolic NAFLD’. Insulin resistance co-segregated with de novo ceramide synthesis, consistent with findings in mice published in two recent reports in *Cell Metabolism* (Turpin et al., 2014; Hla and Kolesnick, 2014) that identified the same molecular species as the key mediator of insulin resistance.

We have also recently characterized the human liver lipidome in two other genetic types of NAFLD not associated with insulin resistance i.e. ‘TM6SF2 NAFLD’ and ‘MBOAT7 NAFLD’. We completed a three-arm mechanistic overfeeding study comparing the effect of a high sugar/high saturated/high polyunsaturated fat diet on routes of synthesis of liver fat. We found saturated fat to increase insulin resistance and liver fat more than the two other diets. In addition, we observed that saturated fat caused an increase in the same ceramide species we identified in the liver in ‘Metabolic NAFLD’.

Panu Luukkanen, MD, is preparing his Doctor of Medical Sciences (DMSc) thesis on the human liver lipidome in different types of NAFLD.

Jenni Hyysalo completed her M.D. degree and is currently finalizing her DMSc thesis, a study comparing the serum lipidome in ‘PNPLA3 NAFLD’
and ‘Obese/Metabolic NAFLD’ as well as a study examining the impact of genetic variation in apoCIII on NAFLD. The thesis includes the first population-based study addressing the prevalence of NASH based on clinical parameters and PNPLA3 genotype at rs738409. In a systematic review, the authors examined the literature to identify data on the definition of normal liver fat using liver histology and different imaging tools, as well as to analyze whether NAFLD caused by genetic variation in PNPLA3 or TM6SF2 is associated with insulin resistance. Previously, she has shown that subcutaneous adipocyte size is associated with liver fat content in 119 non-diabetic subjects independent of obesity, fat distribution, age, gender and PNPLA3 genotype. In cooperation with the Institute of Health and Welfare and an EU project Elucidating Pathways of Steatohepatitis, she determined the reference value in two population-based studies for Homeostasis Assessment Model of Insulin Resistance (HOMA-IR), a surrogate of insulin resistance, and determined the inter-laboratory variation of HOMA-IR in seven European laboratories.

Susanna Lallukka, MD, is preparing her DMSc thesis on heterogeneity of NAFLD. She showed that coagulation factors are increased in subjects with NAFLD not carrying risk variants (‘Metabolic NAFLD’), but not in subjects with NAFLD and PNPLA3 variant (‘PNPLA3 NAFLD’). The increase in coagulation factor activities was shown to be related to increased gene expression in liver samples (factors VIII, IX and fibrinogen) in subjects with ‘Metabolic NAFLD’. Adipose tissue inflammation was correlated with coagulation factor activities in ‘Metabolic NAFLD’. Thus, obesity/insulin resistance rather than an increase in liver fat per se is associated with a pro-coagulant plasma profile. This reflects adipose tissue inflammation and increased hepatic production of coagulation factors. Dr. Lallukka also wrote an extensive systematic review on whether NAFLD predicts type 2 diabetes. The review included 20 prospective studies in which NAFLD was diagnosed by ultrasonography or liver enzymes. ‘Metabolic NAFLD’ predicted T2DM independent of age and obesity.

Docent You Zhou, PhD has been helping with all bioinformatics analyses in the group. He recently started an independent academic position in Cardiff, UK, and continues to work with the group, especially in the area of statistical analyses. Dr. Zhou published an important paper identifying novel markers in NASH in Clinical Gastroenterology and Hepatology.

Research nurses Anne Salo and Aila Karioja-Kallio have continued to provide superb and essential work in performing the clinical studies and laboratory work.

**Publications 2016**


Our group aims to elucidate how cells maintain specific lipid compositions of organelles and sense their internal lipid status to control vital cellular processes such as signal transduction and vesicle transport. A novel concept, that of membrane contact sites (MCSs), zones of close contact between the limiting membranes of intracellular organelles (Figure), has moved into the focus of biomedical research. Such contacts are formed by virtually every organelle type within cells and provide high capacity and specificity platforms for the inter-organelle transport of small molecules, Ca^{2+} and other signals. In this context, the family of cytoplasmic oxysterol-binding protein (OSBP) homologs, ORPs, identified by the group are of high interest (Figure). A major project in the group aims to unravel the function of these proteins and other new MCS protein components, as well as the roles of MCSs in diseases involving aberrant lipid transport or signaling.

In 2016, major activity in the group was directed at understanding the role of MCSs in cell signaling, particularly in endothelial cells, a cell type centrally involved in common diseases such as atherosclerosis and cancers. Moreover, we investigated the functional roles of ORPs in human lipoprotein metabolism, atherosclerosis and leukemias. We showed that ORP1L, located at endoplasmic reticulum-late endosome interfaces, plays a role in cellular cholesterol efflux and provided evidence that its rare mutations associate with low HDL-cholesterol levels (Motazacker et al. 2016). Ongoing work elucidates
the role of ORP proteins and their interaction partners in endothelial cell functions, angiogenesis and the biogenesis of von Willebrand factor, a key controller of coagulation and thrombus formation. Our novel finding suggests that endothelial ORP2 controls angiogenesis in vitro (Kivelä et al., in preparation). Generation of an ORP2 knock-out mouse model for in vivo study, and phenotypic analysis of ORP2 knock-out hepatocytes generated by using the CRISPR/Cas9 gene editing technique (Kentala et al., in preparation) are underway.

Collaboration with the group of Prof. D. Yan (Jinan University, Guangzhou, China) produced key findings on the function of ORP4L: This protein, not present in normal T-cells but abundantly expressed in acute lymphoblastic T-leukemia (T-ALL) cells, assembles a new G-protein coupled signaling complex that allows efficient bioenergetics of the malignant T-cells via oxidative phosphorylation. Knocking down ORP4L inhibits the proliferation of T-ALL cells in vivo (Zhong et al., 2016a). ORP4L is also expressed in macrophages, and its knock-out resulted in a reduction of atherosclerotic lesions in LDL-receptor deficient mice, due to enhanced apoptosis of lesion macrophages (Zhong et al., 2016b). These observations suggest that ORP4L could be introduced as a potential new therapy target.

A second major activity in the group in 2016 focused on elucidating the role of novel molecular machineries in the development of obesity, non-alcoholic fatty liver disease (NAFLD), insulin resistance, and type 2 diabetes. We finalized two studies addressing the role of microRNAs in NAFLD liver (Soronen et al., 2016) and in visceral adipose tissue of obese subjects (Mysore et al., 2016). Moreover, we initiated a study of the regulation of Angiopoietin-like 8 (ANGPTL8), an important controller of lipid and glucose homeostasis, by adipose tissue microRNA species known to be induced in obese and type 2 diabetic subjects (Mysore et al., in preparation).

Publications 2016


Main research activities

In brief, the group has investigated the molecular mechanisms related to intracellular lipid storage as well as disturbances in these processes associated with human diseases. We have also established new imaging modalities to improve the detection of lipids and their associated pathologies in human cells and tissues. These studies have, in part, been conducted in collaboration with the groups of Prof. Hannele Yki-Järvinen (Pirhonen et al., 2016) and Prof. Vesa Olkkonen (Motazacker et al., 2016) from the Minerva Foundation Institute for Medical Research. Below, some of the highlights are discussed.

Seipin, an endoplasmic reticulum (ER) membrane protein, is mutated in severe congenital lipodystrophy (BSCL2) characterized by virtual absence of adipose tissue accompanied by fatty liver and insulin resistance. The cellular lipid storage sites, lipid droplets, are generated in the ER and are functionally connected to the ER. Seipin is somehow involved in regulating the size of lipid droplets, but its precise function is not well understood. We found that seipin regulates the stability of contacts between lipid droplets and the ER (Salo et al., 2016). Seipin is normally associated with nascent ER-lipid droplet contacts but in its absence, these contacts are malformed or even completely missing. This is accompanied by defective trafficking of protein and lipid cargo into growing lipid droplets. It seems likely that dysfunctional ER-lipid droplet contacts are at least in part responsible for the lipid droplet growth defect and lack of adipose tissue in BSCL2 lipodystrophy.

Using label-free coherent anti-Stokes Raman scattering (CARS) and second-harmonic generation (SHG) imaging, we demonstrated that SHG imaging detects fibrillar collagen in non-alcoholic fatty liver disease (NAFLD) more sensitively than routine histological fibrosis staging (Pirhonen et al., 2016). Moreover, this method enables quantitative assessment of early fibrosis in an observer-independent manner. In this pilot study, 32 NAFLD biopsies were investigated. In future studies, we aim
to employ the method in larger NAFLD patient cohorts for sensitive, quantitative assessment of fibrosis.

We also tested another imaging technique, hyperspectral simulated Raman scattering (SRS), in collaboration with the group of Prof. Potma (Univ. of California Irvine) for imaging intracellular storage of specific lipid species. We demonstrated that highly deuterated cholesterol can be used to differentiate between free cholesterol, cholesteryl ester and triacylglycerol accumulating lipid droplets in cells (Alfonso-Garcia et al., 2016).

Publications 2016


n 2016, the research activity of the group continued with the primary focus on the regulation of glucose metabolism in human skeletal muscle, using primary human muscle cells as a research model.

In collaboration with Docent Riikka Kivelä at Wihuri Research Institute, we identified that the transcription factor Prox1 is an important regulator of myoblast differentiation and determinant of slow muscle fibre type (Kivelä et al., 2016).

We collaborate closely with the FUSION-study (Finland-United States Investigation of NIDDM Genetics, head principal investigator (PI) Prof. Michael Boehnke, University of Michigan, Ann Arbor, USA), with the head of the group, Docent Heikki Koistinen being one of the FUSION PIs.

Large-scale sequencing of exomes and whole genomes of type 2 diabetes (T2D) cases and controls revealed that variants associated with T2D were common and most fell within regions previously identified in genome-wide association studies (GWAS). These data do not support the hypothesis that rare variants play a major role in T2D (Fuchsberger et al., 2016).

In a large collaborative effort we examined if genetic variants affect body shape as a composite phenotype that is represented by a combination of anthropometric traits (body mass index, height, weight, waist and hip circumference, waist-to-hip ratio). With this strategy, six novel loci affecting body shape were identified (Ried et al., 2016). In addition, another collaboration revealed two novel loci, BCL2 and FAM19A2, to be associated with insulin sensitivity (Walford et al., 2016).

In the FUSION tissue study, we collected muscle samples from 271 well-phenotyped Finnish participants with glucose tolerance ranging from normal (NGT) to newly-diagnosed T2D. To understand how T2D status, metabolic traits, and genetic variation influence gene expression, we performed
high-depth strand-specific mRNA-sequencing and dense genotyping. Computational integration of these data with epigenome data and transcriptome data across diverse tissues revealed that the tissue-specific genetic regulatory architecture of skeletal muscle is highly enriched in muscle stretch/super enhancers, including some that overlap with T2D GWAS variants. In one such example, T2D risk alleles residing in a muscle stretch/super enhancer are linked to increased expression and alternative splicing of muscle-specific isoforms of ANK1. Differences in muscle gene expression relate closely to glucose tolerance phenotype, and people with recently diagnosed T2D have an altered expression of several gene groups such as genes involved in endoplasmic reticulum protein localization, translational elongation or cellular respiration (Scott et al., 2016).

In a large international collaboration, which also included subjects from the FUSION- and METSIM (PI Prof. Markku Laakso) cohorts, a missense variant of AKT2 has been identified. AKT2 is an important effector in the insulin signal transduction pathway, and this AKT2 variant is specific for Finns and very rare in non-Finnish Europeans. It is associated with higher fasting insulin concentrations and predisposes people to T2D (unpublished observation). Since the METSIM-cohort contains several carriers of this variant, we started a collaboration with Prof. M. Laakso, University of Eastern Finland, and Prof. Pirjo Nuutila, Turku PET Centre, to study the role of this signaling variant in the pathogenesis of insulin resistance. Given the particular relevance of AKT2 in glucose metabolism in skeletal muscle, we have created primary muscle cell cultures from carriers of the AKT2 variant and wild type controls for detailed in vitro studies that are currently ongoing at Minerva. These data will complement the in vivo investigations and provide further mechanistic evidence of the pathophysiological significance of the Finnish AKT2 variant.

In 2016, Heikki Koistinen was chosen as the Group Teacher of the Year 2015 by the Medical Bachelor’s Association and medical students at the University of Helsinki.

Thesis completed in the group in 2016:

The following Master’s thesis was accepted at University of Helsinki this year:


Publications 2016


Main research activities

We study trophic factor signaling in neurodegenerative diseases, such as Huntington’s (HD) and Parkinson’s disease (PD). We focus on mitochondrial functions and endoplasmic reticulum (ER) stress as well as protein degradation pathways, such as the ubiquitin proteasome and autophagy systems, and how these are altered in disease and in neurons at risk. We employ various biochemical, proteomic, molecular biology and cell biology methods for our studies, including neuronal cultures, and genetically modified mice. The majority of the group is housed in Medium, Faculty of Medicine of the University of Helsinki, and the group is actively engaged in research at Minerva. During 2016, there were three main areas of research in the group.

1. PPARγ and PGC-1α in neuronal signaling and neuroprotection

Peroxisome proliferator activated receptor-gamma (PPARγ) is a nuclear receptor involved in the control of cell metabolism and inflammation in different tissues. Drugs acting on PPARγ are used for treatment of human metabolic disorders, such as type-2 diabetes. However these drugs can also have beneficial effects in various brain disorders (Patrone et al., 2014) but the mechanisms are not fully understood. PGC-1α, a transcriptional coactivator for PPARγ, is neuroprotective, as shown in the neurotoxin-induced mouse model of PD (Mudo...
et al., 2012) and in excitotoxic neuronal injury induced by stimulation with the glutamate receptor agonist, kainic acid in hippocampus (Mäkelä et al., 2016a). To ascertain which proteins are altered after the injury, we studied PGC-1α transgenic mice with overexpression of the protein in brain neurons. Using quantitative proteomics we observed significant changes in mitochondrial proteins as well as in other proteins that are neuron-specific in the PGC-1α mice. The significance of these proteins in neuronal functions is currently under investigation.

Furthermore, using cultured human dopaminergic cells, we obtained evidence that PPARγ agonist-mediated neuroprotection is associated with increases in PGC-1α expression and enhanced mitochondrial function (Mäkelä et al., 2016b). Adenylate cyclase-6 (AD6) was identified as a crucial molecule regulated by PPARγ agonism resulting in an increase in protein kinase A-cyclic AMP signaling and activation of the the transcription factor CREB in the dopaminergic neurons (Mäkelä et al., 2016b).

2. Neurotrophic factors and ER stress in neurodegeneration

Increased ER stress and disturbed protein handling (proteostasis) play a role in many human neurological diseases. We have shown previously that there is an activation of ER stress, and particularly the inositol-requiring enzyme 1α (IRE1α) signaling pathway, in HD (Hyrskyluoto et al., 2014). We hypothesized that ER stress signaling can be regulated in neurons by neurotrophic factors and compounds acting on IRE1α and other signals. Using cellular models for HD and PD, we have studied proteins of the saposin family of factors and showed that they can reduce ER stress and exert neurotrophic action under specific conditions. We are currently studying this observation further and examining if the proteins act by an intracellular or extracellular mechanism and how the ER stress reduction and neurotrophic action may be connected. In addition, we study the role of cABL in mutant huntingtin-expressing cells and examine whether drugs used in human chronic lymphoma may provide neuroprotection in HD as in PD (see Figure; Lindholm et al., 2016).

3. p75NTR in control of cell lipid metabolism and gene expression

The neurotrophin p75 receptor (p75NTR) binds neurotrophins such as nerve growth factor (NGF) and pro-NGF and is expressed both in the brain and peripheral tissue. We have described a signaling pathway for p75NTR in the regulation of cholesterol and lipid uptake in cells (Pham et al., 2016). This pathway is based on pro-NGF-mediated activation of the transcription factor, Sterol regulator element binding protein-2 (SREBP2) following caspase-3 cleavage, which causes induction of low-density lipoprotein receptors (LDLRs). In a mouse model of fatty liver the expression levels of NGF and pro-NGF were increased together with LDLRs expression, suggesting a physiological role of p75NTR and its ligands in dyslipidemias. We further noted that caspase-2 plays a crucial role in LDLR regulation by binding caspase-3 at intracellular membranes. We are currently studying the precise molecular mechanisms of this process, including the role of the signaling molecule p38 MAP kinase. Furthermore, to reveal tissue-specific effects of p75NTR, we are using gene profiling of brain and other tissues from control and p75NTR gene deleted mice.

Publications 2016


Thesis completed in the group in 2016

The following doctoral thesis was accepted at the University of Helsinki this year:
TARGETED GENE-EXPRESSION ANALYSIS

Main research activities

The aim of the research group is to develop novel diagnostic assays based on PCR and Next Generation Sequencing. Technical development is focused on applications where there are specific difficulties in utilizing conventional PCR, as well as on improvements in sequencing pre-amplification.

We have developed a novel technique (ExBP-RT) for ultra-sensitive detection of expressed mutations. This technique provides a means for early detection of malignant transformation in pre-malignant conditions as well as for disease monitoring from plasma samples. (Ho, TH et al, Nucl Acids Res. 2015). During the past year we have applied the ExBP-RT technique to study the occurrence of expressed KRAS and BRAF mutations in endoscopic biopsy samples, as a means for detection of early signs of malignant transformation in paediatric patients with inflammatory bowel disease, as well as in adult patients treated for oesophageal atresia in their early childhood. Our results indicate that tissue expression of mutations associated with malignant transformation can occur years, or even decades before clinical manifestation and could provide valuable markers for selection of patients at risk for follow-up programmes. Another ongoing study focuses on prognostic molecular events in colorectal cancer. We have investigated RNA and protein expression of several potential markers and ExBP-RT has been successfully utilized to detect KRAS and BRAF mutations in a large material of formalin-fixed, paraffin-embedded (FFPE) surgical specimens. We are currently working in collaboration with our previous PhD-student, Dr. Tho Ho, now situated at Vietnam Military Medical University (VMMU) to develop assays based on ExBP-RT for detection of other known Ras mutations.

During the past year, we have continued development of a novel PCR amplification technology called Heat Pulse Extension PCR (HPE-PCR). This technique can overcome difficulties in amplifying long GC rich and repetitive sequences by pulsatile temperature cycling during the extension step of PCR. We have previously demonstrated the utility of this technique by amplifying repetitive expansions in Fragile X syndrome as well as in Type I Myotonic Dystrophy. The technique has been adopted into routine clinical use at HUSLAB of the Helsinki University Central Hospital. Currently, we are focusing on improving the reaction efficiency further in order to enable amplification of longer repeat expansions that are present in Amyotrophic Lateral Sclerosis (ALS). The HPE-PCR technique has proven effective for reduction of GC-bias in sequencing pre-amplification. This improves the sequencing coverage of poorly amplifying regions. European and US patents have been granted for the HPE-PCR technology and collaboration with industrial partners has been established.

Group members
Jakob Stenman, M.D., Dr.Med.Sci., Docent, Head
Ho Huu Tho, M.D., Dr.Med.Sci.
Kien Dang, M.D.

External funding
Wilhelm och Else Stockmanns Foundation
Main research activities

Our team has studied leukocyte telomere length (LTL) in more than 6,000 subjects. Over the years, we have reported significant associations of LTL with 1) alcohol consumption, 2) physical activity, 3) body mass index, and 4) insertion/deletion (I/D) polymorphism of the angiotensin-I converting enzyme (ACE) gene.

During 2015-2016, in cooperation with Prof. Ilkka Pörsti, University of Tampere, we measured LTL in 522 healthy Finnish subjects participating in a study on haemodynamics. Preliminary results show the known relation of LTL to age and gender, but no association of heart rate, or a number of other variables, with LTL. The results will be analyzed and published in 2017.

A study focusing on telomeres and cardiovascular health in the young was started in 2012 in collaboration with Prof. Antti Jula (Institute of Health and Welfare, Turku), as a sub-study of STRIP (Sepelvaltimotaudin Riskitekijöiden Interventio Projekti). The study comprised >500 subjects, each, in the intervention and control groups, with follow-up covering early childhood to maturity. This longitudinal study has been ongoing during 2013–2016, and results will be analyzed and submitted for publication during 2017. Preliminary results suggest that healthy life style intervention is associated with slower telomere attrition compared with controls.

Publication 2016

Professor Ralph Gräsbeck, one of the founders of the Minerva Institute for Medical Research, died January 22, 2016 at the age of 85.

Ralph, born July 6, 1930, got his MD degree (Licentiate of Medicine) at the University of Helsinki at the remarkably young age of 22 years. While working as a young doctor at the IVth Department of Internal Medicine he was inspired by his chief and mentor, professor Bertel von Bonsdorff, to study the physiology and pathophysiology of vitamin B12. This lead to his MD thesis (1956), in which he described the presence and vitamin B12 transporting function of "intrinsic factor" in human gastric juice. In 1960 Ralph independently, at the same time as the Norwegian pediatrician Olga Imerslund, described a hereditary vitamin B12 malabsorption syndrome presently called Imerslund-Gräsbeck syndrome. Importantly, Ralph introduced the crucial concept of "reference values" used routinely in clinical chemistry worldwide. He was author of more than 160 scientific articles. Ralph was appointed lecturer (docent) in clinical chemistry at the University of Helsinki in 1959. He was head physician of the central laboratory at Maria Hospital 1960-1990.

Ralph was a member of several national and international scientific societies and institutional boards. Besides many other honors he was in 1966 awarded the Jahre price for young investigators at the University of Oslo. He was appointed professor honoris causa 1982 and doctor of honor at the Poincaré university of Nancy.

Ralph was one of the founders of the Minerva Foundation Institute for Medical Research in 1959. He was chief of the institute 1971–1993 and active researcher at Minerva almost until his death. Ralph was also one of the founders of the clinical laboratory Medix in 1964 (now Yhtyneet Medix Laboratories), and in 1985 of the company Medix Biochemica. Both companies are successful and offer crucial financial support for Minerva Foundation and its research institute. Ralph was a positive and dynamic person with an open and exceptionally creative mind and a vast knowledge of both his field of scientific interest but of life in a broad sense as well.

Frej Fyhrquist


Reviews


Other publications

