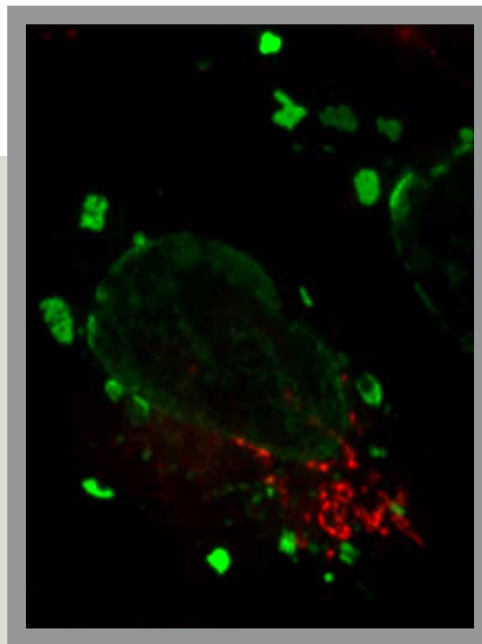




MINERVA FOUNDATION
&
MINERVA FOUNDATION INSTITUTE
FOR MEDICAL RESEARCH



Scientific Report 2014



Front page image: A bimolecular fluorescence complementation (BiFC) image revealing the interaction site of the Oxysterol-binding protein (OSBP)-related protein ORP9 and the VAMP-associated protein VAPA in HuH7 human hepatoma cells. ORP9 is C-terminally fused to the N-terminal half of the Venus fluorescent protein, and VAPA is C-terminally fused to the C-terminal half of Venus. Shown in green is the BiFC interaction between the ORP9 and VAPA proteins and in red the Golgi complex stained with Golgi marker GM130 (courtesy of Marion Weber-Boyyat/Lipid signaling and homeostasis).

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SUMMARY OF RESEARCH AND ACTIVITIES DURING THE YEAR 2014

Minerva Foundation Institute for Medical Research is a privately owned research institute located at the Biomedicum Academic Medical Center Helsinki. The Institute, the history of which dates back to 1959, combines basic biomedical research with clinical investigation relevant for common diseases. The objectives of study of fundamental molecular mechanisms of disease are addressed by a range of approaches from cultured cells and genetically manipulated animal models to investigation of human patients.

The overarching aims are to generate a new knowledge base as well as innovations for the development of future diagnostic approaches, preventive measures and treatments for common diseases such as diabetes, cardiovascular diseases and neurodegenerative disorders. The work carried out in the eight research groups of the Institute during the year 2014 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 their students and salaries of their personnel. Despite the current challenging financial situation, during 2014 Minerva scientists succeeded in raising a substantial amount of external funds, covering 44% of the total Institute budget.

To enhance the research impact and maximise the gain from the investment in the Institute, it is essential to continually encourage integration and research collaboration both within the Institute and with other organizations located at the campus. One area of development towards this goal is generation of common technology cores in collaboration with neighboring institutions. For example, in 2014, Minerva participated in the shared purchase of a Vevo 2100 ultrasound imaging system for small animal models.

To promote the exchange of information and seed new collaborations, the Institute organized a one-day international symposium, Lipid Storage and Signaling in Disease on September 22, with six invited international speakers, each a leading figure in his/her field, and two presentations by Minerva researchers (see page 26). Moreover, a new seminar series, Minerva Discussion Forum, was initiated. This small scale forum with invited lecturers is arranged approximately once per month and aimed at creating new contacts with scientists working close to the areas of emphasis of the Institute. In addition to building external collaboration, the internal integration and spirit of the Institute were promoted by weekly internal meetings and a recreational event organized at Kaisankoti, Espoo, in June.

Despite the present limitations in external funding, the work at the Institute in 2014 thrived. Minerva scientists published a total of 33 articles in international peer-reviewed journals, with an impact factor median of 5.03. In addition, three doctoral theses were finalized and defended during the year.

EVENTS AT MINERVA 2014

Seminars and Symposia

BERND HELMS:

Lipid droplet dynamics: identification of proteins involved in the synthesis and remodeling of neutral lipids during hepatic stellate cell activation, January 30th, Biomedicum Helsinki

MINERVA FOUNDATION – MEDIX SYMPOSIUM:

Lipid storage and signaling in disease, September 22nd, Biomedicum Helsinki

Quentin Anstee, Newcastle University, UK: *Genetic modifiers of non-alcoholic fatty liver disease (NAFLD)*

Tobias Hartmann, Saarland University, Germany: *Alzheimer's disease: The lipid connection*

Anna Krook, Karolinska Institutet, Sweden: *Regulation of skeletal muscle insulin sensitivity*

Cesare Patrone, Karolinska Institutet, Sweden: *Palmitate decreases adult neural stem cell viability*

Susan Pyne, University of Strathclyde, UK: *The role of sphingosine 1-phosphate in cancer*

Kid Törnquist, Minerva Foundation Institute for Medical Research, Finland: *Interactions between the TRPC1 ion channel, sphingosine 1-phosphate receptor 3, and VEGF receptor 2 in thyroid cancer cells*

Tobias Walther, Harvard School of Public Health and Harvard Medical School, USA: *The phase of fat: Mechanisms and consequences of neutral lipid storage*

Hannele Yki-Järvinen, Minerva Foundation Institute for Medical Research, Finland: *Subtypes and diagnosis of NAFLD*

Doctoral Dissertations

Jere Paavola: *Aberrant intracellular calcium cycling in the heart – mechanistic insights into catecholaminergic polymorphic ventricular tachycardia and heart failure*. March 28th, 2014. (Cardiovascular research)

Leena Juurinen: *Insulin therapy in type 2 diabetes: Initiation, impact on liver fat, role of combination therapy with glitazones or glitazones*. May 16, 2014. (Endocrinology)

Alise Hyrskyluoto: *Endoplasmic reticulum stress, regulation of autophagy and ubiquitin-proteasome system in cellular models of Huntington's disease*. September 12th, 2014. (Neuroscience)

ADMINISTRATION

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 from January 1 – December 31, 2014. During this period, the board of trustees included the following persons:

Professor Jim Schröder, chair
Professor Caj Haglund, vice chair
Professor Johan Eriksson
Professor Per-Henrik Groop
Professor Carola Grönhagen-Riska
Professor Carl-Gustaf Nilsson
M.Sc. (Econ. & Bus. Adm.) Thomas Ramsay
M.Sc. Ann-Christine Sundell, secretary
Docent Carina Wallgren-Pettersson
M.Sc. (Econ. & Bus. Adm.) Carl-Magnus Westermarck

The administration was handled by following groups:

Financial Committee

Jim Schröder, chair
Carola Grönhagen-Riska
Thomas Ramsay
Ann-Christine Sundell
Carl-Magnus Westermarck

Nomination Committee

Jim Schröder, chair
Per-Henrik Groop
Carola Grönhagen-Riska
Caj Haglund

Scientific Committee

Per-Henrik Groop, chair
Johan Eriksson
Caj Haglund
Carl-Gustaf Nilsson
Carina Wallgren-Pettersson

THE MINERVA FOUNDATION INSTITUTE FOR MEDICAL RESEARCH

Board of Directors

Docent Vesa Olkkonen, chair
Professor Elina Ikonen
Docent Heikki Koistinen
Professor Dan Lindholm
Docent Jakob Stenman
Docent Ilkka Tikkanen
Professor Kid Törnquist
Professor Hannele Yki-Järvinen
Carita Estlander-Kortman
Cia Olsson, secretary



TIKKANEN

Group Members

Ilkka Tikkanen, M.D., Dr.Med.Sci., Docent, Head
 Miika Laine, M.D., Dr.Med.Sci., Docent
 Päivi Lakkisto, M.D., Dr.Med.Sci., Docent
 Jere Paavola, M.D., Dr.Med.Sci spring 2014
 Hanna Forsten, V.M.D.
 Heli Segersvärd, M.D.
 Juuso Siren, M.D.
 Mikko Hänninen, M.B.
 Karri Kalervo, M.B.
 Suneeta Narumanchi, M.Sc.
 Katariina Immonen, B.Sc., laboratory technician
 Riikka Kosonen, M.Sc., laboratory technician
 Sanni Perttunen, B.Sc., laboratory technician

External funding

The Aarne Koskelo Foundation
 The Finnish Foundation for Cardiovascular Research
 The Finnish Foundation for Laboratory Medicine
 The Finska Läkaresällskapet
 The Liv och Hälsa Foundation
 Research Funding of the Helsinki-Uusimaa Hospital District
 (government special state subsidiary for health sciences)

CARDIOVASCULAR RESEARCH

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 the development of heart failure to identify potential, new targets for novel cardiovascular medicines.

We have shown previously that heme oxygenase-1 (HO-1) and carbon monoxide (CO) have potential roles in post-myocardial infarction (MI) cardiac repair. Our novel findings demonstrated that treatment with a specific CO-donor, CORM-3, improves both structural and functional cardiac recovery after myocardial injury. These effects were associated with increased expression of angiogenic factors and altered expression of several miRNAs involved in myocardial homeostasis, remodeling, and repair. Induction of the HO-1-CO pathway may prove to be a useful tool to protect against pathological myocardial changes and development of heart failure after MI, thereby facilitating cardiac recovery.

In collaboration with Docent Hannele Laivuori, University of Helsinki, we studied the association of HO-1 gene (*HMOX1*) polymorphisms with an increased risk of preeclampsia. Our finding that the long allele of a guanine-thymine microsatellite repeat seemed to predispose one to a late-onset, less severe form of preeclampsia supported the role of HO-1 in the pathogenesis of preeclampsia.

The role of HO-1 in cardiac arrest patients was studied in collaboration with Docent Ville Pettilä, Helsinki University Hospital (HUCH), utilizing patient material obtained from a 12-month prospective observational multicenter study (FINNRESUS-CI) from 21 Finnish intensive care units. We were

able to show that plasma HO-1 predicts 90-day mortality and long-term neurologic outcome after out-of-hospital ventricular fibrillation.

During 2014, our group has participated in the COST Action BM1005, European Network on Gasotransmitters in which Päivi Lakkisto served as a member of the Management Committee.

In collaboration with Professors Heikki Ruskoaho and Eero Mervaala, University of Helsinki, we studied the effects of selected microRNAs on cardiac regeneration using the cardiac cryoinfarction model in adult zebrafish. First, we validated protocols for efficient delivery of microRNAs and synthetic anti-microRNAs, or antagomirs, to the adult zebrafish. This approach enabled us to study molecules and pathways related to cardiac regeneration in a fast-growing, affordable model organism with an amazing regenerative capacity. Additionally, we developed a drug-induced cardiac hypertrophy/failure model in embryonic zebrafish. This model was utilized in collaboration with Professor Risto Kerkelä and coworkers, University of Oulu, to study the role of cardiac transcription factors and microRNAs in the pathogenesis of ventricular hypertrophy. Furthermore, the role and function of septin7b in the zebrafish heart was studied in collaboration with Dr. Surjya Dash and Professor Sanna Lehtonen, University of Helsinki. With easy delivery of drugs and methodology for gene silencing, these models provide a robust platform for studying the development of cardiac hypertrophy and failure, as well as factors affecting recovery of cardiac function from heart failure.

We have also been working in several collaborative clinical studies that continued in 2014. For example, in collaboration with Docent Veli-Pekka Harjola, Helsinki University Hospital, and Dr. Yvan Devaux, Centre de Recherche Public de la Santé, Luxembourg, we have been searching for potential miRNAs associated with the outcome of cardiogenic shock patients (CardShock project). The effects of drug treatment on the progression of disease in aortic stenosis patients have been investigated in collaboration with Professor Markku Kupari, Helsinki University Hospital (ROCKAS study). In collaboration with Docent Ville Pettilä, Helsinki University Hospital, potential new biomarkers of acute kidney injury (FINNAKI study) have been studied.

Despite effective treatment with currently available drugs, the prognosis of severe systolic heart failure is poor. One possible explanation for the progression of cardiac failure may be dysregulation of

cardiac reparative mechanisms. Our novel findings on cardiac regenerative potential open a new avenue for preventing and treating chronic heart failure after myocardial injury. By combining treatments promoting cardiac regeneration and repair with current established therapies for cardiovascular diseases, the results of the treatments could improve significantly in the future.

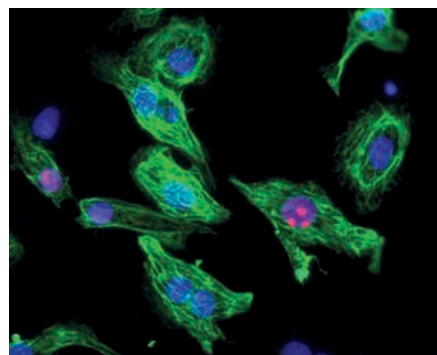


Figure. ki67+ cultured neonatal rat cardiomyocytes.

Publications

- Dash SN, Lehtonen E, Wasik AA, Schepis A, **Paavola J**, Panula P, Nelson WJ, Lehtonen S. Sept7b is essential for pronephric function and development of left-right asymmetry in zebrafish embryogenesis. *J Cell Sci.* 2014; 127(Pt 7):1476–86.
- Eräranta A, Törmänen S, Kööbi P, Vehmas TI, **Lakkisto P**, **Tikkanen I**, Moilanen E, Niemelä O, Mustonen J, Pörsti I. Phosphate binding reduces aortic Angiotensin-Converting Enzyme and enhances nitric oxide bioactivity in experimental renal insufficiency. *Am J Nephrol.* 2014; 39(5):400–8.
- Kaartokallio T, Klemetti MM, Timonen A, Uotila J, Heinonen S, Kajantie E, Kere J, Kivinen K, Pouta A, **Lakkisto P**, Laivuori H. Microsatellite polymorphism in the heme oxygenase-1 promoter is associated with nonsevere and late-onset preeclampsia. *Hypertension.* 2014; 64(1):172–7.
- Tikkanen I**, Narko K, Zeller C, Green A, Salsali A, Broedl UC, Woerle HJ; on behalf of the EMPA-REG BP Investigators. Empagliflozin reduces blood pressure in patients with type 2 diabetes and hypertension. *Diabetes Care.* 2014; Sep 30 (Epub).
- Zhou Y**, Wohlfahrt G, **Paavola J**, **Oikkonen VM**. A vertebrate model for the study of lipid binding/transfer protein function: Conservation of OSBP-related proteins between zebrafish and human. *Biochem Biophys Res Co.* 2014; 446(3):675–80.

Thesis completed in the group in 2014

The following doctoral thesis was accepted at the University of Helsinki this year:

Jere Paavola: Aberrant intracellular calcium cycling in the heart – mechanistic insights into catecholaminergic polymorphic ventricular tachycardia and heart failure. March 28, 2014.



TÖRNQUIST

Group members

Kid Törnquist, Ph.D., Professor, Head
Veronica Kalhori, M.Sc.

In addition the following researchers were working at Åbo Akademi University:

Basit Butt, M.D.
Mohammad Yasir Asghar, Phil.lic.
Kati Kemppainen, M.Sc.
Melissa Magnusson, M.Sc.
Ilari Pulli, M.Sc.

The group is part of the Centre of Excellence in Cell Stress and Molecular Ageing (2010–2014, Åbo Akademi University).

External funding

The Liv och Hälsa Foundation
The Sigrid Juselius Foundation
Åbo Akademi University

CELLULAR PHYSIOLOGY

Main research activities

The research group focuses on investigating 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 (TRPC)-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.

Our investigations using rat thyroid cells have shown that TRPC2, a member of the transient receptor potential (TRP) superfamily of ion channels, is an important regulator of thyrotropin receptor expression, calcium homeostasis, calcium-activated chloride channels (Anoctamins), and migration. In a novel study, we have now shown that a previously detected, phosphatase-regulated ion channel in rat thyroid cells is, in fact, the TRPC2 ion channel. This important link indicates that TRPC2 is under the regulation of protein kinase A. Furthermore, although most cells express several members of the TRPC family, rat thyroid cells express only TRPC2, highlighting the many-faceted role of this channel in thyroid physiology.

In normal, as well as cancerous, human thyroid cells, several members of the TRPC ion channel family are expressed. Since some members may have redundant effects and knock-down of one channel may give results difficult to interpret or have no effect, it is challenging to pinpoint the exact functions of the individual receptors. However, current efforts are aimed to understand the importance of TRPC channels in human thyroid cancer cells, especially on migration. These ongoing studies suggest that members of the TRPC channel family are important regulators of the expression

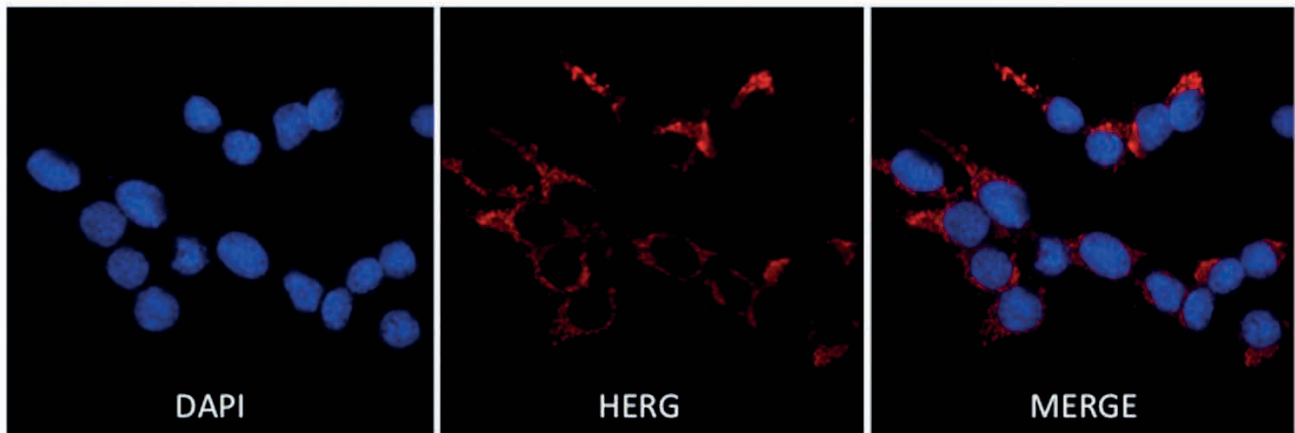


Figure. Staining of anaplastic C643 thyroid cancer cells with the nuclear DAPI stain, and for the HERG potassium potassium channel.

of receptors crucial for migration of the cancer cells. TRPC channels are permeable to both sodium and calcium ions, and it is already known that regulation of cell migration is dependent on the entry of calcium ions and not the depolarization obtained through the entry of sodium ions. Finally, we are also interested in understanding the interactions of these channels with S1P signaling and the regulation of the expression of matrix-metalloproteinases and hypoxia-inducible factor 1- α .

Ongoing work in our group also aims at understanding 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. Of special interest are calcium signals in the caveolae, endoplasmic reticulum and mitochondria. Together with our collaborators, we have developed novel tools to enhance these still ongoing studies.

Publications

Sukumaran P, Asghar MY, Phil L, Löf C, **Viitanen T**, **Törnquist K**. Phosphatase inhibitor calyculin A activates TRPC2 channels in thyroid FRTL-5 cells. *Calcium Signaling*. 2014; 1(2):1–7.

Törnquist K, Sukumaran P, Kemppainen K, Löf C, **Viitanen T**. Canonical transient receptor potential channel 2 (TRPC2): old name-new games. Importance in regulating of rat thyroid cell physiology. *Pflug Arch Eur J Phy*. 2014; Nov;466(11):2025–34.

Other publications

Pullil I, Löf C, **Törnquist K**. Utilizing Hidex Sense for mitochondrial and cytosolic calcium measurements using the luminescent calcium reporter protein aequorin and the fluorescent calcium indicator Fura-2 AM. 2014; Technical Report for HIDEX Corp.



YKI-JÄRVINEN

Group members

Hannele Yki-Järvinen, M.D., Dr.Med.Sci., F.R.C.P, Professor, Head
 Nidhina Haridas, Ph.D.
 Leena Juurinen, M.D., Dr.Med.Sci. spring 2014
 Anna Kotronen, M.D., Dr.Med.Sci., Docent
 Ksenia Sevastianova, M.D., Dr.Med.Sci.
 You Zhou, Ph.D.
 Hua Bian, M.D.
 Panu Luukkonen, M.D.
 Sanja Sädevirta, M.D.
 Antti Hakkarainen, M.Sc. Techn.
 Jarkko Soronen, M.Sc.
 Jenni Hyysalo, medical student
 Susanna Lallukka, medical student
 Elina Petäjä, medical student
 Aila Karioja-Kallio, laboratory technician
 Anne Salo, laboratory technician
 Mia Urjansson, laboratory technician

External funding

The Academy of Finland
 EU - European Medical Information Framework (EMIF)
 EU- H2020
 The Liv och Hälsa Foundation
 The Novo Nordisk Foundation
 Research Funding of Helsinki-Uusimaa Hospital District (EVO)
 The Sigrid Jusélius Foundation

ENDOCRINOLOGY

Main research activities

During 2014, we estimated the prevalence of non-alcoholic steatohepatitis (NASH) in a population-based study using scores validated against liver histology. Clinical and biochemical characteristics, *PNPLA3* (patatin-like phospholipase domain-containing protein 3) genotype at rs738409, and serum cytokeratin 18 fragments were measured in 296 patients who underwent a liver biopsy to discover a 'NASH score'. The score was validated in an Italian cohort comprising 380 mainly non-bariatric surgery patients. The cut-offs were utilized in the Finnish population-based D2D type 2 diabetes study involving 2849 subjects in an effort to estimate the population prevalence of NASH. The final 'NASH score' model included *PNPLA3* genotype, AST (aspartate aminotransferase) and fasting insulin. The model predicted NASH with equal accuracy, sensitivity and specificity in Finns and Italians. Based on the NASH score, the population prevalence of NASH in 45–74 year old Finnish subjects was 5%.

We also characterized circulating triacylglycerol (TAG) signatures in non-alcoholic fatty liver disease (NAFLD) associated with the I148M variant in *PNPLA3* ('*PNPLA3* NAFLD') and with obesity ('obese NAFLD'). A total of 372 subjects were divided into groups based on *PNPLA3* genotype or obesity. Absolute and relative deficiencies of distinct circulating TAGs were observed in the *PNPLA3*(148MM/148MI; minor allele carrier) group compared with the *PNPLA3*(148II) group. Obese and 'nonobese' groups had similar *PNPLA3* genotypes, but the obese subjects were insulin-resistant. Liver fat was similarly increased in obese and *PNPLA3*(148MM/148MI) groups. Relative concentrations of TAGs in the obese subjects versus nonobese displayed multiple changes. The changes closely resembled those between obese subjects with NAFLD but without the *PNPLA3*(I148M) allele

versus subjects with the I148M variant and NAFLD. We concluded that the etiology of NAFLD markedly influences circulating TAG profiles. ‘Obese NAFLD’ is associated with multiple changes in TAGs, which can be attributed to obesity/insulin resistance rather than increased liver fat content *per se*. Together with Docent Vesa Olkkonen, we have studied the cellular mechanisms underlying PNPLA3 NAFLD by overexpression of the PNPLA3(I148M) variant in human hepatocytes, [(13)C]glycerol labeling, and mass spectrometric lipidome analysis. We found that a defect in TAG remodeling activity likely contributes to the TAG accumulation observed in cells expressing PNPLA3(I148M).

A Chinese endocrinologist, Dr. Bia Huan spent one year in our research group at Minerva to study factors regulating liver volume in humans. She established an equation to predict liver volume in humans and used that equation to examine the impact of dietary interventions on liver volume. She found that liver volume decreases much more rapidly during a low carbohydrate than a low fat hypocaloric diet, a finding that has implications in trying to reduce liver volume before bariatric surgery.

Publications

- Bian H**, Hakkarainen A, Lundbom N, **Yki-Järvinen H**. Effects of dietary interventions on liver volume in humans. *Obesity* (Silver Spring). 2014; 22(4):989–95.
- Hyysalo J**, Gopalacharyulu P, **Bian H**, Hyötyläinen T, Leivonen M, Jaser N, Juuti A, Honka MJ, Nuutila P, **Olkkonen VM**, Oresic M, **Yki-Järvinen H**. Circulating triacylglycerol signatures in nonalcoholic fatty liver disease associated with the I148M variant in PNPLA3 and with obesity. *Diabetes*. 2014; 63(1):312–22.
- Hyysalo J**, Männistö VT, **Zhou Y**, Arola J, Kärjä V, Leivonen M, Juuti A, Jaser N, **Lallukka S**, Käkälä P, Venesmaa S, Simonen M, Saltevo J, Moilanen L, Korpi-Hyövalti E, Keinänen-Kiukaanniemi S, Oksa H, Orho-Melander M, Valenti L, Fargion S, Pihlajamäki J, Peltonen M, **Yki-Järvinen H**. A population-based study on the prevalence of NASH using scores validated against liver histology. *J Hepatol*. 2014; 60(4):839–46.
- Mori MA, Thomou T, Boucher J, Lee KY, **Lallukka S**, Kim JK, Torriani M, **Yki-Järvinen H**, Grinspoon SK, Cypess AM, Kahn CR. Altered miRNA processing disrupts brown/white adipocyte determination and associates with lipodystrophy. *J Clin Invest*. 2014; 124(8):3339–51.
- Ruhanen H**, **Perttilä J**, **Hölttä-Vuori M**, **Zhou Y**, **Yki-Järvinen H**, **Ikonen E**, Käkälä R, **Olkkonen VM**. PNPLA3 mediates hepatocyte triacylglycerol remodelling. *J Lipid Res*. 2014; 55(4):739–46.
- Yki-Järvinen H**. Non-alcoholic fatty liver disease as a cause and a consequence of metabolic syndrome. *Lancet Diabetes Endocrinol*. 2014; Nov;2(11):901–910.
- Yki-Järvinen H**, Bergenstal R, Ziemien M, Wardecki M, Muehlen-Bartmer I, Boelle E, Riddle MC; on behalf of the EDITION 2 study investigators. New insulin glargine 300 units/ml versus glargine 100 units/ml in people with type 2 diabetes using oral agents and basal insulin: glucose control and hypoglycemia in a 6-month randomized controlled trial (EDITION 2). *Diabetes Care*. 2014; Dec;37(12):3235–43.

Thesis completed in the group in 2014

The following doctoral thesis was accepted at the University of Helsinki this year:

Leena Juurinen: Insulin therapy in type 2 diabetes: Initiation, impact on liver fat, role of combination therapy with glitazones or glinides. May 16, 2014.



OLKKONEN

Group members

Vesa Olkkonen, Ph.D., Docent, Head
 Marion Weber-Boyvat, Ph.D.
 P.A. Nidhina Haridas, Ph.D.
 Henriikka Kentala, M.Sc.
 Raghavendra Mysore, M.Sc.
 Hanna Ruhanen, B.Sc.
 Eeva Jääskeläinen, laboratory technician

External funding

The Academy of Finland (M.W.-B.)
 The Diabetes Research Foundation
 The Finnish Foundation for Cardiovascular Research
 The Liv och Hälsa Foundation
 The Magnus Ehrnrooth Foundation
 The Sigrid Jusélius Foundation

LIPID SIGNALING AND HOMEOSTASIS

Main research activities

The group aims to elucidate how cells maintain specific lipid compositions of organelles and sense their internal lipid status to control a number of vital cellular processes, including signal transduction and vesicle transport. Oxysterols are oxidized derivatives of cholesterol that act as potent regulators of cellular lipid metabolism, signaling and differentiation. In this context, the cytoplasmic oxysterol-binding protein homologs, ORPs, are of major interest. The project aims to unravel the function of these proteins as lipid sensors and transporters with major impacts on a variety of downstream processes and their roles in diseases involving skewed lipid signaling.

In 2014, major activity was directed at understanding the spatial and temporal regulation of the interaction of ORPs with their endoplasmic reticulum (ER) anchors, the VAMP-associated proteins (VAPs). The results demonstrated that ORP sterol liganding modifies the interaction with VAPs and the subcellular distribution of ORP-VAP complexes. Several ORP-VAP complexes localize at membrane contact sites, which play central roles in the inter-organelle transport of lipids, control of Ca^{2+} fluxes, and signaling processes. A complex of ORP2 with VAPA at endoplasmic reticulum-lipid droplet junctions (Figure 1) was shown to impact the cellular metabolism of triacylglycerols (Weber-Boyvat et al. 2014). Collaborative efforts with the group of M. Van Eck (Leiden, the Netherlands) provided evidence for a proatherogenic role of macrophage ORP8 (Van Kampen et al. 2014), and work with D. Yan (Guangzhou, China) demonstrated that ORP8 interacts with the kinetochore protein Astrin/SPAG5, facilitating oxysterol-mediated interference of the cell cycle (Zhong et al. 2014).

In addition, we carried out a collaborative project on the molecular etiology of non-alcoholic fatty liver disease (NAFLD) with the Endocrinology

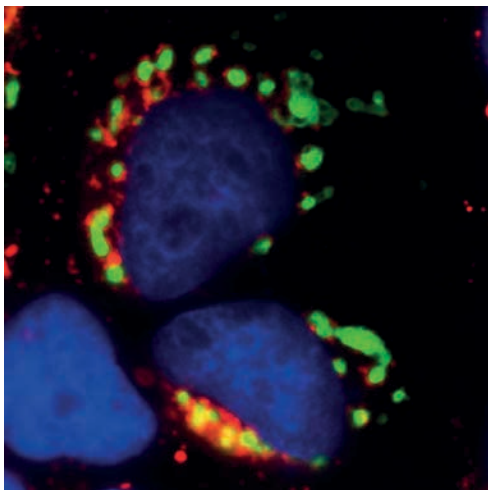
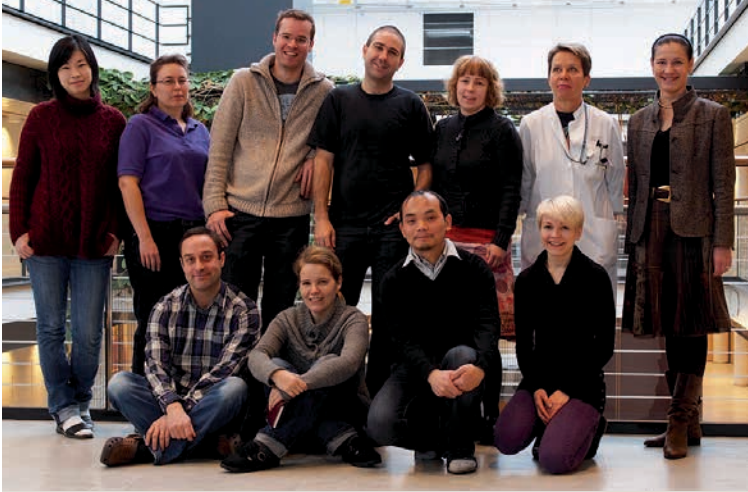


Figure. A bimolecular fluorescence complementation (BiFC) image revealing the interaction site of the oxysterol-binding protein (OSBP)-related protein ORP2 and the VAMP-associated protein VAPA in HuH7 human hepatoma cells. ORP2 is C-terminally fused to the N-terminal half of the Venus fluorescent protein, and VAPA is C-terminally fused to the C-terminal half of Venus. Shown in green is the BiFC interaction between the ORP2 and VAPA proteins, in red, lipid droplets stained with BodipyC12 and in blue, the nucleus stained with DAPI.

group led by Prof. Hannele Yki-Järvinen. NAFLD affects more than 20% of the population in Western countries and predisposes one to insulin resistance/metabolic syndrome, liver disease, type 2 diabetes and cardiovascular complications. During 2014, we finalized work suggesting that the PNPLA3 protein underlying a genetic form of NAFLD facilitates the remodeling of cellular TAGs, an activity defective in the PNPLA3 I148M variant associated with elevated liver fat content (Ruhanen et al. 2014). In addition, we focused on the role of microRNAs in NAFLD (manuscript in preparation) as well as the insulin-mediated regulation and function of angiotensin-like proteins 3, 4 and 8, important controllers of lipid metabolism and insulin sensitivity (manuscript submitted for review). Finally, a new project aimed at understanding the function of *TM6SF2* was initiated. A polymorphism of this gene is associated with high liver fat content but a reduction of circulating beta-lipoproteins and provides protection from myocardial infarction.

Publications

- Brown AJ, **Ikonen E, Oikkonen VM**. Cholesterol precursors: more than mere markers of biosynthesis. *Curr Opin Lipidol*. 2014; 25(2):133–9.
- Ruhanen H, Perttälä J, Hölttä-Vuori M, Zhou Y, Yki-Järvinen H, Ikonen E, Käkälä R, Oikkonen VM**. PNPLA3 mediates hepatocyte triacylglycerol remodelling. *J Lipid Res*. 2014; 55(4):739–46.
- Van Kampen K, **Beaslas O**, Hildebrand RB, Lammers B, Van Berkel TJC, **Oikkonen VM**, Van Eck M. Orp8 deficiency in bone marrow-derived cells reduces atherosclerotic lesion progression in LDL receptor knockout mice. *PLoS One*. 2014; Oct 27;9(10):e109024. doi: 10.1371/journal.pone.0109024. eCollection 2014.
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IKONEN

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The Academy of Finland: Centre of Excellence in Biomembrane Research
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 The Finnish Foundation for Cardiovascular Research
 The Sigrid Jusélius Foundation
 Biocentrum Helsinki, Biocenter Finland
 University of Helsinki, Research excellence and infrastructure funding

MEMBRANE BIOLOGY

Main research activities

Our group aims to understand how cholesterol functions in the cellular context, both under physiological conditions and in human diseases. To this end, we also develop novel imaging techniques to analyze cholesterol and other lipids in cells. Cholesterol is an essential component of cell membranes, where it dictates important biophysical properties of the bilayer and participates in lipid-protein interactions. These aspects are important for understanding the mechanisms that underlie the adverse effects of cholesterol. Besides cardiovascular diseases, disturbances in cholesterol metabolism are implicated e.g., in several neurodegenerative diseases.

Cholesterol metabolism and Alzheimer's disease (AD) are connected, but the molecular mechanisms involved are not well understood. Amyloid precursor protein (APP) undergoes amyloidogenic processing (beta-cleavage), that predisposes a patient to AD, in a cholesterol-dependent manner. Moreover, increasing evidence suggests that APP itself regulates cholesterol metabolism. We have recently discovered a new link between APP and cholesterol that is relevant both physiologically and in AD: APP regulates the key transcription factor of cholesterol metabolism, sterol-regulatory element binding protein 2 (SREBP2) via its secretory ectodomain fragments. This regulation can be either positive or negative, depending on APP non-amyloidogenic vs. amyloidogenic (alpha vs. beta) processing. The physiologically dominant, APPs-alpha fragment stimulates SREBP2, leading to enhanced cholesterol synthesis and LDL receptor levels. In contrast, APP secretory beta fragment suppresses SREBP2 signaling. In line with this finding, in familial AD patients with increased beta-cleavage, serum cholesterol synthesis markers were decreased and fibroblast LDL-receptor levels were reduced (Wang et al., 2014).

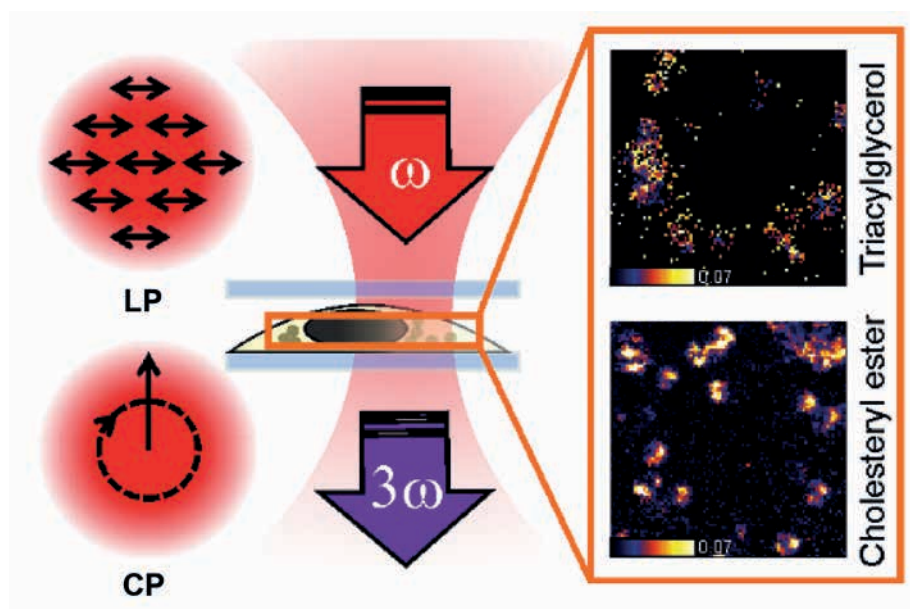
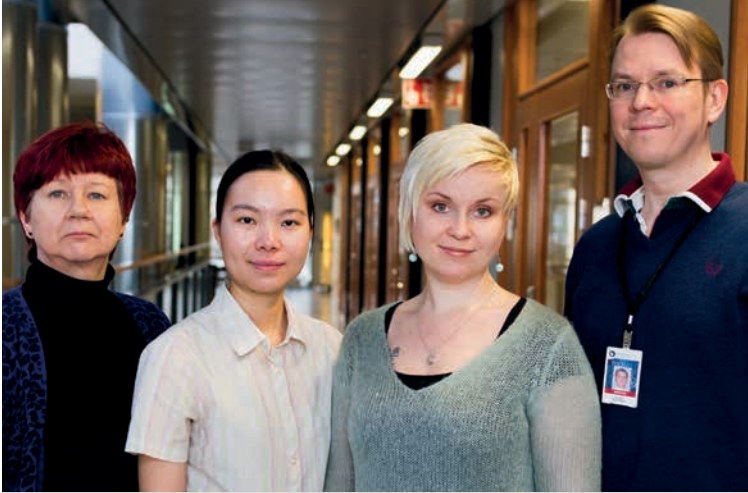


Figure. The principle of third-harmonic generation (THG) microscopy for label-free imaging of cellular lipid storage. Left: Both linearly polarized (LP) and circularly polarized (CP) light can be used to generate signals from lipid droplets. Right: Representative images of single cells with either triacylglycerol or cholesteryl ester deposition. Cholesteryl ester storage generates a more pronounced THG signal, with individual lipid droplets visualized. Courtesy of Dr. Godofredo Bautista, Tampere University of Technology (for details see Bautista et al., *Biophys J* 107:2230).

Cells can store excess lipids in cellular lipid droplets. The major storage lipids inside lipid droplets are cholesteryl esters (CE) and triacylglycerols (TAG). Lipid droplets can be visualized in cells without exogenous labels by non-linear imaging techniques. In collaboration with physicists, we have recently discovered that one such technique, polarized third-harmonic generation (THG) microscopy, can differentiate between native TAG- and CE-enriched lipid droplets in mammalian cells. This distinction is based on the differential ordering of the two lipid classes (Bautista, Pfisterer et al., 2014). The degree of lipid ordering is thought to play an important role in the mobility and enzymatic processing of lipids in lipid droplets. This technique may, in the future, be useful for differentiating lipid storage types in a label-free fashion.

Publications

- Bautista G, **Pfisterer SG**, Huttunen MJ, Ranjan S, **Kanerva K**, **Ikonen E**, Kauranen M. Polarized THG microscopy identifies compositionally different lipid droplets in mammalian cells. *Biophys J*. 2014; Nov 18;107(10):2230–6.
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KOISTINEN

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External funding

The Academy of Finland
 Jalmari and Rauha Ahokas Foundation
 Finnish Diabetes Research Foundation
 The Finska Läkaresällskapet
 The Liv och Hälsa Foundation
 The Novo Nordisk Foundation
 Research Funding of Helsinki-Uusimaa Hospital District (EVO)
 The Sigrid Jusélius Foundation

METABOLISM

Main research activities

In 2014, the primary focus of research activity in the group was on the regulation of glucose and fatty acid metabolism in skeletal muscle. To this end, the team established a collection of primary human muscle cells from clinically characterized subjects from different glucose tolerance phenotypes, including normal glucose tolerance (NGT), impaired fasting glucose (IFG), impaired glucose tolerance (IGT), and type 2 diabetes (T2D). A variety of biochemical and functional analyses with these collections are currently ongoing.

In FUSION (Finnish United States Investigation on NIDDM Genetics) Tissue Biopsy study we collected muscle, adipose and skin samples from a cohort of >300 clinically characterized Finnish people across all glucose tolerance stages. Sampling was performed in 2009–2013. Analysis of the skeletal muscle transcriptome by RNA sequencing of *vastus lateralis* muscle biopsies from 279 subjects was completed in 2014. With these patient materials, we performed dense genotyping and imputation, as well as constructed and sequenced strand-specific mRNA-seq libraries. Using expression quantitative trait loci (eQTL), we identified >8000 genes, some of which appear to be disease-state specific. Multiple eQTLs are in high linkage disequilibrium with genome-wide association studies single nucleotide polymorphisms for T2D, pinpointing genes as candidates for a role in T2D risk. Further analyses of FUSION Tissue Biopsy samples are currently ongoing.

As a part of a large international collaboration, whole-exome sequencing data from peripheral blood DNA of >17,000 persons was analysed. The presence of detectable somatic mutations rose in frequency with advancing age, with prevalence being highest in age group of people >90 years of age. The highest proportion of somatic mutations occurred in three genes: *DNMT3A*, *TET2*, and *ASXL1*, and the presence of a variant increased not only the

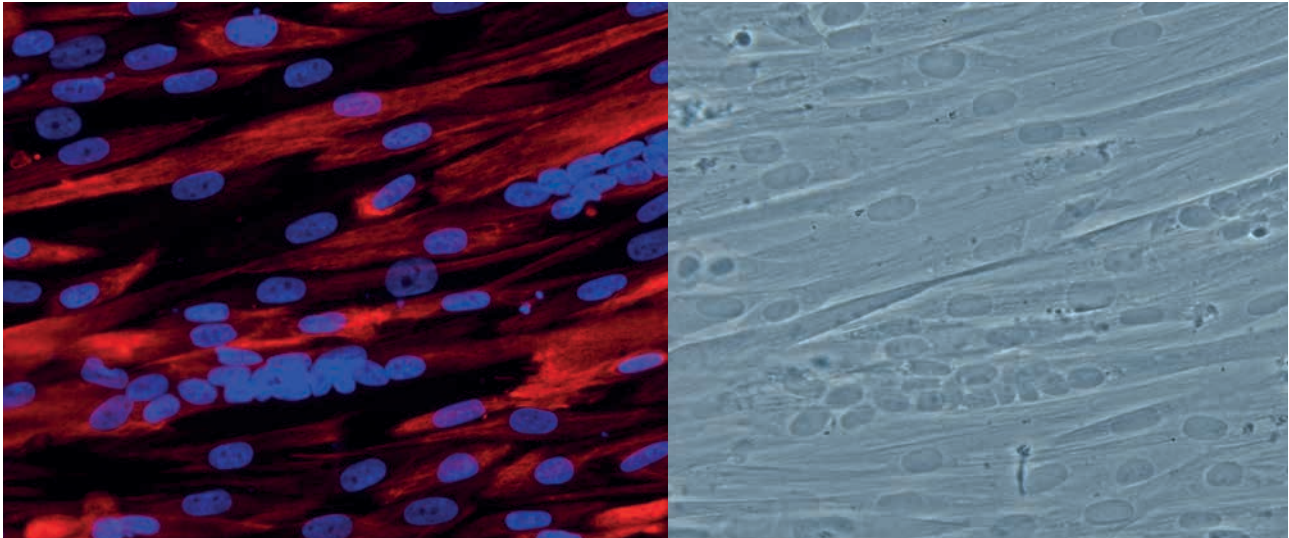


Figure. Human myotubes after seven days of differentiation. For immunocytochemistry, cells were stained with DNA binding dye DAPI to show nuclei (blue) and muscle-specific antibody desmin (red) to show the myotubes. The photograph was taken at 20X magnification using a Leica fluorescence microscope.

risk of hematological malignancy, but increased all-cause mortality and the risk of cardiovascular disease, as well.

Publications

Jaiswal S, Fontanillas P, Flannick J, Manning A, Grauman PV, Mar BG, M.D., Lindsley RC, Mermel CH, Burt N, Chavez A, Higgins JM, Moltchanov V, Kuo FC, Kluk MJ, Henderson B, Kinnunen L, **Koistinen HA**, Ladenvall C, Getz G, Correa A, Banahan BF, Gabriel S, Kathiresan S, Stringham HM, McCarthy MI, on behalf of T2D-GENES; Boehnke M, on behalf of GoT2D; Altshuler D, on behalf of SIGMA T2D, Tuomilehto J, Haiman C, Groop L, Atzmon G, Wilson JG, Neuberg D, Altshuler D, Ebert BL. Age-related clonal hematopoiesis associated with adverse outcomes. *New Engl J Med*. 2014; 25;371(26):2488–98.



LINDHOLM

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 Kristiina Söderholm, laboratory technician

External funding

The Academy of Finland
 The Finska Läkaresällskapet
 The Liv och Hälsa Foundation
 The Magnus Ehrnrooth Foundation
 The Sigrid Jusélius Foundation

NEUROSCIENCE

Main research activities

We study basic mechanisms underlying neurodegenerative diseases and nerve cell damage in the brain. Our specific areas of focus are protein ubiquitination and the role of mitochondrial and endoplasmic reticulum (ER) stress pathways as well as autophagy in the pathogenesis and models of brain diseases. Furthermore, we examine neurotrophic factors, such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) and their tyrosine kinase receptors, Trk and p75, in regulation of cell-specific events. We employ various proteomic and gene expression methods, cell culture and animal models of disease and genetically modified mice. Although the majority of the group is housed in the Institute of Biomedicine, Faculty of Medicine, the group is actively engaged in research at Minerva.

Molecular Mechanisms underlying Neurodegenerative Diseases

1. Role of Usp14 in protein aggregation diseases

Mitochondria dysfunction and ER stress play a role in human degenerative diseases afflicting the brain. Using a cellular model, we have previously shown that Sigma-1 receptors present in mitochondria-associated ER membrane structures (MAMs) play a role in Huntington's disease (HD) by counteracting ER stress. We have now shown that aggregates of mutant huntingtin (Htt) protein are decreased by expression of the deubiquitinating enzyme (DUB) Usp14. Usp-14 is widely expressed in brain neurons and had been linked previously to nerve cell signaling as shown in an ataxia (axJ) mouse model lacking Usp14. We found that Usp-14 plays a role in clear-

ing of mutant Htt protein aggregates by binding to the ER protein, IRE1 α (Hyrskyluoto et al, 2014). Using a mouse model for HD, we further showed that the Usp14-IRE1 α interaction is specifically reduced in striatum of an animal model of HD suggesting a functional role of Usp14 in HD pathogenesis (Hyrskyluoto et al, 2014). Recently we observed that Usp14 expression increases autophagy flux in neuronal cells via the autophagy-associated protein LC3B in autophagosomes. Our further studies are focused on understanding the molecular mechanisms of the Usp14-mediated autophagy flux and the role that Usp14 may play in other neurological diseases including Parkinson's disease (PD).

2. Role of PGC-1 in neuroprotection and control of mitochondria functions in neurons

With regard to PD, we also study the transcriptional coactivator protein, peroxisome proliferator activated receptor-gamma (PPAR-gamma) coactivator 1- α (PGC-1) that is a master regulator of mitochondrial and oxidative stress in cells. Previously, using transgenic mice overexpressing PGC1 in the brain, we showed that dopaminergic neurons in the substantia nigra are resistant to the neurotoxin MPTP (Mudo et al 2012). In addition, we recently showed that brain neurons in the PGC-1 transgenic mice are partially protected against excitotoxic injury induced by the glutamate receptor agonist kainic acid. Gene profiling and proteomic studies of brain tissue from control and PGC-1 transgenic mice revealed an interesting pattern of changes. These changes were most prominent in mitochondria-associated proteins that contribute to increased nerve cell viability in the PGC-1 transgenic mice. These genes and proteins will be the objectives of future studies.

PGC-1 acts by binding the PPAR-gamma receptor in cell nuclei to regulate various genes. We have therefore analyzed the effects of chemical compounds and drugs that are known to influence PPAR-gamma signaling. These drugs are usually employed for treatment of metabolic disorders such as type-2 diabetes, and we are studying whether they may also have beneficial effects in brain disorders (Patrone et al., 2014). For many of these studies we are using the Seahorse XF Analyzer that permits testing of mitochondrial functions living neu-

rons in real-time. As a result, we have obtained evidence that some growth factors (Mäkelä et al., 2014) as well as bona fide neurotrophic factors (unpublished) regulate the levels and activity of PGC-1 in neuronal cells. We are currently exploring these mechanisms in more detail.

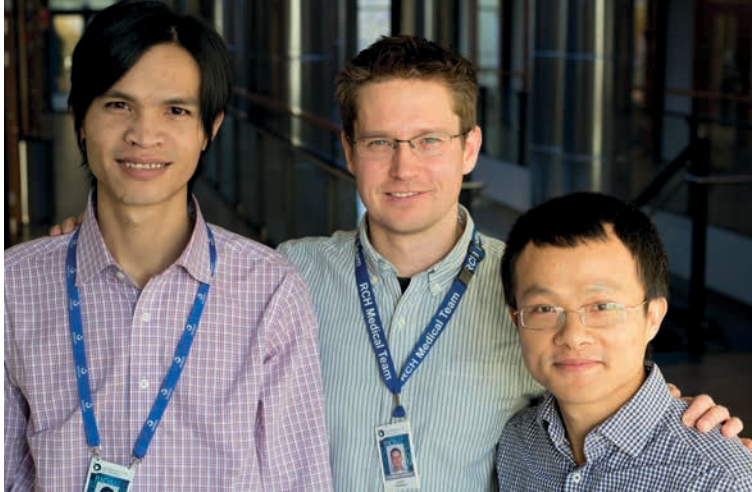
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Thesis completed in the group in 2014

The following doctoral thesis was accepted at the University of Helsinki this year:

Aiise Hyrskyluoto: Endoplasmic reticulum stress, regulation of autophagy and ubiquitin-proteasome system in cellular models of Huntington's disease. September 12, 2014.



STENMAN

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External funding

The Finska Läkaresällskapet
 The Liv och Hälsa Foundation

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 pre-sequencing amplification.

During the past year we have worked intensively on a novel technique for ultra-sensitive detection of expressed *KRAS* mutations in patients with colorectal cancer. This technique could provide a means not only for disease monitoring from plasma samples, but also for early detection of malignant transformation in pre-malignant conditions, such as Barrett's esophagus and Primary Sclerosing Cholangitis, where there are specific difficulties with currently available diagnostic tests. Our first paper describing this technique has recently been published. We have applied this technique for studying the occurrence of expressed *KRAS* and *BRAF* mutations in pediatrics patients with inflammatory bowel disease and patients treated for oesophageal atresia in their early childhood (manuscripts under preparation). In the coming year we are planning to study a large sample series consisting of 800 colon cancer FFPE (formalin-fixed, paraffin-embedded) samples.

We have continued development of a novel PCR amplification technology called Heat Pulse Extension PCR (HPE-PCR). Specifically, we have shown that difficulties in amplifying long GC rich and repetitive sequences can be overcome by using pulsatile temperature cycling during the extension step of each PCR cycle to destabilize secondary structures in the template. 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. Currently, we are focusing on improving the reaction efficiency further in order to enable amplification of longer repeat expan-

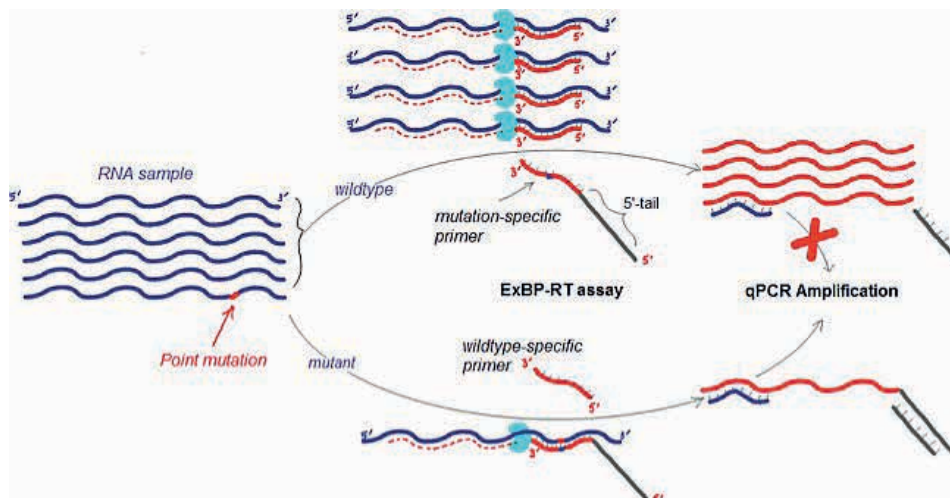


Figure. Schematic of the Extendable Blocking Probe reverse transcription (ExBP-RT) method that enables ultra-sensitive detection of expressed mutation through allele-specific reverse transcription. The method was published in *Nucleic Acids Research* in January 2015.

sions that are present in Amyotrophic Lateral Sclerosis (ALS). We have also achieved very promising results in utilizing HPE-PCR for reduction of GC-bias in sequencing pre-amplification and thus improving the coverage of poorly amplifying regions. European and US patents were granted for the HPE-PCR technology during 2014.

Publications

Ho TH, Dang KX, Lintula S, Hotakainen K, **Feng L, Oikkonen VM**, Verschuren EW, Tenkanen T, Haglund C, Kolho KL, Stenman UH, **Stenman J**. Extendable blocking probe in reverse transcription for analysis of RNA variants with superior selectivity. *Nucleic Acids Res*. 2014 Nov 5 (Epub).



FYHRQUIST

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The Finska Läkaresällskapet
The Liv och Hälsa Foundation

TELOMERE RESEARCH

Main research activities

The team has studied leucocyte telomere length (LTL) in more than 5000 subjects to-date and examined LTL in comparison with various behaviors and medical and genetic profiles. For example, in the Helsinki Businessman Study, LTL was inversely related to smoking and body mass index during a long follow-up period, from 1964–1973 to 2002–2003, during which DNA for telomere measurement was isolated from blood leucocytes of 622 participants. Moreover, we discovered associations of LTL with 1) alcohol consumption, 2) physical activity, and 3) insertion deletion (I/D) polymorphism of the angiotensin-I converting enzyme (ACE) gene.

A new study, focusing on telomeres and cardiovascular health in the young, was started in 2012 in collaboration with Professor A. Jula (Institute of Health and Welfare, Turku) as a sub-study of the STRIP project (Sepelvaltimotaudin Riskitekijöiden Interventio

Projekti) comprising >500 subjects, each in the intervention and control groups, with follow-up covering early childhood to maturity. This study has been ongoing during 2013–2014, and results will be analyzed during early 2015. Preliminary results suggest that healthy life style intervention is associated with slower telomere attrition compared with controls.

DVM Anders Eriksson has published a study in Cavalier King Charles spaniels with mitral regurgitation showing the predictive value of NT-proANP measurements in the evaluation of congestive heart failure.

Publications

Eriksson AS, Häggström J, Pedersen HD, Hansson K, Järvinen AK, Haukka J, Kvarn C. Increased NT-proANP predicts risk of congestive heart failure in Cavalier King Charles spaniels with mitral regurgitation caused by myxomatous valve disease. *J Vet Cardiol.* 2014; 16:141–154.

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MINERVA FOUNDATION SYMPOSIUM AND MEDIX PRIZE 2014 AWARD CEREMONY

Lipid Storage and Signaling in Disease

September 22, 2014

Minerva Foundation and researchers of the Minerva Institute organized a one-day symposium on Sept 22, 2014. Six invited international lecturers and two of Minerva Institute group leaders addressed in their presentations distortions of lipid signaling and storage in metabolic disease, neurodegenerative disorders and cancer.



Medix Prize 2014 was awarded to prof. Elina Ikonen's group from University of Helsinki, Institute of Biomedicine. The certificate of honor was handed out by dean Risto Renkonen to Elina Ikonen and Kristiina Kanerva.



Quentin Anstee, Newcastle University, UK:
Genetic modifiers of non-alcoholic fatty liver disease (NAFLD)



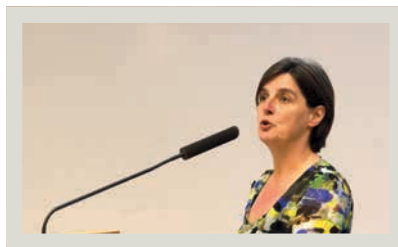
Tobias Hartmann, Saarland University, Germany:
Alzheimer's disease: The lipid connection



Anna Krook, Karolinska Institutet, Sweden:
Regulation of skeletal muscle insulin sensitivity



Cesare Patrone, Karolinska Institutet, Sweden:
Palmitate decreases adult neural stem cell viability



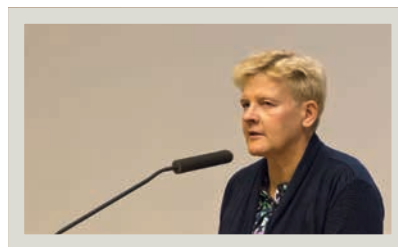
Susan Pyne, University of Strathclyde, UK:
The role of sphingosine 1-phosphate in cancer



Kid Törnquist, Minerva Foundation Institute for Medical Research, Finland: *Interactions between the TRPC1 ion channel, sphingosine 1-phosphate receptor 3, and VEGF receptor 2 in thyroid cancer cells*



Tobias Walther, Harvard School of Public Health and Harvard Medical School, USA:
The phase of fat: Mechanisms and consequences of neutral lipid storage



Hannele Yki-Järvinen, Minerva Foundation Institute for Medical Research, Finland:
Subtypes and diagnosis of NAFLD



