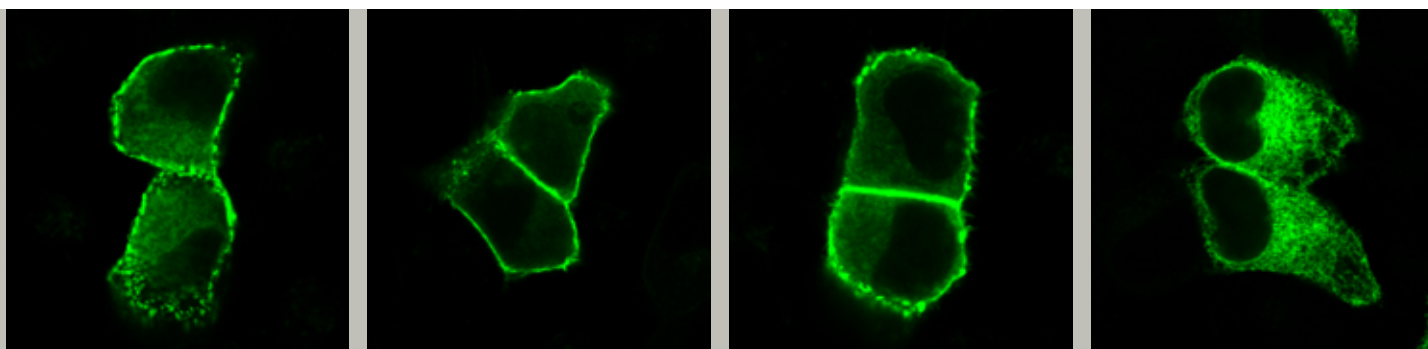
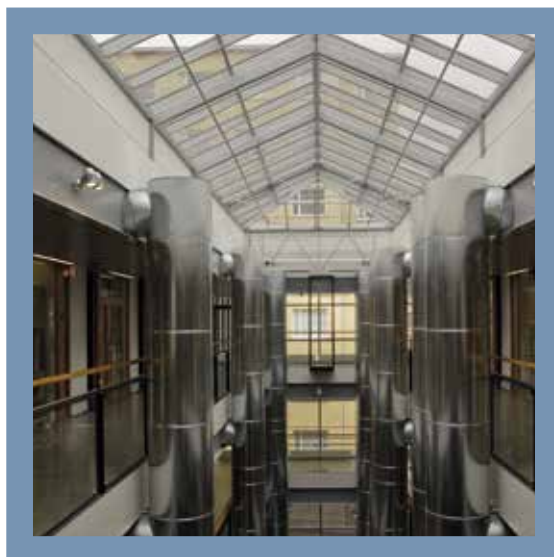


MINERVA FOUNDATION
AND
MINERVA FOUNDATION INSTITUTE
FOR MEDICAL RESEARCH



SCIENTIFIC REPORT 2012





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Front page image: Green fluorescent protein (GFP) fusions of human OSBP-related protein 8 (ORP) and its mutant forms expressed in the HuH7 hepatoma cell line (courtesy of Terhi Vihervaara/Unit of Lipid signaling and homeostasis)

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SUMMARY OF RESEARCH AND ACTIVITIES DURING THE YEAR 2012

The Minerva Foundation Institute for Medical Research is a privately owned research institute located at the Biomedicum-Helsinki Research Center on the Meilahti campus of the University of Helsinki. The Institute focuses on creating a fruitful combination of basic biomedical research and clinical investigations relevant for common disease. The objectives of research range from the study of fundamental molecular mechanisms in cultured cell models and genetically manipulated animals to the investigation of human patient materials. The aim is to generate new knowledge bases and 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 2012 is summarized in this report.

The funds of Minerva Foundation are directed at maintaining and further developing a research infrastructure that serves, in the most effective way, the substantive activities of the groups, and encourages the scientists to maximize efforts to acquire external research funds. During 2012, the Institute budget was covered 48% by funds from the Minerva Foundation and 52% by external, competitive research funds.

To enhance the research impact and maximize the investment gain of the Institute, it is necessary to constantly enhance integration within Minerva and to facilitate the employment of up-to-date technologies in the research. Therefore, in 2012 the

groups took concerted action to acquire modern recombinant virus tools for the knockdown or overexpression of the disease associated gene products under study. With targeted financial support from the Liv och Hälsa Foundation, the new viral tools were obtained and an isolated class 2 cell culture unit was equipped for their use. To further facilitate integration and provide the other research organizations at the Meilahti campus information on the work carried out at Minerva, three symposia were organized: On Sept 28th, all Minerva research groups presented snapshots of their research at an open Minerva symposium. In addition a minisymposium with two lectures by Minerva's distinguished guest lecturers and two lectures by junior scientists at Minerva was arranged on Oct 4th. Furthermore, a seminar to honor the impressive scientific career of one of Minerva's founders, Prof. Ralph Gräsbeck, was held at the Biomedicum Faculty Club on Oct 29th. A major challenge is to achieve a critical mass in core research areas that further boosts the scientific impact of the Institute. In 2012, research of cellular lipid metabolism and cardiovascular diseases was reinforced by the addition of a new research group, that of Membrane Biology, led by academy professor Elina Ikonen.

The work of the research groups in 2012 was highly productive. Minerva scientists published 54 articles, 45 of which appeared in citation ranked international peer reviewed journals, with a mean impact factor of 4.21. In addition, three Ph.D. degrees were completed in the research groups of Minerva.

EVENTS AT MINERVA 2012

Seminars and symposia

MINERVA SYMPOSIUM

September 28th, Biomedicum Helsinki 1

MINERVA MINISYMPOSIUM

Cellular Lipid Metabolism and Metabolic Disease
October 4th, Biomedicum Helsinki 1

MINERVA SEMINAR

RALPH GRÄSBECK: "Hooked to vitamin B12 since 1955"
October 29th, Biomedicum Helsinki 1

Dissertations

Paulina Skrobuk: Glucose and lipid metabolism in human skeletal muscle. September 7, 2012. (Metabolism)

Terhi Vihervaara: The role of oxysterol binding proteins in macrophages. October 5, 2012. (Lipid Signalling and Homeostasis)

Noora Putkonen: Organelle specific mechanisms of neuronal cell death. October 26, 2012. (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, 2012, to December 31, 2012. 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
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Carl-Magnus Westermarck

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Jim Schröder, chair
Per-Henrik Groop
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Caj Haglund

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Per-Henrik Groop, chair
Johan Eriksson
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THE MINERVA FOUNDATION INSTITUTE FOR MEDICAL RESEARCH

Board of Directors

Docent Vesa Olkkonen, chair
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Docent Ilkka Tikkanen
Professor Kid Törnquist
Professor Hannele Yki-Järvinen
Carita Estlander-Kortman
Cia Olsson, secretary



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 Päivi Lakkisto, M.D., Dr.Med.Sci.
 Hanna Forsten, V.M.D.
 Jere Paavola, M.D.
 Heli Segersvärd, M.D.
 Juuso Siren, M.B.
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Funded by the Minerva Foundation, Finska
 Läkaresällskapet, the Liv och Hälsa Foundation,
 the Finnish Foundation for Cardiovascular
 Research, the Finnish Medical Foundation / the
 Finnish Medical Society Duodecim, the Aarne
 Koskelo Foundation, the Orion-Farmos Research
 Foundation, the Ida Montin Foundation, the Oskar
 Öflund Foundation, and Hospital District of Helsinki
 and Uusimaa research grants (HUS/EVO).

CARDIOVASCULAR RESEARCH

Main research activities

The group studies mechanisms and repair of end organ damage in cardiovascular and renal diseases. The group is investigating the local expression and effects of components of the renin-angiotensin-aldosterone system, and other vasoactive factors and apoptosis in hypertension, heart failure, and progression of renal damage. In addition, the cardiovascular and renal protective properties of new cardiovascular drugs was evaluated. During recent years, the groups 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 cardiac function after MI, by promoting neovascularization and increase in the number of cardiomyocytes in the infarct area. Induction of 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.

The role of local, paracrine mechanisms of cardiac regeneration was studied in collaboration with Professor Ari Harjula's research group by utilizing modified myoblast cell sheet transplantation methodology. Overexpression of vascular endothelial growth factor, VEGF, was shown to improve mesenchymal stem cell sheet transplantation therapy for acute myocardial infarction.

In collaboration with Professor Ehrlich and Associate Professor Qyang, we established a murine embryonic stem cell (mESC) line for production of homogeneous, functional ventricular cardiomyocytes. We used immunohistochemistry, current clamp recordings, real-time intracellular calcium imaging, isoproterenol stimulation, and external electrical stimulation to characterize these cells, which may be used for basic research and cardiac repair.

In collaboration with Docent Aalto-Setälä, we used induced pluripotent stem cell (iPSC)-derived cardiomyocytes to study the cellular pathomechanisms of catecholaminergic polymorphic ventricular tachycardia (CPVT). These cardiomyocytes,

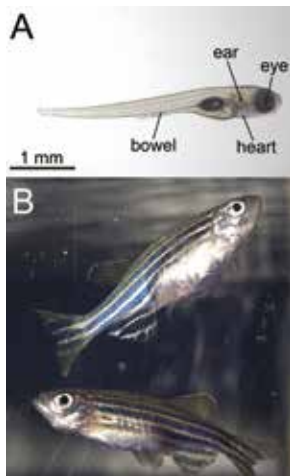


Figure. (A) Zebrafish larva at 9 days post-fertilization. (B) Adult zebrafish.

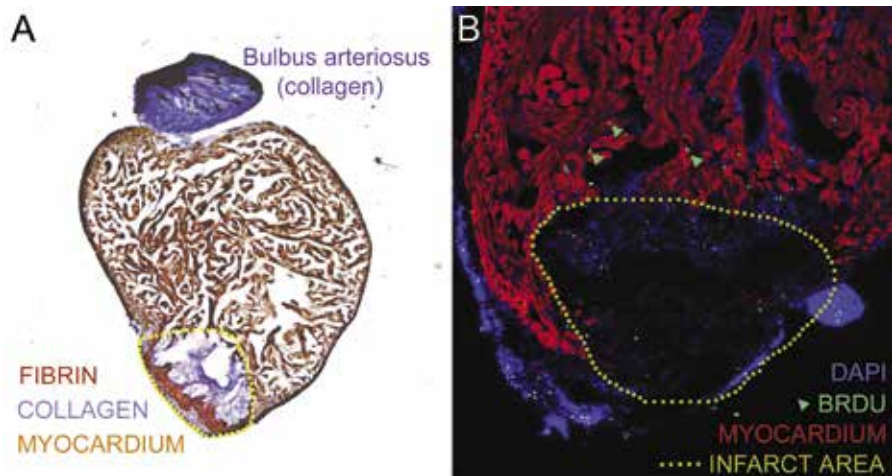


Figure. Cryoinjury-induced myocardial infarction. (A) AFOG-stained sagittal section of an adult zebrafish ventricle 14 days post-injury (dpi) showing myocardium (orange), collagen (blue), and fibrin (red). Infarct area indicated by the broken yellow line. (B) Immunohistochemistry on a sagittal section 14 dpi showing myosin heavy chain (myocardium, red), DAPI (nuclei, blue), and BRDU-positive cells (green arrowheads), indicating cells that are newly formed after the cryoinjury.

which carry a mutation in the cardiac ryanodine receptor (RyR2), showed abnormal calcium handling and arrhythmias induced by adrenergic stimulation. Patch-clamp recordings of CPVT cardiomyocytes showed both delayed afterdepolarizations (DADs) and early afterdepolarizations (EADs). These results, with the cell model, recapitulated the changes seen in monophasic action potential and 24h-ECG recordings in the same patient. Thus, our cell model of CPVT provides a platform to study basic pathophysiology, to screen drugs, and to optimize drug therapy.

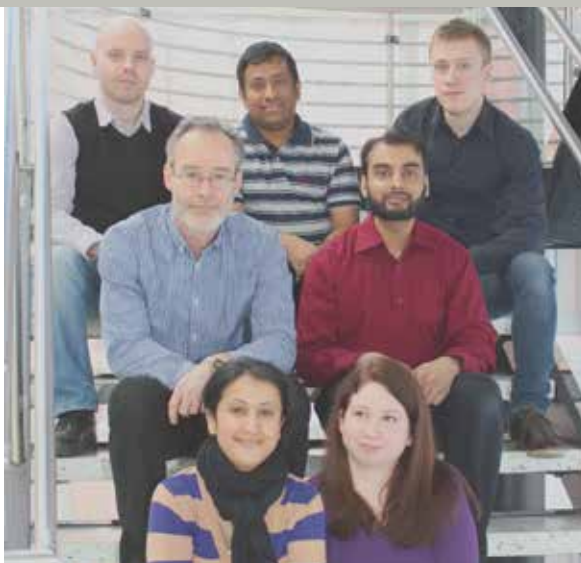
We also set up a cardiac cryoinfarction model in adult zebrafish. This method enables the study of molecules and pathways related to cardiac regeneration in a fast-growing, affordable model organism with an amazing regenerative capacity. We conducted pilot studies to optimize methodology for immunohistochemistry and histology, as well as analyses involving the effect of carbon monoxide (CO) on cardiac regeneration.

In collaboration with Professor Konttinen, we investigated the effects of histamine 3 receptor (H_{3R}) on intracellular calcium cycling and contractility in skeletal muscle cells. H_{3R} is highly expressed and appears to regulate the contractile state of these cells.

Despite effective treatment with currently available drugs, prognosis of severe systolic heart failure is poor. One possible explanation for the progression of cardiac failure may be dysregulation of cardiac reparative mechanisms. The novel findings on cardiac regenerative potential open a completely new avenue for preventing and treating chronic heart failure after myocardial injury. By combining treatments promoting cardiac regeneration with current established therapies for cardiovascular diseases, the results of the treatments could improve significantly in the future.

Publications

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- Kujala K, **Paavola J**, Lahti A, Larsson K, Pekkanen-Mattila M, Viitasalo M, Lahtinen AM, Toivonen L, Kontula K, Swan H, **Laine M**, Silvennoinen O, Aalto-Setälä K. Cell model of catecholaminergic polymorphic ventricular tachycardia reveals early and delayed afterdepolarizations. *PLoS One* 2012; 7(9):e44660
- Paavola J**. Zebrafish as a model organism in cardiovascular research. *Sydänääni* 2012; 3 (review)
- Tikkanen I**. Munuaisten denervatio – uusi hoitokeino hoitoresistenttiin hypertensioon. *Erikoislääkäri* 2012 (review)



► Group Members

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

Christoffer Löf, Ph.D.
Kati Kemppainen, M.Sc.
Pramod Sukumaran, M.Sc.
Yasir Asghar, M.Phil.
Ilari Pulli, M.Sc.

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

Collaborators

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Funded by The Sigrid Juselius Foundation, the Liv och Hälsa Foundation, and Åbo Akademi University. Christoffer Löf was funded by the Turku Doctoral Program of Biomedical Sciences.

CELLULAR PHYSIOLOGY

Main research activities

The main interests of the group are to understand how calcium signaling is regulated in various cell types, how different lipid metabolites (especially the sphingomyelin metabolite sphingosine 1-phosphate (S1P)) participate in this process; and how S1P modulates thyroid cancer cell migration and proliferation, and interacts with channel function.

A substantial amount of work has been done to understand calcium signaling in thyroid epithelial cells. In addition to receptor-mediated entry mechanisms, the regulation of store-operated calcium entry is the focus of our investigations. During the last year, we have investigated the mechanisms by which a member of the canonical transient receptor potential (TRPC) cation channel family is regulated. We have shown that rat thyroid FRTL-5 cells exclusively express the TRPC2 channel. Very little is known in regard to the physiological significance of this channel, and the first reports have been published. In brief, TRPC2 regulates a multitude of signaling mechanisms, and participates in, at least, the regulation of proliferation, migration and TSH receptor signaling. In regard to the regulation of the TSH-receptor expression, TRPC2 has a physiologically interesting function: calcium signaling through TRPC2 participates in a negative feedback mechanism by reducing cAMP production and stabilizing TSH receptor expression. TSH receptor signaling, in turn, regulates TRPC2 function. Of interest is also the importance of stromal interaction molecules (STIM), the endoplasmic reticulum calcium sensors and their redistribution to endoplasmic reticulum-plasma membrane (ER-PM) junctions and interactions with the calcium channel subunit ORAI1. TRPC2 is constitutively active in the cells, feeding extracellular calcium for storage into the endoplasmic reticulum. In addition, TRPC2 regulates PKC isoform expression, and SERCA activity. Furthermore, the importance of TRPC2 in regulating the electrophysiological properties of thyroid cells have been investigated. TRPC2 regulates the function of anoctamin 1, an anion channel participating in the regulation of iodine transport in thyroid cells. Taken together, our investigations have revealed a novel role for TRPC ion channels. Furthermore, the importance of TRPC channels in human thyroid cells (both normal and cancer cells) are also under investigation. In these cells, the channel

expression profile seems more complex than in the experimental rodent model.

Investigations of the importance of S1P in regulating thyroid cancer cell migration has continued. These investigations have pointed out that the S1P receptor profile may not be useful for diagnostic purpose, as thyroid tumor cells with very similar receptor profiles may show diametrically opposite migratory responses after stimulation with S1P. This is evident using thyroid cancer cell lines of both follicular and anaplastic origin.

We are also interested in the regulation of the HERG potassium channel. Our data show that HERG is expressed in both normal thyroid cells and in thyroid cancer cells, but regulates proliferation in cancer cells only. In addition, HERG channels regulated migration of thyroid cancer cells, and some S1P receptor subtypes enhance the internalization of HERG channels, suggesting a useful mechanism when considering interventions for blocking the migration of cancer cells.

Publications

Asghar MY, Kemppainen K, **Viitanen T**, **Törnquist K**: Sphingosine 1-phosphate and human ether-a'-go-go related gene (HERG) potassium channels modulate migration in human anaplastic thyroid cancer cells. *Endocr Relat Cancer* 2012; 19(5):667–80

Löf C, Sukumaran P, **Viitanen T**, Vainio M, Näsman J, **Törnquist K**: Canonical transient receptor potential 2 channel as regulator of rat thyroid FRTL-5 cell function: Evidence of a feedback regulatory mechanism between TSH receptor expression and channel function. *Mol Endocrinol* 2012; 26:2046–2057

Sukumaran P, **Viitanen T**, Löf C, Kankaanpää P, Kemppainen K, Näsman J, **Törnquist K**: Canonical transient receptor potential 2 and calcium signaling in rat thyroid FRTL-5 cells: Importance of protein kinase C δ and STIM2. *J Biol Chem* 2012; 287:44345–44360

Viitanen T, Sukumaran P, Löf C, **Törnquist K**. Functional coupling of TRPC2 cation channels and the calcium-activated anion channels in rat thyroid cells: Implications for iodide homeostasis. *J Cell Physiol* 2012; Epub Sep 27



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Funded by Sigrid Jusélius Foundation 2011-2014,
EVO foundation large grant 2011-2013, EVO foundation small grant 2012-2012, Liv och Hälsa Foundation.

ENDOCRINOLOGY

Main research activities

During 2012, the group continued its studies addressing diagnosis, pathophysiology and treatment of non-alcoholic fatty liver disease (NAFLD). Regarding diagnosis, we have performed lipidomics and metabolomics analysis of 700 subjects whose liver fat has been quantitated by ¹H-MRS or liver biopsy and found that a combination of three markers performs as well as the NAFLD score in predicting NAFLD in humans. A patent application and manuscript have been submitted. Regarding pathophysiology, we have continued studies addressing the physiological function and clinical significance of the rs738409 C->G single nucleotide polymorphism in the patatin-like phospholipase domain-containing 3 (PNPLA3; adiponutrin) gene, that leads to a missense mutation (1148M) in the protein and increased liver fat content but not insulin resistance. Furthermore, we have made the novel observation that PNPLA3 is regulated by ChREBP in *in vitro* studies. Also, in our studies, we showed that three weeks of carbohydrate overfeeding markedly increases liver fat content in the face of a trivial change in body weight and the relationship between changes in liver fat and serum triglycerides differs between subjects homozygous for the variant allele compared to those lacking the mutant allele. Finally, we have shown that *in vivo*, PNPLA3 is upon insulin infusion the 3rd most upregulated gene out of over 20 000 genes studied.

Publications

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- Bergholm R, **Sevastianova K**, Santos A, **Kottrönen A**, **Urbansson M**, Hakkarainen A, Lundbom J, Tiikkainen M, Rissanen A, Lundbom N, **Yki-Järvinen H**. CB(1) blockade-induced weight loss over 48 weeks decreases liver fat in proportion to weight loss in humans. *Int J Obes (Lond)* 2012; Epub Jul 17
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- Hyötyläinen T, Mattila I, Wiedmer SK, Koivuniemi A, Taskinen MR, **Yki-Järvinen H**, Orešič M. Metabolomic analysis of polar

metabolites in lipoprotein fractions identifies lipoprotein-specific metabolic profiles and their association with insulin resistance. *Mol Biosyst* 2012; 8(10):2559-65

- Perttilä J**, Huaman-Samanez C, Caron S, Tanhuanpää K, Stals B, **Yki-Järvinen H**, **Olkkonen VM**. PNPLA3 is regulated by glucose in human hepatocytes, and its I148M mutant slows down triglyceride hydrolysis. *Am J Physiol Endocrinol Metab* 2012; 302(9):E1063-9
- Sevastianova K**, **Santos A**, **Kottrönen A**, Hakkarainen A, Makkonen J, Silander K, Peltonen M, Romeo S, Lundbom J, Lundbom N, **Olkkonen VM**, Gylling H, Fielding BA, Rissanen A, **Yki-Järvinen H**. Effect of short-term carbohydrate overfeeding and long-term weight loss on liver fat in overweight humans. *Am J Clin Nutr* 2012; 96(4):727-34
- Soronen J**, Laurila PP, Naukkarinen J, Surakka I, Ripatti S, Jauhiainen M, **Olkkonen VM**, **Yki-Järvinen H**. Adipose tissue gene expression analysis reveals changes in inflammatory, mitochondrial respiratory and lipid metabolic pathways in obese insulin-resistant subjects. *BMC Med Genomics* 2012; 5(1):9



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 Terhi Vihervaara, M.Sc., Ph.D. fall 2012
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Funded by The Academy of Finland, the European Union 7th Framework Programme, the Sigrid Jusélius Foundation, the Novo Nordisk Foundation, the Finnish Foundation for Cardiovascular Research, the Liv och Hälsa Foundation, and the Magnus Ehrnrooth Foundation.

LIPID SIGNALLING AND HOMEOSTASIS

Main research activities

The major activity of the group focuses on cellular mechanisms to sense lipid status and relay this information to regulate (i) expression of the genes and activities of enzymes of lipid metabolism as well as (ii) other vital processes under the control of lipid cues. Oxysterols are oxidized derivatives of cholesterol that act as potent regulators of cellular lipid metabolism, signaling and differentiation processes. We have identified a family of 12 human genes that encode cytoplasmic oxysterol-binding proteins, ORPs. The project aims to elucidate the function of these proteins as lipid sensors controlling a variety of downstream processes and their roles in diseases involving skewed lipid signaling.

In 2012 we carried out the first detailed analysis of the impacts of ORP genetic manipulation on the macrophage lipidome, revealing a multitude of subtle common or gene-specific lipid alterations, the strongest of which involve cholesterol and cholesterol esters (Vihervaara et al. 2012). A detailed study of macrophage ORP8 demonstrated a functional role of the protein in migration, apparently via its interaction with the nucleoporin Nup62 (Béaslas et al. 2012). These and other new findings of macrophage oxysterols and their binding proteins were summarized in a thematic review published in *Current Opinion in Lipidology* (Olkkonen 2012). As a new opening, we mapped the expression of all ORP family genes in human adipose depots and reported the first evidence for roles of ORPs (ORP8 and ORP11) in adipogenic differentiation (Zhou et al. 2012). A 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 implied that ORP sterol liganding modifies the interaction with VAPs and the formation of membrane contact sites (MCS), which play central roles in the inter-organelle transport of lipids and in the control of Ca^{2+} fluxes as well as other signaling processes. The recent work (Weber-Boyvat et al., in preparation), and collaborative efforts with the group of D. Yan (Guangzhou, China) provided new evidence for a function of ORP-VAP complexes at the MCSs, with implications for human diseases including dyslipidemias, neurologic disorders, and cancer.

A collaborative project on the molecular mechanisms underlying non-alcoholic fatty liver disease (NAFLD) is carried

out with the group of Endocrinology led by Prof. Hannele Yki-Järvinen. NAFLD affects more than 20% of the population in Western countries and predisposes to insulin resistance/metabolic syndrome as well as steatohepatitis and liver cirrhosis. During 2012, we identified eight microRNAs upregulated in livers of subjects with NAFLD and assessed their function in the control of hepatic gene expression (Soronen et al., manuscript in preparation). In addition, a number of collaborative studies with the group of Prof. H. Yki-Järvinen focusing on the molecular etiology of NAFLD were completed. Moreover, a study aimed at elucidating the functions of new, highly insulin-responsive genes in human subcutaneous adipose tissue in adipocyte physiology was initiated.

Theses completed in the group in 2012

The following Ph.D. thesis was accepted at the University of Helsinki this year:

Terhi Vihervaara: The role of oxysterol binding proteins in macrophages. October 5, 2012.

Selected publications

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Funded by Academy of Finland, EU FP7
 LipidomicNet, EUROCORES Programme
 EuroMembrane, Sigrid Jusélius Foundation,
 Biocentrum Helsinki, Biocenter Finland.

MEMBRANE BIOLOGY

Main research activities

Our group focuses on understanding how cholesterol functions in the cellular context, both under physiological conditions and in human diseases. Cholesterol is an essential component of cell membranes and a precursor of vital metabolites, including steroid hormones, bile acids and oxysterols. However, when in excess, cholesterol predisposes to major health burdens. The hallmarks of elevated cholesterol side effects are cardiovascular diseases, such as heart infarct and stroke. Disturbed cholesterol metabolism may also accompany the development of other diseases, including neurodegenerative diseases and cancers.

Cholesterol has a rigid four-ring structure that displays high affinity for saturated lipids that are typically found in sphingolipids. In human pathophysiology, this is reflected in several conditions that cause the co-accumulation of cholesterol and sphingolipids in cells. The predilection site for lipid storage is in the endo-lysosomal compartments. One of the key proteins involved in late endosomal/lysosomal lipid export is the Niemann-Pick C1 (NPC1) protein. This protein has been suggested to be either a primary cholesterol transporter or a sphingosine transporter. We have recently established novel assays for monitoring sphingolipid metabolism and transport in the endo-lysosomal compartments (Blom et al., Traffic 2012). Using this system, we found that NPC1 does not play a significant role in late endosomal/lysosomal sphingosine export.

We have also carried out transcriptional profiling to identify novel proteins involved in the handling of excess cholesterol that enters the endo-lysosomal compartments of macrophages when they scavenge modified lipoproteins. It is apparent that the actin cytoskeleton plays a key role in controlling the entry of material into cells. Yet, how the actin cytoskeleton is orchestrated upon cholesterol entry into macrophages is not well understood. Based on microarray analyses of wild-type and NPC1-deficient macrophages, we have identified the first actin regulator that controls macrophage foam cell formation (Hölttä-Vuori et al., Circ Res 2012). This protein, Coronin-1A, regulates the dynamic association of actin with endosomes that contain engulfed lipoproteins. The endosomal actin association, in turn, slows down the communication of endosomes and thereby limits macrophage lipoprotein uptake and degradation.

under cholesterol excess. Thus, in the absence of Coronin-1A, macrophage foam cell formation is enhanced. These findings are of importance for understanding the regulatory mechanisms of foam cell formation.

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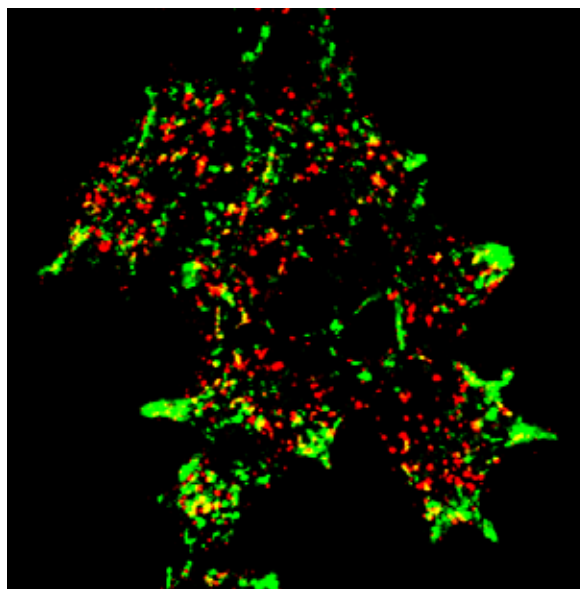
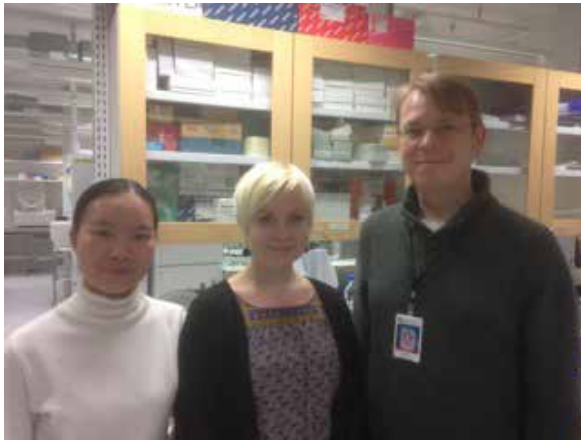


Figure. Delivery of fluorescently labeled acetylated LDL to lysosomes in Coronin-1A-silenced Raw264.7 macrophages. Green: FITC dextran (lysosomes). Red: Dil-labeled acetylated LDL, 15 min labeling.



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 Finska Läkaresällskapet, Finnish Diabetes Research
 Foundation, the Liv och Hälsa Foundation, the
 Novo Nordisk Foundation, and the Sigrid Jusélius
 Foundation.

METABOLISM

Main research activities

In 2012, Paulina Skrobuk finished her PhD studies and defended her thesis, entitled "Glucose and lipid metabolism in skeletal muscle" on 7th September 2012. PhD-student Selina Mäkinen and post-doctoral researcher Yen Nguyen joined the team, and the research activity of the group continued with primary focus on the interaction between fatty acid and glucose metabolism in skeletal muscle, using isolated human skeletal muscle strips and primary human muscle cells.

In 2012 we reported the effects of putative insulin-sensitizing compound resveratrol on muscle metabolism. Surprisingly, resveratrol did not restore insulin action in primary human myotubes exposed to palmitate, but acted as a metabolic inhibitor in an acute setting. Resveratrol treatment caused a decrease in both glucose and lipid metabolism, impaired proximal insulin signaling and increased activation of endoplasmic reticulum stress. Further analysis demonstrated that resveratrol acted as an inhibitor of AMP-activated protein kinase, a central regulator of metabolic pathways.

Moreover, sample collection for projects "MAMA – The Molecular Mechanisms of Insulin Resistance in Gestational Diabetes (PI docent Heikki Koistinen)" and "FUSION-Finnish United States Investigation on NIDDM Genetics (PI prof. Jaakko Tuomilehto)" has been actively ongoing, and will continue in 2013.

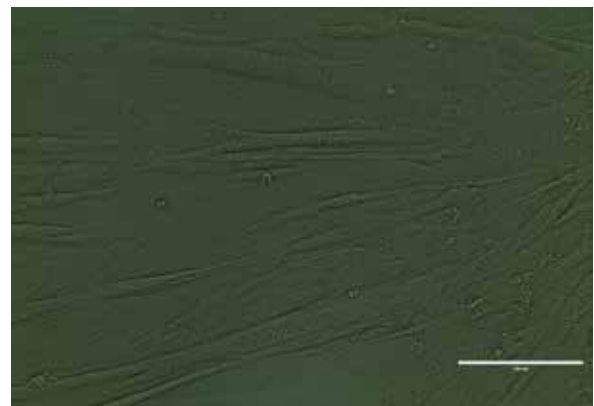


Figure. Human skeletal myotubes differentiated for 5 days. Brightfield image 40x, scale bar 100 μ m.

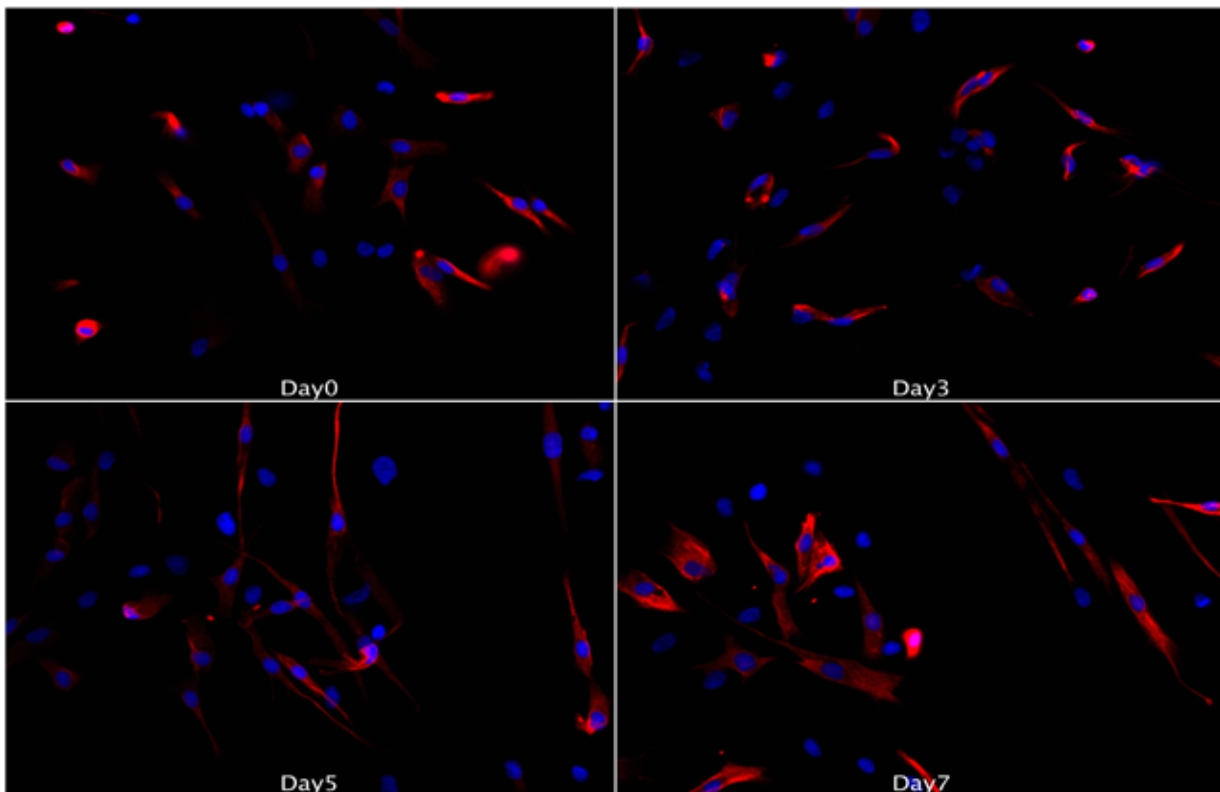


Figure. Immunofluorescence image (20x) of primary human skeletal myoblasts during differentiation, stained with anti-desmin (red) and DAPI (blue).

Publications

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Skrobuk P, von Kraemer S, Semenova MM, Zitting A, Koistinen HA: Acute exposure to resveratrol inhibits AMPK activity in human skeletal muscle cells. *Diabetologia* 2012; 55:3051-60

Theses completed in the group in 2012

The following Ph.D. thesis was accepted at the University of Helsinki this year:

Paulina Skrobuk: Glucose and lipid metabolism in human skeletal muscle. September 7, 2012.



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The group is a member of the Nordic Autophagy Network (<http://nordicautophagy.org/>) and is also member of the Brain&Mind Doctoral Programme and the Helsinki Biomedical Graduate Programme (HBGP).

Funded by Academy of Finland, Sigrid Jusélius Foundation, Liv och Hälsa Foundation, Finska Läkaresällskapet, Magnus Ehrnrooth and Minerva Foundation.

NEUROSCIENCE

Main research activities

We study basic mechanisms underlying neurodegenerative diseases and excitotoxic cell damage in the brain. We focus on protein ubiquitination and the role of mitochondrial and endoplasmic reticulum (ER) stress pathways as well as autophagy in the pathogenesis of brain diseases with the aim to combat cell death and degeneration. We also study neurotrophic factors, such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) and their signaling in regulation of cell-specific events. The majority of the group are housed in the nearby Institute of Biomedicine, Faculty of Medicine, but the group is also actively engaged in research at Minerva.

Signaling Mechanisms and Organelle Dysfunctions in the Regulation of Cell Death in Neurodegenerative Diseases

We study the role of mitochondria and ER stress in models of Huntington's disease (HD) and in Parkinson's disease (PD), showing that cytoprotective autophagy plays a role in cell viability, and that autophagy can be regulated by the upstream mTOR/GADD34 pathway in a cellular model of HD (Hyrskyluoto et al 2012). Using generated transgenic mice overexpressing the transcriptional coactivator protein, peroxisome proliferator activated receptor-gamma (PGC-1) we showed that dopaminergic neurons in the substantia nigra in these mice are resistant to the neurotoxin MPTP as a model for PD (Mudo et al 2012). We are currently looking closer into the pathways and are going to study proteins mediating neuroprotection using gene profiling and studies of human dopaminergic neurons in culture (Lindholm et al 2012). Along with this we are interested in searching for compounds that may influence the activity and/or levels of PGC-1 in the dopaminergic system to arrive at putative novel factors to affect mitochondrial metabolism and neuronal survival. In this context, we, in collaboration showed that endogenous histone exert a negative impact on mitochondria by destabilizing the inner and outer membrane (Cascone et al, 2012). In an attempt to understand the role of Deubiquitinating Enzymes (DUBs) in nerve cell signaling and viability we studied Usp-14 widely expressed in brain neurons. Our main interest is to know how disturbances in synapses

are linked to degenerative diseases and how UPS can influence nerve cell functions.

MIR/IDOL in the Regulation of Membrane Receptors

Receptor ubiquitination by specific E3-ubiquitin ligases has emerged as an important step in the regulation of various membrane proteins and we have cloned a E3 ligase, Mir/Myip that is expressed in neurons. We showed that Mir/Myip, also named Idol (Inducible degrader of the LDLR) influence lipoprotein receptor levels in cells. VLDLR is also connected to signaling by the extracellular protein Reelin that is important for neurodevelopment. Therefore, we are currently studying the role of Myip/Idol in VLDLR more closely. Along this we have previously cloned Msap, an antagonist of Myip/Idol, belonging to the Canopy family of proteins. We recently showed that now showed that Cnpy2/Msap is involved in the regulation of LDLR levels in human hepatocyte cells acting in conjunction with Myip/Idol (Do et al, 2012). We are currently studying this relationship detail using viral constructs for downregulation and overexpression of the protein in different cell types. The results may have potential significance for development of novel therapeutics in metabolic disorders and for cell signaling in the brain.

Regulation of Neural Progenitor Cells (NPCs)

NPCs are highly dividing cells with the capacity for self-renewal with the potential to give rise to both neurons and glial cells. We studied how cell-surface proteins influence cell proliferation of NPCs, focusing on transmembrane serine protease inhibitors, HAI-1 and HAI-2 that are expressed by NPCs. We observed that these molecules are regulated by bone morphogenic proteins (BMPs) important in neurodevelopment and that HAI-1 influences progenitor cell proliferation and glial cell differentiation, depending on brain maturation and age (Koivuniemi et al. submitted). The results will add to our understanding of the regulation of NPCs in the brain and the role of HAI and cell-associated proteases in growth regulation and brain tumors.

Publications

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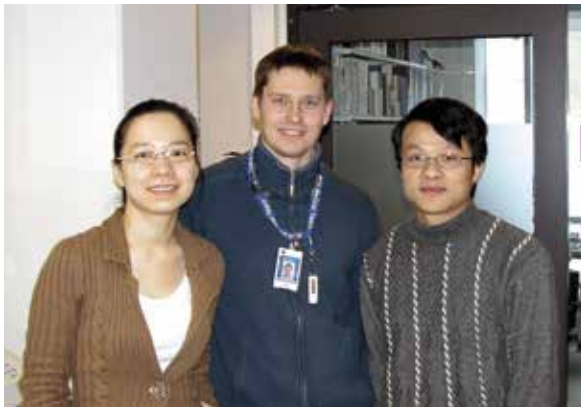
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Theses completed in the group 2012

The following Ph.D. thesis was accepted at the University of Helsinki this year:

Noora Putkonen: Organelle specific mechanisms of neuronal cell death. October 26, 2012.



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Funded by Finska Läkaresällskapet, Medicinska understödsföreningen Liv och Hälsa, Tekes (the Finnish Funding Agency for Technology and Innovation).

TARGETED GENE-EXPRESSION ANALYSIS

Main research activities

The aim of the research group is to develop new gene-expression-based assays for cancer diagnostics with the main focus on colon, prostate and breast cancer. We have extended our research interests to cover genetic and viral diseases for situations where there are specific technical difficulties related to amplification-based techniques.

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 for disease monitoring following treatment, 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.

In addition, a longstanding project of developing multi-gene predictor assays for colon, breast and prostate cancer diagnostics is coming to a conclusion. The PCR-based analyses of all 500 formalin-fixed, paraffin-embedded samples are completed in the prostate cancer arm of this study. Clinical data have been updated from patient records, and we are currently working on statistical data analysis. Preliminary results are promising.

A new research area has been the development of a novel PCR amplification technology called Heat Pulse Extension PCR (HPE-PCR). 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. The utility of this technique has been demonstrated by amplifying repetitive expansions in Fragile X syndrome as well as in Type I Myotonic Dystrophy. (Orpana et al 2012, Orpana et al 2013)

We have also been working in collaboration with FIMM-EMBL group leader Denis Kainov to develop a novel technique for strand-specific gene-expression analysis (Feng et al 2012). The resulting publication was ranked among the top ten peer reviewed articles published in the journal Biotechniques during 2012. The utility of this technique was demonstrated by strand-specific analysis of the + and – strands of the PB2 gene of human influenza virus. (Denisova et al 2012) In this project we have

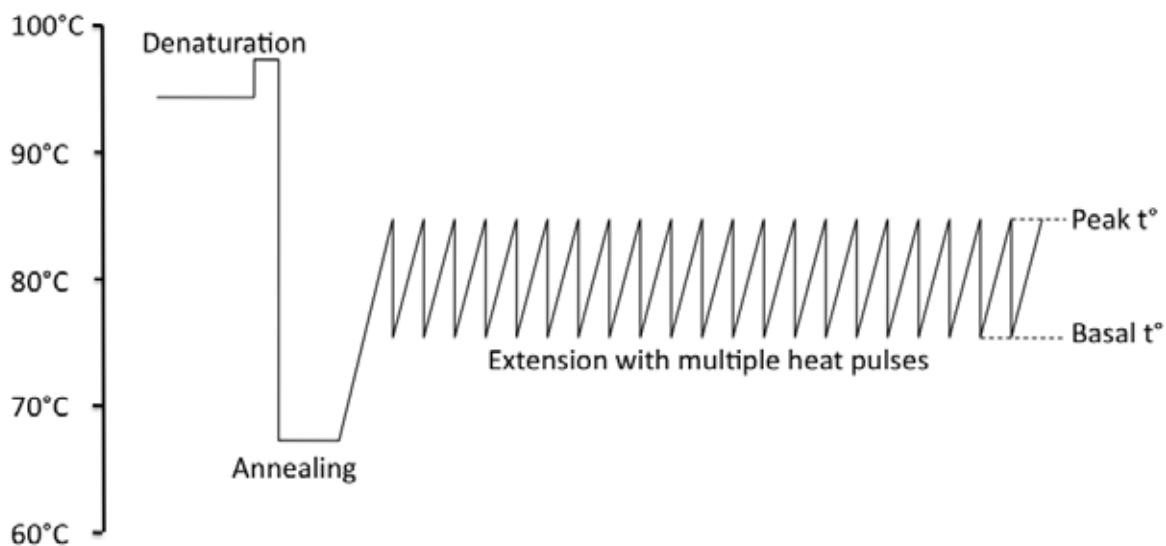


Figure. Schematic cycling profile for Heat Pulse Extension PCR.

been able to utilize our previous experience with developing gene-expression assays for cancer diagnostics.

In the coming year we are planning to expand the research group with another PhD student, and the work with gene-expression based assays for cancer diagnostics will continue. Additional efforts will be focused on innovative assay development for gene expansion diseases as well as point-of-care testing.

Publications

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Feng L, Lintula S, **Ho TH**, Anastasina M, Paju A, Haglund C, Stenman U-H, Hotakainen K, Orpana A, Kainov D and **Stenman J**. Technique for strand-specific gene-expression analysis and monitoring of primer-independent cDNA synthesis in reverse transcription. *Biotechniques* 2012; 52(4):263-70

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TELOMERE RESEARCH



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Antti Jula, Research Professor, National Institute for Health and Welfare, Turku, Finland

Funded by

Finska Läkaresällskapet ,the Liv och Hälsa Foundation, and the Paavo Nurmi Foundation

Main research activities

The team studied leucocyte telomere length (LTL) using Southern blot in more than 4000 subjects. 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, when DNA for telomere measurement was isolated from blood leucocytes of 622 participants (collaboration with Professor T. Strandberg). While studies on LTL have generally been cross-sectional, this was the first published longitudinal study on the relation of LTL to life style. In another study comprising the same subjects, high cholesterol in the beginning combined with low cholesterol at the end was related to the shortest LTL and highest mortality, in accord with the “cholesterol paradox”. Moreover, we discovered associations of LTL with 1) alcohol consumption, 2) physical activity, 3) the insertion deletion (I/D) polymorphism of the angiotensin-I converting enzyme (ACE) gene. A new study, Telomeres and cardiovascular health in the young, was started in collaboration with Professor A. Jula as a substudy of the STRIP project (SepelvaltimoTaudin Riskitekijöiden InterventioProjekti) comprising >500 subjects in the intervention group and >500 subjects in the control group with follow-up covering early childhood to maturity. This study is ongoing during 2013.

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Publications

Fyhrquist F. Kohonneen verenpaineen tiukka hoito diabeteksessa – vähempikin riittää (Tight blood pressure control of hypertension in diabetes – less is enough). *Suomen Lääkärilehti* 2012; 67:672-673

Fyhrquist F, Eriksson A, Saijonmaa O, Nordestgaard BG, Kontula K, de Faire U, Ibsen H, Kjeldsen S, Os I, Björn Dahlöf. Leucocyte telomere length is associated with ACE insertion/deletion polymorphism in hypertensive patients with left ventricular hypertrophy. *J Renin Angiotensin Aldosterone Syst* 2012; Epub Oct 17

Fyhrquist F, Saijonmaa O. Telomere length and cardiovascular aging. *Ann Med* 2012; 44:S138-42

Immonen I, Seitsonen S, **Saijonmaa O, Fyhrquist F.** Leucocyte telomere length in age-related macular degeneration. *Acta Ophthalmol* 2012; Epub May 2

Savela S, **Saijonmaa O, Strandberg TE, Koistinen P, Strandberg AY, Tilvis RS, Pitkälä KH, Miettinen TA, Fyhrquist F.** Physical activity and telomere length measured in old age. *Exp Gerontol* 2012; Epub Feb 22

Strandberg T, Strandberg AY, **Saijonmaa O, Tilvis RS, Pitkälä KH, Fyhrquist F.** Association between alcohol consumption in healthy midlife Long-term alcohol consumption and telomere length in older men. *Eur J Epidemiol* 2012; 27(10):815-22

SENIOR RESEARCHER



Ralph Gräsbeck, M.D., Dr.Med.Sci, Dhc, Professor

Ralph Gräsbeck, 82, remains as the sole representative of the former Biochemistry Unit. His main research topics are the metabolism of vitamin B12 and its reference values. Due to health and age-related reasons, he no longer performs experimental work, but visits the Institute writing reviews and advising doctors and patients.

In September Dr. Gräsbeck gave the opening lecture at a Symposium on vitamin B12 in Nancy, France. The lecture reviewed all of his contributions to the vitamin B12 field, was very well received, and will appear in print in English in the journal *Biochimie*. After the congress, Dr. Gräsbeck shared approximately the same lecture with the personnel of the Minerva Institute.

To celebrate the 50 years of committee work and published recommendations from Central Europe in the reference value field, Dr. Gräsbeck was invited to contribute to an article describing the introduction of the concept of reference values and the first Scandinavian and international activities in the field. The article appeared in vol. 50 of the journal *Clinical Chemistry and Laboratory Medicine*.

With these momentous accomplishments of many years, highlighted in 2012, Minerva celebrates the influential work of Dr. Gräsbeck.

Publications

Gräsbeck R. Hooked to vitamin B12 since 1955: A historical perspective. *Biochimie* 2012; Epub Dec 26

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