



MINERVA FOUNDATION &
MINERVA FOUNDATION INSTITUTE
FOR MEDICAL RESEARCH

60th
anniversary

SCIENTIFIC REPORT 2019



Front page image: Fred Björkstein in the Minerva laboratory
at Konkordia hospital, 1961.

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SUMMARY OF RESEARCH AND ACTIVITIES DURING 2019

The Minerva Foundation Institute for Medical Research is a privately owned research institute located at Biomedicum, Academic Medical Center Helsinki, Finland. The Institute, the history of which dates back to 1959, combines basic biomedical research with clinical investigation relevant to common diseases. Notably, the Minerva Foundation and the Institute celebrated their 60th anniversary in 2019. The 60-year history of Minerva is reviewed on pp. 6–9 of this report.

The overarching aims of the Minerva Institute are to generate 1) new fundamental knowledge and mechanistic insight, 2) innovations for the development of future diagnostic approaches, and 3) preventive measures and treatments for common diseases such as diabetes and cardiovascular diseases, as well as neurodegenerative and neuropsychiatric disorders. The study objectives, rooted in the fundamental molecular mechanisms of disease, are addressed at the Minerva Institute through a spectrum of approaches ranging from studies employing pure proteins and lipids, cultured cells and genetically manipulated animal models, to the investigation of human patients. The research undertaken in the groups of the Institute during 2019 is outlined in this report.

The financial resources of the Minerva Foundation are directed at maintaining and further developing a research infrastructure that serves, in the most effective way possible, the work in the research groups. The groups are responsible for acquiring external funds to cover the costs of special reagents, the stipendium or salary support of doctoral students, and the salaries of other personnel. The amount of external, competitive research funds acquired by the groups reached 1.4 M€ during 2019, covering 62% of the total Institute budget. The year 2019 was highly productive: a total of 50 articles were published, 43 of which were original articles in international peer-reviewed journals, the median impact of the publications being 4.79. In addition, M.Sc. Iryna Hlushchenko defended her doctoral thesis in 2019. During the year the research group of Dr. Taisto Sarkola, designated Cardiovascular disease in the young, was affiliated to the Institute.

The 60th anniversary of the Foundation and the Institute was celebrated in several steps: April 15, the actual birthday, was celebrated at a cocktail and coffee event organized at the Biomedicum Faculty Club. On September 23, the Medix Prize of the Minerva Foundation was awarded exceptionally as two distinct prizes, 20 000 € each, one for basic biomedical research (to Professor Gong-Hong Wei's group, University of Oulu) and the other for clinical research (to Professor Anu Wartiovaara's group, University of Helsinki; pp. 10–11), followed by a dinner hosted by the Foundation's Research Council. On Oct 23, present and past members of the Foundation's executive board celebrated the anniversary. The Foundation's Bror-Axel Lamberg Prize in Endocrinology, 10 000 €, was awarded to Professor Ulla Feldt-Rasmussen (Rigshospitalet, Copenhagen, Denmark) on Oct 31 (p. 12). To promote cohesion between the research groups, the Institute organized on June 4 a summer excursion, on Dec 2 a group leaders' brain-storming session, and on Dec 5 a Christmas party for the personnel.

To conclude, the Institute thrived in 2019 both scientifically and financially, and is determined to make 2020 even more successful.

EVENTS AT MINERVA 2019

Seminars

MINERVA SEMINAR, BIOMEDICUM HELSINKI

Karl Bechter, Department of Psychiatry and Psychotherapy II, University of Ulm, Germany: *Update on the mild encephalitis hypothesis of severe mental disorders – with a look on the role of CSF flow*. April 29, 2019.

BIOMEDICUM HELSINKI SEMINAR

Sander Kersten, Division of Human Nutrition & Health, Wageningen University, the Netherlands: *Regulation of lipid metabolism by angiopoetin-like proteins*. November 4, 2019.

Prizes and Grants

THE MINERVA FOUNDATION'S MEDIX PRIZE

Award Ceremony and Prize Lectures, Biomedicum Helsinki, September 23, 2019.

The winning article on the category of Biomedical Basic Research:

Biology and clinical implications of the 19q13 aggressive prostate cancer susceptibility locus. *Cell*. 2018; 174:576–589.

Authors: Gao P, Xia JH, Sipeky C, Dong XM, Zhang Q, Yang Y, Zhang P, Cruz SP, Zhang K, Zhu J, Lee HM, Suleman S, Giannareas N, Liu S, The PRACTICAL consortium, Tammela TLJ, Auvinen A, Wang X, Huang Q, Wang L, Manninen A, Vaarala MH, Wang L, Schleutker J, Wei GH.

The winning article on the category of Clinical Medicine:

Genetic basis of severe childhood-onset cardiomyopathies. *J Am Coll Cardiol*. 2018; 72:2324–2338.

Authors: Vasilescu C, Ojala TH, Brilhante V, Ojanen S, Hinterding HM, Palin E, Alastalo TP, Koskenvuo J, Hiippala A, Jokinen E, Jahnukainen T, Lohi J, Pihkala J, Tyni TA, Carroll CJ, Suomalainen Wartiovaara A.

Read more on pages 10-11.

THE MINERVA FOUNDATION'S BROR-AXEL LAMBERG PRIZE
IN ENDOCRINOLOGY

Ulla Feldt-Rasmussen, Rigshospitalet, Copenhagen, Denmark
Read more on page 12.

SELMA AND MAJA-LISA SELANDER'S FUND FOR RESEARCH
IN ODONTOLOGY

From Selma and Maja-Lisa Selander's fund 14 grants were
awarded 2019, in all 95.000 euros.

Doctoral Dissertations

Iryna Hlushchenko: Actin regulation in dendritic spines:
from synaptic plasticity to animal behavior and human
neurodevelopmental disorders. University of Helsinki, August 16,
2019. (Cellular Neuroscience)

ADMINISTRATION

THE MINERVA FOUNDATION

The main purpose of the Foundation is to promote re-
search in medicine and biosciences by maintaining the
Minerva Foundation Institute for Medical Research.
This scientific review covers the period from January 1
– December 31, 2019. During this period, the board of
trustees included the following persons:

Professor Caj Haglund, chair
Professor Patrik Finne
Professor Per-Henrik Groop
M.Sc. (Econ. & Bus. Adm.) Kim Karhu
M.Sc. (Econ. & Bus. Adm.) Carl-Magnus Westermarck

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Docent Carina Wallgren-Pettersson

Agent for Minerva Foundation

M.Sc. (Econ. & Bus. Adm.) Patrik Lerche

THE MINERVA FOUNDATION INSTITUTE FOR MEDICAL RESEARCH

Board of Directors

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Docent Pirta Hotulainen
Professor Elina Ikonen
Docent Matti Jauhiainen
Docent Heikki Koistinen
Professor Dan Lindholm
Docent Taisto Sarkola
Professor Ilkka Tikkanen
Professor Kid Törnquist
Professor Hannele Yki-Järvinen
M.Sc. Cia Olsson

MINERVA AT 60 YEARS

This short review will give a brief glimpse into the activities of the Minerva Foundation Institute for Medical Research during the last 60 years. Thanks to generous funding and good management, the research has shown an impressive scope of richness, with many interesting findings.

Early times. In the postwar period, Finland was a poor country and in Helsinki the University hospital was small and worn-down. Therefore, clinical instruction was also given in the city hospitals of Helsinki. The Fourth Department of Internal Medicine, headed by Professor Bertel von Bonsdorff, was located in the Maria City Hospital. In this department, scientific activity was intense, to a great extent due to the inspiring chief who in the 1930s had been trained in cardiology in Göttingen and hematology in Boston. His main topic of interest was megaloblastic anemia caused by the fish tapeworm, a traditional and hotly debated research topic among Finnish medical scientists. Another research theme of the department was goiter, which was endemic in Finland. Both projects utilized radioactive techniques, and the necessary equipment was consequently acquired – at a cost considered to be enormous at the time. This became the first nuclear medicine laboratory in Finland, founded in the late 1940s, and a very early one even on the European scale. In addition, the department was a pioneer in nephrology.

To solve the issue of more space, Professor von Bonsdorff had the idea that the scientists should pool their grants and rent a facility suitable for a laboratory. Such space was found in the small Konkordia hospital, owned by the Methodist congregation. Three small research teams moved there: a parasitological one headed by Wolmar Nyberg (1919-1973), an endocrinological one headed by Bror-Axel Lamberg (1923-2014), and a biochemical one headed by Ralph Gräsbeck (1930-2016). An experienced lawyer pointed out that the laboratory had to be governed by a legal person, otherwise it would have difficulties in dealing with the authorities. Thus, in 1959, a foundation was created, on ridiculously small capital, and given the name the Minerva Foundation Institute for Medical Research (Minerva being the Roman goddess of wisdom and science). A number of distinguished doctors were elected as members of its Board.



Ralph Gräsbeck, 1969

Two institutes in one. A few years later, a welcome source of money was discovered: a fund possessed by the Folkhälsan (People's Health) organization and bequeathed to it by Professor Ossian Schauman and his wife Betsy, intended for the establishment of an institute of genetics. However, war-time inflation had reduced the capital so that it was no longer sufficient for the founding of a true institute. A few rooms of the Minerva Institute were named the Folkhälsan Institute of Genetics, and research in the field was begun under the leadership of Albert de la Chapelle (1933-). In 1962, he produced the first dissertation from the Institute. It dealt with cytogenetic findings in female gonadal dysgenesis. In 1997, the Folkhälsan Institute separated from Minerva to become an independent and relatively large institute, but remained in close contact with Minerva.

Early research. The early research in the Institute was first a continuation of that in the Fourth Department of Medicine. The then state-of-the-art methods used included chromosome analysis (karyotyping), radioimmunoassays of numerous hormones, and sophisticated protein chemical techniques resulting in the isolation and characterization of 'new' proteins, including human intrinsic factor and mitogens. Research on the thyroid contributed to the eradication of endemic goiter and the development of refined diagnostic methods. Interestingly, in the late 1960s, Minerva was the most-quoted Finnish biomedical institution after Helsinki University, being more cited in the literature than our new universities.



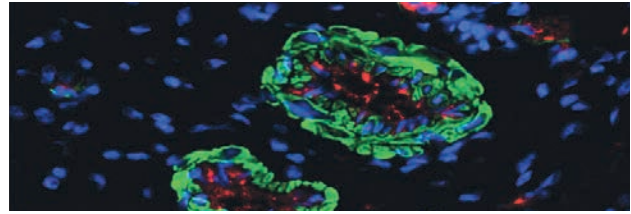
Photo on the left:
Fred Björkstein, 1961.



Photo below:
Working in laboratory
in early 1960s.







Modern times – new challenges. The institute has moved several times, but since 2001 it has been located in the Biomedicum Helsinki building in the vicinity of the Helsinki University Hospital. Biomedicum is a conglomerate of laboratories mostly belonging to Helsinki University but also to other institutions. In 2008, Minerva moved to brand-new facilities with 480 m² of space and located in an expanded part of the Biomedicum complex.

Since the early days, new research teams have established themselves and others have finished or moved elsewhere. Among these are the Unit of Clinical Physiology headed by Frej Fyhrquist, and Unit of Biochemistry headed by Ralph Gräsbeck (1930-2016). New units that have joined the Institute during the last ten years are; Cellular neuroscience (head Pirta Hotulainen), Lipid signaling and homeostasis (head Vesa Olkkonen, head of the Institute), Membrane biology (head Elina Ikonen), and very recently Cardiovascular disease in the young (head Taisto Sarkola). Research at Minerva covers several topics, including studies of membrane contact sites and transport mechanisms, lipid transport and signaling, neuronal function and pathophysiology, endocrinology, cardiovascular and metabolic diseases, and insulin resistance. Throughout the years, Minerva has fostered successful collaborative research and training of researchers as exemplified by the fact that more than 80 doctoral theses have been completed at the Institute. It should be pointed out that most of the researchers hold posts outside the Institute and are able to perform research at Minerva part-time or during leaves of absence.

To foster interactions and ensure appropriate research training, Minerva seminars are arranged on a regular basis. Guest lecturers are invited to these seminars, and the guest seminars have been open to all who are interested at Biomedicum. In addition, the Minerva Foundation has arranged international symposia, either alone or in collaboration with the Wihuri Research Institute and the Brain Research Society Finland.

The history of the first 50 years of Minerva's existence has been published¹⁻³. For more details regarding the research at the Institute, the principal achievements of the present research units are described later in this booklet. Please also see the Institute's web pages (https://minervafoundation.fi/minervainst_index.html).

Minerva and Medix. To investigate interesting patients and diseases, the scientists set up a number of sophisticated laboratory tests such as assays for thyroid hormones, angiotensin II and vitamin B₁₂, as well as for karyotyping. Many of the tests gradually became routine clinical analyses, and Minerva received numerous requests to perform these assays on hospital patients. By law, the Foundation was not permitted to charge for such services. To take over the routine work, in 1964 the scientists decided to found a separate service laboratory. They paid for the shares themselves and donated them to the Minerva Foundation, the Folkhälsan and the Liv och Hälsa (Life and Health) Societies, with the provision that the profit of the company be used to support the research in the Minerva and Folkhälsan Institutes. The company was named Medix, and it grew substantially, becoming one of the largest private clinical reference laboratories in Finland. The profit generated by Medix enabled Minerva to purchase its present laboratory facilities and also covers the salaries of the people servicing the laboratory as a whole.

Medix subsequently began to manufacture biochemicals and reagents. This activity was later taken over by a separate company, Medix Biochemica, which has become one of the largest manufacturers of monoclonal antibodies in the world. The companies have been highly successful and of crucial importance in financing the Minerva Institute. The profits obtained from the companies have also been used to donate the prestigious Medix price of the Minerva Foundation to be awarded by Helsinki University for the best medical publication of the year to originate from Finland. The Minerva Foundation also donates funds for the Bror-Axel Lamberg Prize in endocrinology, and the Ralph Gräsbeck Scholarship for research in laboratory medicine. Furthermore, the Minerva Foundation administers the Selma and Maja-Lisa Selander's Fund for research in odontology, and has participated in funding expensive laboratory equipment for common use at Biomedicum Helsinki/the Meilahti campus.

1. Minerva 30 år. Finska Läkaresällsk Handl 1989; 133 (1):5–87.
2. Minerva 50 år. Finska Läkaresällsk Handl 2009; 169 (2):17-72.
3. Minerva Foundation and the Minerva Foundation Institute for Medical Research. Annual report 2009.

MEDIX PRIZE OF THE MINERVA FOUNDATION

The Minerva Foundation's Medix Prize is a major annual award for internationally recognized medical research in Finland. The Minerva Foundation celebrated its 60th anniversary 2019, and therefore it awarded two prizes instead of the customary one. The award categories were biomedical basic research and research into clinical medicine.

The Minerva Foundation's Medix prize in biomedical basic research was awarded to a research group headed by Professor of Biochemistry and Molecular Medicine **Gong-Hong Wei** at the University of Oulu and the prize for research in clinical medicine was awarded to Academy Professor **Anu Wartiovaara's** research group at the University of Helsinki. Both prizes were €20,000.

The prize is awarded annually for excellent Finnish scientific research published as one article during the previous year. The research is to be in the fields of biomedicine or clinical medicine and performed fully or in its essential parts in Finland. The awardee is selected by a committee consisting of representatives from the universities of Helsinki, Turku, Tampere, Eastern Finland and Oulu, who are appointed for three years.

The Medix prize, awarded by the University of Helsinki, was awarded for the 32nd time. The prize is donated to the University of Helsinki by the Minerva Foundation. The Minerva Foundation maintains the Minerva Foundation Institute for Medical Research at Biomedicum Helsinki.

Research group at the university of oulu awarded for their work into the mechanisms of aggressive prostate cancer

The research group of Professor **Gong-Hong Wei** at the University of Oulu, collaborating with the group of genetic predisposition to cancer led by Professor of Medical Genetics **Johanna Schleutker** at University of Turku, was recognized for the discovery of the genetic mechanism of aggressive prostate cancer.

The awarded study examines the progression mechanisms of aggressive prostate cancer and identified new genes affecting the spreading of the cancer. The group has discovered a novel molecular-level mechanism to explain why some men are more susceptible to the aggressive form of prostate cancer. The research offers new insights into the risk stratification of aggressive prostate cancer and clinical treatment.

Every individual has small genetic variation in their genome. Earlier studies have identified the genomic variant that has been associated with the aggressive progression of prostate cancer. Interestingly, the awarded study also observed this association in a large group of 2,738 Finnish prostate cancer patients. If a man has this genomic variant, he is at a high risk of developing an aggressive form of prostate cancer. "Earlier, a connection was found, but not a mechanism for how and why this particular variant af-

fects prostate cancer. We have now been able to shed light on these mechanisms by using genetic, genomic, molecular and bioinformatic analysis on a large number of samples from prostate cancer patients", says Professor Gong-Hong Wei.

The results cannot yet be utilised in clinical medicine, but according to Professor Wei, this will be possible in the next couple of years. "It is not necessary to treat everyone diagnosed with prostate cancer in the same way, because only few have the life-threatening form of the disease. In the future, scientific research can be used to reliably predict who has the aggressive form of the disease and who will benefit from less intensive therapy or observation."

"The novel genes and mechanisms discovered offer opportunities for completely new, personalised therapeutic strategies of prostate cancer", says Professor Wei.

According to Professor of Cell Biology **Aki Manninen**, prostate cancer is the second most common cancer in men. Over 1.1 million new cases are diagnosed worldwide every year. It is also the fifth leading cause of cancer-related deaths in men, resulting in some 300,000 deaths every year. In Finland, nearly 5,000 new cases are diagnosed every year. "These figures indicate that targeting the best possible treatment to the patients who need it the most is very important for public health. Finding genetic reasons and mechanisms behind cancer is also important because prostate cancer has a higher hereditary element than many other cancer types", says Professor Manninen.

"Although an elderly patient diagnosed with prostate cancer today would not be able to benefit from the results of this research, they may well benefit his children or grandchildren, who may develop a genetically similar prostate cancer in the future", Professor Manninen concludes.

The study of Professor **Gong-Hong Wei's** group was published in Cell. The authors of the awarded article Biology and clinical implications of the 19q13 aggressive prostate cancer susceptibility locus. Cell. 2018; 174:576-589: Gao P, Xia JH, Sipeky C, Dong XM, Zhang Q, Yang Y, Zhang P, Cruz SP, Zhang K, Zhu J, Lee HM, Suleman S, Giannareas N, Liu S, The PRACTICAL consortium, Tammele TLJ, Auvinen A, Wang X, Huang Q, Wang L, Manninen A, Vaarala MH, Wang L, Schleutker J, Wei GH.

Research group from the university of helsinki awarded for research into the genetic bases of childhood-onset cardiomyopathies

The research group of Academy Professor **Anu Wartiovaara** at the University of Helsinki, working in cooperation with the group of paediatric cardiologists headed by **Tiina Ojala** at Helsinki Children's Hospital, was awarded for its research into the genetic basis of severe childhood-onset cardiomyopathies. The results of the

research can be significantly utilised in diagnostics, genetic counselling, the development of therapeutic strategies and treatment decisions.

Being less studied than adult cardiomyopathies, the genetic background of paediatric cardiomyopathies has been poorly understood. Childhood cardiomyopathies are typically severe and may require cardiac transplantation. Often the initial phases of the disease are lifethreatening, but sometimes the disease may spontaneously improve by school-age, in which case intensive conservative care without a cardiac transplantation is the best option.

As both disorders – the terminal and the spontaneously recovering form – are similarly lifethreatening at onset, their different prognoses cannot be identified at this stage on the basis of clinical examination alone. “In Finland, paediatric cardiac transplants are centralised at Helsinki Children’s Hospital. We were therefore able to collect an extensive data on paediatric patients who were considered for cardiac transplant due to a severe cardiac disease over a time period of 21 years. The cohort is globally unique. The average age of diagnosis was four months, and 17 children out of 66 had a cardiac transplant,” says Paediatric Cardiologist Tiina Ojala.

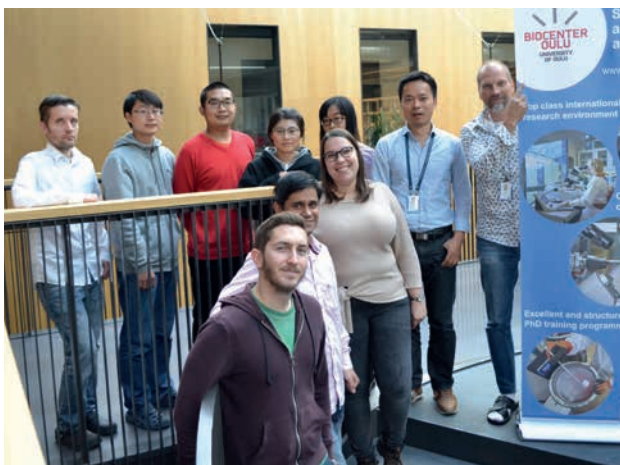
Next-generation DNA sequencing, molecular studies and protein modelling were carried out by doctoral student **Catalina Vasilescu**.

“The results indicate that the genetic background of severe childhood-onset cardiomyopathies is highly heterogenic. Nearly every family had their own specific mutation. We also discovered new genes contributing to childhood cardiomyopathies,” says Academy Professor Anu Wartiovaara.

According to Catalina Vasilescu, the awarded research is an important step towards more personalised treatment. “We managed to identify a significant number of genetic causes in cardiomyopathies of children, and show that the DNA-diagnosis has an impact on therapeutic strategies. Genetic information helps to predict prognosis and facilitates individualised decision-making, such as who will need a cardiac transplantation and who could be best helped with intensive conservative care without transplantation,” she says.

In half of the patients, the mutation was new, occurring during embryonic development and was not found in the parents. In this case, the disease is not hereditary, which means that the parents do not have an elevated risk of having another child with cardiomyopathy. However, if the parents are found to be carriers of the child’s gene defect, the risk for getting a second affected child depends on the type of the mutation. The DNA-diagnosis enables genetic counseling. Also the disease-risk for siblings of a child can be tested, and if a mutation is not found, they can be released from cardiac follow-up.

The study of Academy Professor Anu Wartiovaara’s group was published in the *Journal of the American College of Cardiology*. The authors of the awarded article Genetic Basis of Severe Childhood-Onset Cardiomyopathies. *J Am Coll Cardiol*. 2018; 72:2324-2338: Vasilescu C, Ojala TH, Brilhante V, Ojanen S, Hinderding HM, Palin E, Alastalo TP, Koskenvuo J, Hiippala A, Jokinen E, Jahnukainen T, Lohi J, Pihkala J, Tyni TA, Carroll CJ, Suomalainen Wartiovaara A.



Professor Gong-Hong Wei’s research group studied the genomics and genetic systems of aggressive prostate cancer. Members of the research group from left at the rear: BSc Arto Alatalo, MSc Jihan Xia, MSc Binjie Luo, PhD Xiayun Yang, MSc Qin Zhang, Professor Gong-Hong Wei, Adjunct Professor Aki Manninen. At the front from left: MSc Nikolaos Giannarreas, PhD Karim Ullah, MSc Sara Cruz.



The research group headed by Academy Professor Anu Wartiovaara studied the genetic background of severe childhood-onset cardiomyopathies. Members of the research group, from left: Docent, Specialist in Paediatric Cardiology Tiina Ojala; MD, PhD, Academy Professor, Chief Medical Officer Anu Wartiovaara; MSc, doctoral student Catalina Vasilescu; MD, Specialist in Paediatric Cardiology Anita Hiippala; Docent, Paediatric Cardiology Chief of Department Jaana Pihkala; MD, PhD, Professor, Head of Tertiary Paediatrics Eero Jokinen; MD PhD Eino Palin.

THE MINERVA FOUNDATION'S BROR-AXEL LAMBERG PRIZE IN ENDOCRINOLOGY

The 2019 Bror-Axel Lamberg Prize in Endocrinology was awarded to Professor of Medical Endocrinology Ulla Feldt-Rasmussen at Rigshospitalet, Copenhagen, Denmark. The prize is 10,000€ and this was the second time the prize had been awarded.

The award ceremony took place on Wednesday, October 31, 2019, at the 71th Annual Meeting of the Finnish Endocrine Society. The director of the of the Minerva Foundation Institute for Medical Research, Professor Vesa Olkkonen, delivered the prize.

The Prize Jury's reasons for the award: "Feldt-Rasmussen has made numerous contributions to endocrinology and thyroidology. Impressively, she is the author or co-author of 429 articles, 373 of them original studies, her main field being thyroidology. Her work relates to virtually every aspect of thyroid disorders and their management. In particular, she has made crucial contributions addressing the measurement of thyroglobulin and the interactions of thyroglobulin antibodies with thyroglobulin measurements. She is one of the most distinguished experts in this field."

The Minerva Foundation's Bror-Axel Lamberg Prize in Endocrinology is a 10,000€ prize awarded every other year at the Annual Meeting of the Finnish Endocrine Society. It is awarded to a Scandinavian scientist for innovative, high-quality research in the field of endocrinology. The Finnish Endocrine Society nominates three candidates for the prize from whom the Minerva Foundation selects the winner. The first prize, in 2017, was awarded to Professor of Physiology Jorma Toppari at the University of Turku.



Professor Ulla Feldt-Rasmussen

Bror-Axel Lamberg
(March 1, 1924 –
May 4, 2014)



Bror-Axel Lamberg got his MD degree in 1949, after which his career continued in the Fourth Department of Internal Medicine at the University of Helsinki. Professor Johannes Wahlberg led Lamberg to study the thyroid-stimulating hypophysis hormone TSH. Lamberg was a pioneer in radioimmunoassays and he defended his doctoral thesis in 1953 on using radioactive phosphorus to measure TSH.

After his defense, Lamberg and his co-workers studied the lack of iodine in the Finnish population. His studies played an important part in having iodine added to common salt in Finland, which led to the eradication of endemic goiter. In 1971, Lamberg was appointed professor of endocrinology at the University of Helsinki. He performed his clinical work primarily at the HUS clinics in Meilahti.

Professor Bror-Axel Lamberg was awarded many prizes, including the Matti Äyräpää prize in 1979 and the J.W. Runeberg prize in 1985. During his active career, he acted as a chair and member of many societies and foundations. He was also granted honorary membership of several societies.

Professor Bror-Axel Lamberg was one of the founders of the Minerva Foundation in 1959. The Foundation was formed to maintain the activity of the Minerva Foundation Institute for Medical Research. His endocrinological research team was one of the first to start their research at the newly founded institution at a small hospital, Konkordia, in Helsinki. Professor Bror-Axel Lamberg was the first head of the Institute from 1959 to 1970.

Professor Lamberg was also one of the founders of the clinical service laboratory Medix Ltd. in 1964. From those days Medix has expanded and now consists of several companies offering clinical laboratory services, diagnostic bioreagents and rapid point-of-care tests. These companies are highly successful and offer crucial financial support to the Minerva Foundation and the Research Institute.



Group members

Taisto Sarkola, M.D., Dr.Med.Sci., Docent, Head
My Blomqvist, D.D.S., Dr.Med.Sci.
Johnny Sundholm, M.D., Dr.Med.Sci. autumn 2019
Mari Ylinen, M.D., Dr.Med.Sci.
Linda Litwin, M.D.
Rasmus Olander, M.D.
Essi Karikoski, M.Sc.
Sanna Suonsyrjä, med.stud.
Michelle Renlund, med.stud.
Maria Finne, M.Sc., Research coordinator, starting September 2019

External funding

Foundation for Pediatric Research
Medical Society of Finland
Medicinska understödsföreningen Liv och Hälsa r.f
Sigrid Juselius Foundation
Stiftelsen Dorothea Olivia, Karl Walter och Jarl Walter Perkléns minne
Research Funding of the Helsinki-Uusimaa Hospital District
(state funding for university-level health research)

A new group at Minerva 2019 CARDIOVASCULAR DISEASE IN THE YOUNG

Main research activities

The development of clinical atherosclerosis commonly observed in adult life is a multifactorial lifetime process with its origins in the pediatric age. This process is influenced not only by heredity, but also by disease and cardiovascular risk factors from early childhood or even as early as the fetal stage. The evolution and maturation of the vasculature in the prenatal period, early childhood and later adolescence is, however, to date not well characterized. The impact of disease, treatments and cardiovascular risk factors on the evolution in vascular form and function in children with congenital and acquired diseases is still unclear. This is partly related to shortcomings in methodology developed primarily for adults with more advanced disease progression, and then applied in the pediatric age group were abnormalities are scant and challenging to quantify. There is also lack of data to define normality in healthy populations, partly due to diversity and lack of method validation for the pediatric population, partly due to lack of evidence linking measurement levels with clinical outcomes that typically emerge after several decades in later adulthood. Abnormalities of arterial structure such as intima-media thickness (IMT) are, nevertheless, increasingly used as surrogate markers for subclinical cardiovascular disease, and endothelial dysfunction, IMT, and different measures of arterial stiffness have become therapeutic targets for the prevention of cardiovascular disease progression in younger and younger patients.

Our group has developed a novel non-invasive method based on very-high-resolution ultrasound (VHRU, 25-55 MHz) frequencies that provides the opportunity to study vascular morphology (intima-media and adventitia thickness separately) non-invasively in almost microscopical detail in vivo in adults and children of all sizes and ages, including preterm neonates. Our research group studies the evolution of the cardiovascular structure and func-

tion in different childhood diseases and family populations spanning from the prenatal age to young adulthood, and with an emphasis on transgenerational transformation of cardiovascular risk, aims to elucidate the long-term impact of disease and cardiovascular risk for the development of clinical atherosclerosis.

We are located at the Children's Hospital in Helsinki and our group has been affiliated with the Minerva Institute from August 2019.

The main projects worked on during 2019 are presented below.

Arterial structure and function in children and adults with inflammatory vascular disorders

During the past year we have refined our VHRU method and shown that it is able to non-invasively quantify intima layer thickness in adult populations with a thickened intima layer (>0.06 mm) from 40 years of age. The intima thickness in younger populations is, however, below the axial resolution of the method. We have also shown that VHRU is useful as a diagnostic tool in the acute phase of giant cell arteritis of the temporal artery with good accuracy in detecting a thickened intima due to transmural arterial wall inflammation. The VHRU methodology then provides the opportunity for both diagnosis and follow-up of these patients in the outpatient clinic. We also intend to continue to recruit children with Kawasaki Disease to our ongoing study to assess the clinical utility of VHRU assessment in vasculitis in children.

Fetal cardiovascular programming of cardiovascular disease (RADIEL, SIVELU and FINNCARE studies)

We have since 2012 been recruiting families with a maternal history of obesity, gestational and pregestational diabetes, and pre-eclampsia within the RADIEL, SIVELU and FINNCARE longitudinal study cohorts. So far, we have studied 174 children

during the newborn stage (SIVELU) and 290 mother-child dyads at 6 years from delivery (RADIEL and SIVELU) with detailed characterization of the maternal and child cardiovascular phenotype, including extensive ultrasound examinations of the blood vessel and heart structure and function, arterial stiffness, blood pressure, blood glucose and lipids, and body anthropometrics and composition. During the newborn stage and early childhood, the cardiovascular structure has mainly been predicted by growth of the child anthropometrics and lean body mass (fat free mass), with no relations found to be related with cardiovascular risk. However, the children show signs of early weight and elevated blood pressure in early childhood consistent with a transgenerational transmission of cardiovascular risk, although this is not detectable in the heart and vessels at this stage. In 2019, we finalized the SIVELU 6y recruitment and started to recruit families in the FINNCARE maternal pre-eclampsia study cohort (N about 20 at the end of the year). We have further launched a randomized 12-month controlled intervention trial in collaboration with Dr. Tiina Jääskeläinen and Dr. Hannele Laivuori to address whether we are able to impact on child and maternal blood pressure disease progression.

Studies on children with DM1, coarctation of the aorta, and Mulibrey Nanism, and long-term cancer survivors

Together with Dr. Mari Pulkkinen and co-workers, in 2015-2018 we have randomized 47 DM1 adolescents with poor glucose control to standard educational care (SE, control) or in addition motivational interviewing (MI+SE) in a 12-month MIAD trial (NCT02637154). The study includes a comprehensive assessment of glucose variability and cardiovascular health assessments at baseline and follow-up. We have found no effect of MI on glucose or vascular health, and the manuscripts are currently under preparation.

We also have an ongoing longitudinal population-based national cohort study, coordinated by postdoc Dr. Mari Ylinen, of 300 children with repaired coarctation of the aorta, looking into predictors and vascular and cardiac effects of hypertension long-term. The results have been presented in abstract form at Nordic and Baltic conferences during 2019.

We are also studying vascular aspects in long-term childhood cancer survivors in collaboration with Dr. Kirsi Jahnukainen, with the project managed by postdoc Johnny Sundholm.

Mulibrey Nanism is a peroxisomal disease particularly prevalent in the Finnish population, with onset in early childhood, and patients suffering from severe growth disturbance and heart failure. We are conducting a case-control study to characterize the parameters of pericardial constriction, cardiomyopathy, and vasculopathy.

Oral Health and Dental Care in Children with Congenital Heart Disease (ORALPEDHEART)

With postdoc Dr. My Blomqvist and Ph.D. student Essi Karikoski we are conducting a randomized educational intervention trial to study if early information, counseling, and intensified support will lead to a) increased awareness of the importance of maintaining good oral health, b) prevent the development of poor oral health and dental caries, c) lead to less dental fear, and d) lead to higher oral health-related quality of life in children with major congenital heart disease (NCT03329170). Recruitment started in 7/2017 and so far the study has included 85 controls and 50 patients. The recruitment is planned to be finalized in 12/2020 with final follow-up oral health assessments completed by the end of 2023 at 3 years of age.

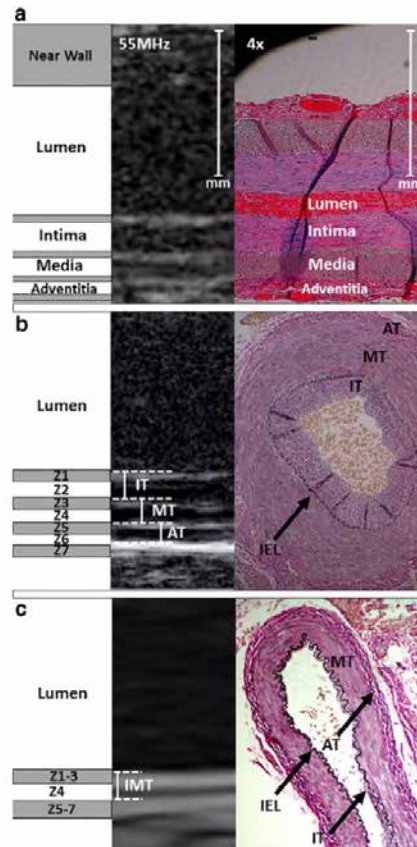


Figure. Non-invasive vascular very-high resolution ultrasound to quantify artery intima layer thickness: validation of the four-line pattern. From Sundholm JKM, Paetau A, Albäck A, Pettersson T, Sarkola T. *Ultrasound Med Biol.* 2019; 45:2010-2018.

Thesis completed in the group in 2019

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

Johnny Sundholm: Non-invasive vascular structure and pathology using very-high resolution ultrasound. University of Helsinki, October 11, 2019.

CARDIOVASCULAR RESEARCH

Main research activities

Our research group studies mechanisms of end-organ damage in cardiovascular and renal diseases. In addition, we evaluate the cardiovascular and renal protective properties of new cardiovascular drugs and treatments. During recent years, the group's research has focused on exploring the molecular mechanisms of cardiac injury and repair after myocardial infarction (MI) and in heart failure, to identify potential targets for cardiovascular medicines and novel biomarkers. In these studies, a translational approach is applied, combining methods and findings from both basic science and clinical research in order to improve the evaluation and treatment of cardiovascular diseases. The main projects worked on during 2019 are presented below.

Role of heme oxygenase-1 (HO-1) in cardiovascular diseases

HO-1 and its reaction products, carbon monoxide (CO), biliverdin, and bilirubin, have a variety of cardiovascular protective properties. The promoter region of *HMOX1* contains a guanine-thymine (GT) microsatellite repeat. A long GTn repeat decreases HO-1 expression and is associated with cardiometabolic diseases and pre-eclampsia. We continued the collaboration with Prof. Per-Henrik Groop and the FinnDiane Study Group, HU, Helsinki University Hospital (HUCH), and the Folkhälsan Institute of Genetics, to study the role of HO-1 and HO-1 gene polymorphisms in the development of cardiovascular and renal complications in type 1 diabetes.

Molecular mechanisms and RNA biomarkers of cardiac injury and heart failure

The prognosis of chronic heart failure is still poor, despite optimal therapy with currently available cardiovascular drugs. Thus, deeper understanding of cellular and molecular mechanisms of cardiac failure and repair is needed to advance discovery of novel drug target molecules, as well as novel biomarkers for more personalized diagnostics and outcome prediction.

Septins are small GTPases that are associated with actin and are important in the organization of the cytoskeleton. We have shown in collaboration with Prof. Sanna Lehtonen, HU, that septin7b, the zebrafish or-



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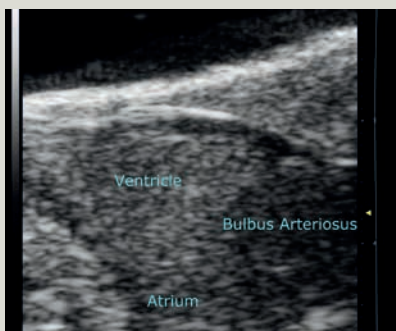


Figure. Echocardiography in adult zebrafish.

tholog of human septin7, is essential for the subcellular organization of cardiomyocytes and cardiac function in zebrafish. Moreover, knock-down of septin7b diminished the expression of retinaldehyde dehydrogenase 2 (raldh2), which catalyzes the synthesis of retinoic acid shown to modulate ischemic injury and stimulate cardiac regeneration. Our studies elucidating the role of septins in cardiac recovery after myocardial injury continued during 2019.

VeZF1 encodes a nuclear protein containing six zinc finger motifs of the C2H2-type (krüppel-like) and a proline-rich transcriptional transactivation domain. The role of *VeZF1* in stress-induced cardiac hypertrophy and development of heart failure in embryonic zebrafish was studied in collaboration with Prof. Risto Kerkelä, University of Oulu. We employed loss of function methods using antisense oligomer molecules that block the translation or splicing of *VeZF1*. We were able to demonstrate that *VeZF1* regulates the development of stress-induced cardiac hypertrophy by modulating β MHC expression and cardiac contractile function by stabilizing cardiomyocyte calcium cycling. Our results indicate a clinically relevant role for *VeZF1* in the heart.

MicroRNAs are short noncoding RNA molecules widely involved in the regulation of gene expression. The role of miRNAs after cardiac injury and in heart failure was studied utilizing both experimental and clinical materials. The let-7 family of miRNAs is expressed during embryonic development and plays an important role in cell differentiation. We investigated the role of let-7 in heart regeneration after injury in adult zebrafish in collaboration with Prof. Heikki Ruskoaho, HU. Let-7 inhibition increased cardiomyocyte proliferation and epicardial activation, and resulted in faster recovery of cardiac function and faster fibrinolysis after cryoinjury.

Circulating miRNAs have emerged as potential diagnostic and prognostic biomarkers of cardiovascular disease. Cardiogenic shock (CS) is a severe state of inadequate systemic tissue perfusion due to low cardiac output, often resulting in multi-organ failure. In-hospital mortality is close to 40% even with current treatment. New biomarkers to stratify CS patients according to their risk and to optimize treatment are needed. Based on initial microarray screening, followed by quantitative RT-PCR, several novel cardiovascular miRNAs of interest were identified. We were able to show, in collaboration with Adj. Prof. Veli-Pekka Harjola, HUCH, and Dr. Yvan Devaux, the Luxembourg Institute of Health, Luxembourg, that high plasma levels of miR-423-5p independently predict mortality in patients with acute cardiogenic shock.

During 2019, our group has participated in the COST Action CA17129 Catalysing transcriptomics research in cardiovascular disease (CardioRNA), in which Päivi Lakkisto served as a member of the Management Committee.

Clinical hypertension

High blood pressure is the leading risk factor for death worldwide. Despite developments in antihypertensive therapies during recent years, treatment results are still unsatisfactory.

Our clinical hypertension research has focused on new treatment strategies for resistant hypertension. In addition, we have participated in international collaboration to elucidate the blood pressure lowering and vascular effects of sodium glucose co-transporter 2 (SGLT2) inhibitors, novel glucose-lowering drugs with cardiovascular and renal protective properties, in the treatment of type 2 diabetes.

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 Iryna Hlushchenko, M.Sc., Ph.D. autumn 2019
 Amr Abouelezz, M.Sc.
 Pushpa Khanal, M.Sc.
 David Micinski, M.Sc.
 Aruna Ghimire, M.Sc.
 Maria Anastasiadou, B.Sc., M.Sc. January 2020

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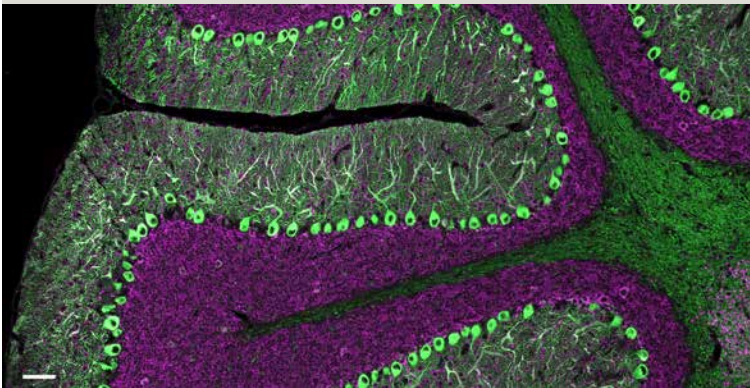


Figure. Picture shows a part of cerebellum of the adult mouse brain slice. MIM protein is shown in green and MAP2 (general neuron marker) in magenta. MIM is highly expressed in Purkinje cells in cerebellum. Bar, 50 μ m.

CELLULAR NEUROSCIENCE

Main research activities

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

A neuron typically extends a long thin axon to transmit information to target cells and several shorter dendrites that receive input from other cells through specialized connections known as synapses. The axon initial segment (AIS) is the site of action potential initiation. At same time, the AIS serves as a barrier between dendrites and axons, by sorting vesicles and proteins to the axon or somatodendritic compartment. The majority of excitatory synapses in the central nervous system exist on small bulbous structures on dendrites known as dendritic spines. The dendrites of a single neuron can contain hundreds to thousands of spines. During learning, new spines will appear. At the same time, other spines and synapses will be removed. Thus, dendritic spines can be considered 'memory units' or 'stuff that memories are made of'. Through adding or removing, or strengthening or weakening, these units, the brain modulates its function. By reorganizing synaptic pathways, new skills are stored in the brain. Precise control of the dendritic spine morphology and density, as well as the length and location of AISs, are critical for normal brain function.

Accordingly, both aberrant spine morphology and non-functional AISs are linked to many neurological diseases. The actin cytoskeleton is a structural element underlying the proper morphology of dendritic spines as well as the proper structure of the AIS.

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

In 2019, the main aim of the group was to publish articles to complete two PhD theses. Iryna Hlushchenko defended her PhD thesis in August 2019, Amr Abouelezz will defend in February 2020. In 2019, the two final publications for Iryna's thesis were accepted, the first studying actin-severing protein gelsolin in synaptic plasticity (Hlushchenko and Hotulainen, 2019). Iryna showed that a protein called gelsolin relocates to dendritic spines upon LTD-type stimulation but not upon LTP-type stimulation. This study gives insights into how neurons can distinguish between two types of Ca signaling. The last paper for Iryna's thesis was the main project of postdoc Rimante Minkeviciene, where she studied the overall effects of MIM protein deficiency for brain function and anatomy (Minkeviciene et al., 2019). The main findings were that MIM is expressed in the hippocampus and cortex only in the first weeks after birth but expression continues in Purkinje Cells in the cerebellum throughout the life. We showed that MIM is required for proper learning and motor coordination in adult mice. In addition, brain ventricles were significantly enlarged in MIM knockout mice. For Amr Abouelezz's PhD thesis, a study elucidating the basic characteristics of actin rings in axon initial segment (AIS) was accepted for publishing (Abouelezz et al., 2019). In this study, Amr showed that actin rings exhibit very slow dynamics and are resistant to Latrunculin treatment. Latrunculin is an actin monomer-binding drug which sequesters actin monomers,

thus inhibiting actin polymerization. Latrunculin treatment depolymerizes actin in dynamic actin structures. However, if actin structures are stable, Latrunculin's effect is very modest. A second article for Amr's thesis studying actin regulation in AIS has been submitted and revised but not yet accepted (Abouelezz et al., BioRxiv).

In our current projects, we are revealing the roles of the actin-regulating proteins Rif, Gas7, DAAM1 and myosin XVI in neurons.

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

Thesis completed in the group in 2019

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

Iryna Hlushchenko: Actin regulation in dendritic spines: from synaptic plasticity to animal behavior and human neurodevelopmental disorders. August 16, 2019.

Publications 2019

Abouelezz A, Micinski D, Lipponen A, Hotulainen P. Sub-membranous actin rings in the axon initial segment are resistant to the action of latrunculin. *Biol Chem.* 2019; 400:1141–1146.

Hlushchenko I, Hotulainen P. Chemical LTD, but not LTP, induces transient accumulation of gelsolin in dendritic spines. *Biol Chem.* 2019; 400:1129–1139.

Minkeviciene R, Hlushchenko, Virenque A, Lahti L, Khanal P, Rauramaa T, Koistinen A, Leinonen V, Noe FM, Hotulainen P. MIM-deficient mice exhibit anatomical changes in dendritic spines, cortex volume and brain changes in motor coordination and learning. *Front Mol Neurosci.* 2019; 12:276.



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External funding

The Sigrid Juselius Foundation
The Liv och Hälsa Foundation
Magnus Ehrnrooth Foundation

CELLULAR PHYSIOLOGY

Main research activities

Our research group investigates calcium and lipid signaling in thyroid and other human tumor-derived cancer cells. At present, we are interested in the importance of stromal interacting molecule 1 (STIM1) and Orai1, two important proteins regulating intracellular calcium storage and signaling. Furthermore, we are also investigating the interactions between calcium signaling and the sphingomyelin metabolite sphingosine 1-phosphate (S1P) on the regulation of thyroid cancer cell migration and invasion. Preliminary results indicate that knock-down of either STIM1 or Orai1 potently modulates the behavior of thyroid cancer cells (e.g. attenuation of invasion, migration, and also proliferation). Furthermore, knock-down of these proteins also modulates the expression of S1P receptors. Our results suggest that knock-down of STIM1 or Orai1 results, in part, in similar actions to those that were previously shown for the knock-down of the transient receptor potential canonical 1 (TRPC1) ion channels. A manuscript is presently being compiled on the results.

Our current investigations in our group also aim to understand compartmentalized calcium signaling in different types of cells, and how these signals are modulated by sphingolipid signaling. 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, mitochondria, and endosomes. Our investigations have shown that sphingosine kinase 1 (SphK), i.e. the kinase that phosphorylates sphingosine to S1P, potently enhances mitochondrial calcium signaling when cells are stimulated through G protein-coupled receptors. The increase in mitochondrial calcium seems to be derived entirely from the endoplasmic reticulum (ER). Mitofusin 2, which modulates ER-mitochondria contact sites (MAMs), seems to be of importance, as overexpression of SphK cleaves

mitofusin 2, apparently by a calpain-mediated mechanism. Furthermore, overexpression of putative calpain-cleaved MFN2 N- and C-terminal fragments increases mitochondrial matrix calcium during agonist stimulation. The results are interesting, as microdomains at the MAMs are of importance in oncogenesis. In the investigated cells, overexpression of SphK enhanced both oxygen consumption and migration.

We have also participated in a collaboration with professor Jessica Risenholm regarding the use of mesoporous silica nanoparticles (MSNs) as drug delivery systems in nanomedicine. In the investigation, the MSNs were coated with polydopamine, to enhance sustained and pH-responsive drug release. Furthermore, the ability of the nanoparticles to deliver hydrophobic (FTY720, i.e. Fingolimod) and hydrophilic (doxorubicin) drugs was tested. The use of methotrexate was also tested. In addition, the effect of the particle shape (rods and spheres) was investigated. To enhance cellular uptake, the nanoparticles were further coated with a polyethylene imine (PEI)-polyethylene glycol (PEG) copolymer. The in vitro tests were performed using follicular thyroid cancer ML-1 cells. Both particles showed effective cellular uptake and intracellular drug release. Interestingly, rods showed more effective cellular uptake and delayed intracellular drug release, compared with spheres. Furthermore, the used nanoparticles effectively induced the cell death and attenuated the invasiveness of thyroid cancer cells. The very promising results obtained in this study have prompted us to further test and use nanoparticles in our research on thyroid cancer cells.

Our group has ongoing collaborations with Prof. Vesa Olkkonen (Minerva), Assoc. Prof. Diana Toivola (ÅAU), and Prof. Jessica Rosenholm (ÅAU).

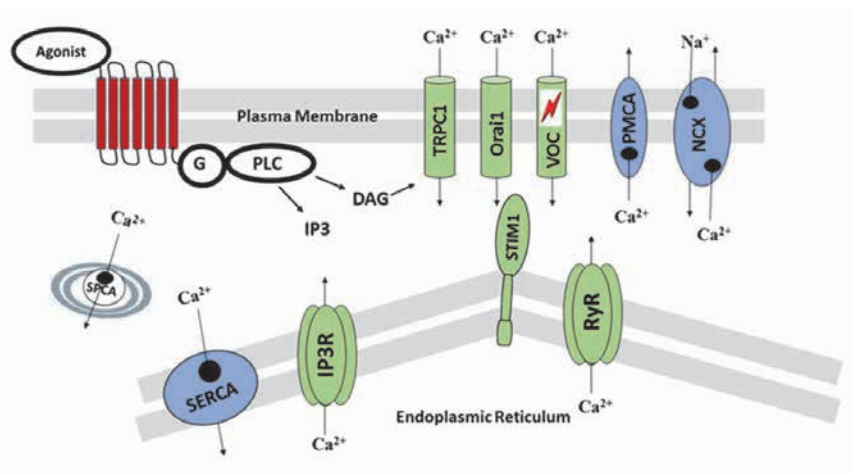


Figure. Mechanisms of calcium signaling. Upon activation of a GPCR receptor by a ligand, phospholipase C (PLC) is activated, which generates two second messengers; DAG and IP3. DAG is capable of activating TRPC channels in the plasma membrane and calcium influx is triggered. IP3 diffuses through the cytoplasm and binds to IP3 receptors on the ER membranes. This binding enables ER depletion, resulting in a rapid calcium transient. The depletion of ER is sensed by STIM1 proteins which act as sensors. STIM1 makes a complex with Orai1 channels in the plasma membrane and induces store operated calcium entry through Orai1. Voltage-operated calcium channels open in response to a depolarization of the plasma membrane in excitable neural and muscle cells. Calcium in the cytoplasm activates ryanodine receptors and calcium is released from the ER. To avoid a calcium flood in the cytoplasm, the pumps SERCA and PMCA, and the sodium calcium exchangers (NCX) are activated which export calcium out of the cell or into the ER. The secretory pathway calcium ATPase (SPCA) transports calcium ions into the Golgi apparatus.

Publications 2019

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ENDOCRINOLOGY

Main research activities

We have continued our studies addressing the pathogenesis of subtypes of non-alcoholic fatty liver disease (NAFLD) in humans. Susanna Lallukka showed that coagulation factor activities and production from the liver are increased in NAFLD attributable to metabolic syndrome and insulin resistance ('Metabolic NAFLD'). This contrasts with subjects with increased liver fat content due to the PNPLA3 I148M variant ('PNPLA3 NAFLD') who do not have increased activity of coagulation factors compared to those without the variant. Thus, obesity/insulin resistance rather than an increase in liver fat per se is associated with a pro-coagulant plasma profile. Dr. Lallukka also showed that adipose tissue is inflamed in Metabolic NAFLD but not PNPLA3 NAFLD. In addition, Dr. Lallukka determined that baseline liver fat content predicts NAFLD and advanced fibrosis after an 11-year period more accurately than metabolic measurements.

Dr. Luukkonen characterized the molecular heterogeneity of NAFLDs in the human liver. His work showed that ceramides but not other bioactive lipids are markedly increased in Metabolic NAFLD but not PNPLA3 NAFLD. We therefore examined whether overfeeding of polyunsaturated as compared to saturated fat or carbohydrate (which if consumed in excess is converted into saturated fat) influences the pathways, bioactive mediators of insulin resistance, and magnitude of overfeeding-induced changes in intrahepatic triglycerides in 38 overweight subjects. We used a combination of state-of-the-art in vivo (imaging, stable isotope tracers and hyperinsulinemic-euglycemic clamp) and ex vivo (plasma lipidome, adipose tissue transcriptome and gut microbiome) techniques. Three weeks of overfeeding of saturated fat increased hepatic TGs more than that of unsaturated fat by increasing adipose tissue lipolysis. Moreover, the saturated fat-enriched diet



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The Academy of Finland
Biomedicum Helsinki Foundation
EU/ EFPIA: Innovative Medicines Initiative Joint Undertaking (EMIF),
2013–2018 (grant 116125-2)
EU H2020: Elucidating Pathways of Steatohepatitis (EPoS), 2015–2019
(project 634413)
EU H2020: Liver Investigation: Testing Marker Utility in Steatohepatitis
(LITMUS), 2017-2022 (project 777377)
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Suomen Lääketieteen Säätiö

increased insulin resistance, circulating ceramides and gram-negative gut bacteria. Simple sugars increased hepatic TGs by stimulating hepatic de novo lipogenesis. Each diet had distinct effects on the adipose tissue transcriptome. These results showed that the effect of overfeeding on the hepatic metabolism depends on the dietary macronutrient composition. Saturated fat may be metabolically more harmful than unsaturated fat or simple sugars. The data support the hypothesis that insulin resistance in Metabolic NAFLD is a consequence of a saturated fat-induced increase in ceramides.

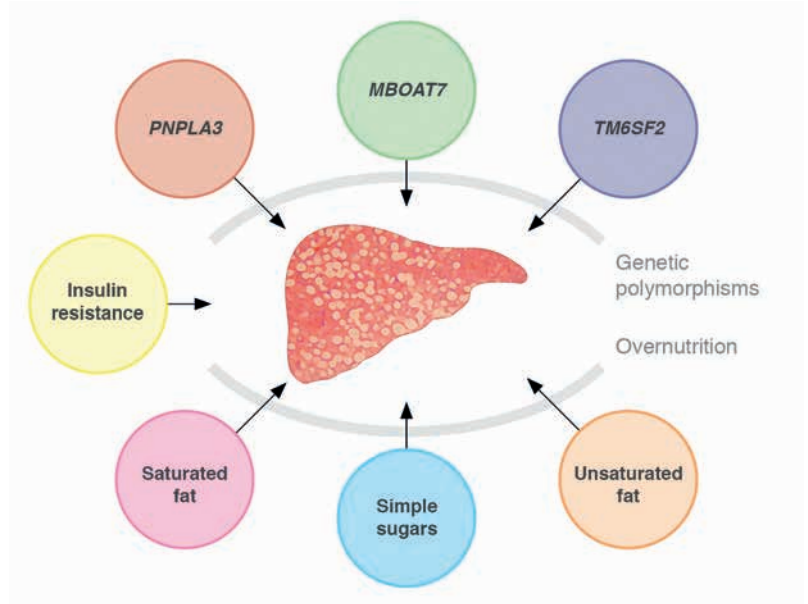


Figure. Heterogeneity of non-alcoholic fatty liver disease is mediated by multiple factors including dietary macronutrient composition, insulin resistance and common genetic polymorphisms in genes such as *PNPLA3*, *MBOAT7* and *TM6SF2* (courtesy of Siiri Luukkonen).

We are currently a partner in three EU-funded projects:

EU H2020: Liver Investigation: Testing Marker Utility in Steatohepatitis (LITMUS)

A project to develop better test for liver disease is a pioneering European research project (total funding €34 million), which aims to lead to new diagnostic tests to assess patients with non-alcoholic fatty liver disease (NAFLD) and identify those most at risk for developing severe inflammation and liver scarring.

EU H2020: Elucidating Pathways of Steatohepatitis (EPoS)

EPoS is a 48-month project funded by the European Commission within the Horizon 2020 Framework Programme.

EU/ EFPIA: Innovative Medicines Initiative Joint Undertaking (EMIF)

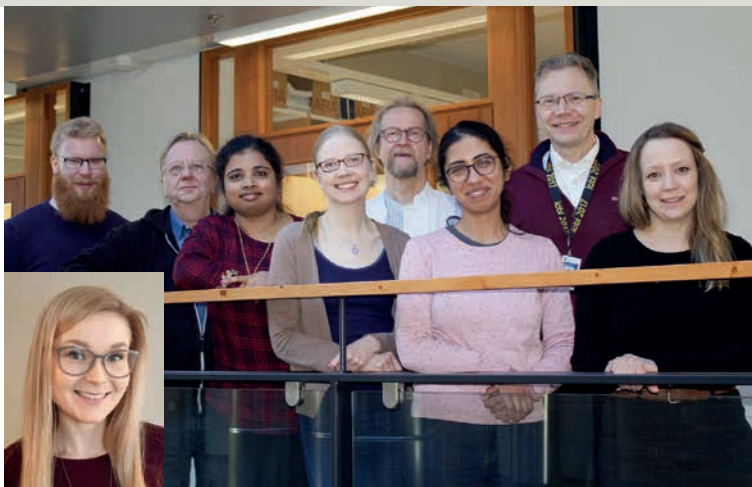
EMIF is a project studying the metabolic consequences of obesity. These consequences include NAFLD.

Publications 2019

Lahelma M, Sädevirta S, Lallukka-Brück S, Sevastianova K, Mustelin L, Gylling H, Rockette-Wagner B, Kriska AM, **Yki-Järvinen H.** Effects of weighted hula-hooping compared to walking on abdominal fat, trunk muscularity, and metabolic parameters in overweight subjects: a randomized controlled study. *Obes Facts.* 2019; 12:385–396.

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Luukkonen PK, Nick A, Hölttä-Vuori M, Thiele M, **Isokuortti E, Lallukka-Brück S, Zhou Y,** Hakkarainen A, Lundbom N, Peltonen M, Orho-Melander M, Oresic M, Hyötyläinen T, Hodson L, **Ikonen E, Yki-Järvinen H.** Human *PNPLA3*-I148M variant increases hepatic retention of polyunsaturated fatty acids. *JCI Insight.* 2019; 4(16).



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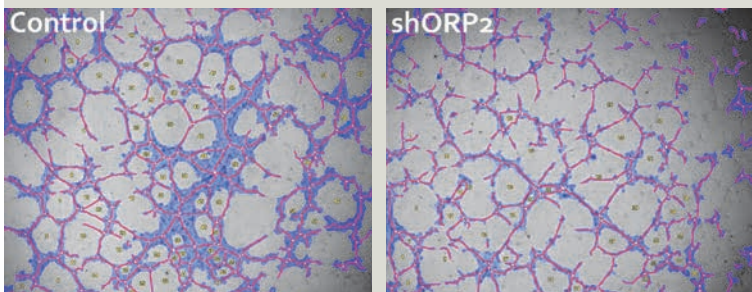


Figure. Impact of ORP2 knock-down (shORP2) in human umbilical vein endothelial cells (HUVECs) on angiogenic tube formation *in vitro*.

LIPID SIGNALING AND HOMEOSTASIS

Main research activities

One of the major interests of the group is how cells maintain the specific lipid compositions of their organelles and sense their internal lipid status to control vital cellular processes such as signal transduction, vesicle transport, motility, and proliferation. The concept of membrane contact sites (MCSs), zones of close contact between the limiting membranes of intracellular organelles, has in recent years moved into the focus of biomedical research. A major project in the group aims to unravel the function of lipid transfer proteins and other MCS components in endothelial cells (ECs), a cell type with a crucial role in practically all common diseases.

In 2019, major activity in the group was directed at understanding the role of two MCS components, the OSBP-related protein ORP2, a counter-current transporter of cholesterol and PI(4,5)P₂, and Protrudin/ZFYVE27 in characteristic EC functions such as angiogenesis. Our findings revealed crucial roles for functions of ORP2 in VEGF signaling, its knock-down in primary human ECs resulting in defective VEGFR2 signaling and inhibition of angiogenic tube formation (Figure). Moreover, study of ORP2/Osbp12 knock-out mice demonstrated a moderate but significant defect in retinal angiogenesis *in vivo* (manuscript submitted). A similar study on Protrudin function in ECs *in vitro* and *in vivo* is in progress. In collaborative studies, we elucidated the function of ORP4L in the energy metabolism of CD34⁺CD38⁻ leukemia stem cells of acute myeloid leukemia (AML) patients, identifying ORP4L as a putative candidate target for new leukemia therapies (Zhong et al., 2019), and revealed that ORP4L couples the IP₃ generated by phospholipase C to endoplasmic reticulum Ca²⁺ release (Cao et al., 2019). We further participated in elucidating the functions of ORP10, the phosphatase Sac1 and the four-phosphate-adaptor protein FAPP1 at ER-trans-Golgi contacts (Venditti et al., 2019a,b), as well as the function of ORP11 in intracellular cho-

lesterol transport and oxysterol generation in macrophages (Arnal-Levron et al., 2019).

A second major activity in the group in 2019 focused on the dysregulation of adipocyte metabolism and communication with other cell types relevant for human disease. We focused on a miRNA associated with both cancers and metabolic disease, miR-221-3p, and produced data suggesting that its expression in adipocytes plays an important role in the communication between adipocytes and breast cancer cells during tumor development (manuscript in preparation). Furthermore, study of human subjects with loss-of-function mutations in Angiopoietin-like 3 (ANGPTL3), and experiments with cultured hepatocytes, demonstrated key roles of this protein in both lipoprotein metabolism in vivo (Tikkanen et al., 2019) and in the intra-hepatocellular lipid metabolism (manuscript submitted). Our observations support the potential of this gene/protein as a future cardiovascular therapy target.

Other activities have been targeted to understanding the complexity of upstream stimulatory factor 1 (USF1), a transcription factor that is not only involved in lipid metabolism but is also indispensable for the proper maintenance of mammalian spermatogenesis (Faisal et al., 2019). In addition, we have continued lipid/lipoprotein research and described a specific, rapid isolation method for human LDL to be used in lipidomic/functional assays (Liangsupree et al., 2019). Finally, in a large collaboration study we demonstrated that lipidomic profiles capture information beyond traditionally used lipids in clinical work and identify genetic variants modifying lipid levels and risk of CVD (Tabassum et al., 2019).

Selected publications 2019

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MEMBRANE BIOLOGY

Main research activities

Our group focuses on the molecular mechanisms of intracellular lipid transport and storage in mammalian cells as well as disturbances in these processes associated with human diseases. The research relates to the basic pathogenic mechanisms of lysosomal lipid storage diseases, non-alcoholic fatty liver disease, atherosclerosis, and lipodystrophies. In this context, we also develop novel techniques for lipid cell biology, including lipid imaging, analysis of rapid metabolic fluxes of lipids, and manipulation of cells and their lipid constituents at improved spatio-temporal resolution.

The group is broadly networked both nationally and internationally, as reflected by our research. During 2019, we have published papers with collaborating research groups from the Minerva Foundation Institute for Medical Research (Luukkonen et al., 2019), the Academy of Finland Centre of Excellence in Biomembrane Research (Li et al., 2019), and the Institute of Biotechnology, University of Helsinki (Salo et al., 2019), as well as with several international academic (Heybrock et al., 2019; Salo et al., 2019) and biopharmaceutical company (Gungör et al., 2019) collaborators. We also published an editorial summarizing recent progress in the area of HDL-cholesterol uptake into cells (Ikonen and Kanerva, 2019). A major technological breakthrough during 2019 was the establishment of a rapid protein degradation system (Figure 1, Li et al., 2019). This principle has already been successfully employed in our recent work (Salo et al., 2019) and is currently being further developed in the group.

Improved rapid protein degradation methodology

The principle of auxin-inducible degen (AID) technology was originally invented over ten years ago. It employs the plant hormone auxin for targeted degradation of proteins in eukaryotic cells. In this system, the protein to be degraded is tagged with a degradation peptide or degen. This is recognized by a

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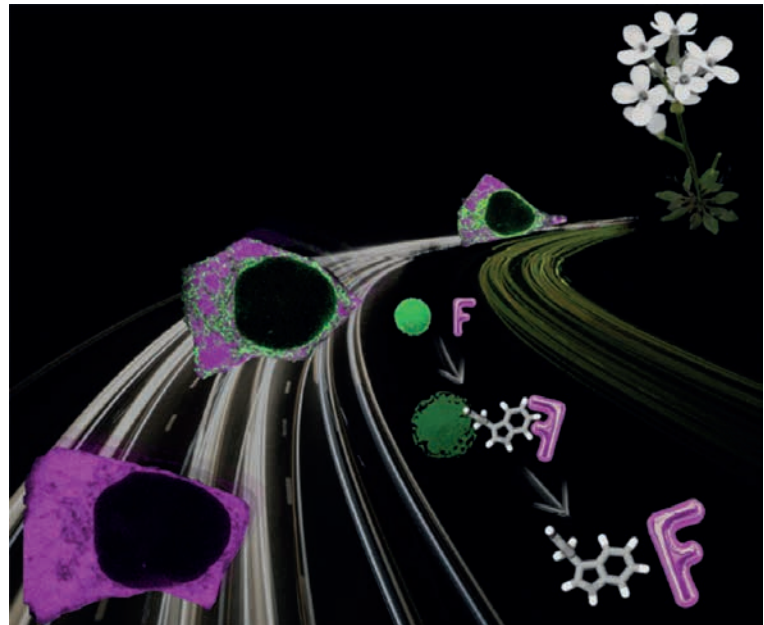
The Academy of Finland: Centre of Excellence in Biomembrane Research (ProLipids),
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 University of Helsinki, Research Excellence and Infrastructure Funding (HiLIFE)

Figure. Artist's view of the auxin-inducible protein degradation principle. The plant-derived small molecular hormone auxin (white compound) induces rapid, F-box receptor protein (magenta) driven degradation of the target protein that carries a degron sequence (green). Image courtesy of Kristiina Kanerva.

chimeric plant-eukaryotic degradation machinery in an auxin-dependent manner. However, the utility of the system has been hampered by basal protein degradation that occurs before addition of the inducer, auxin, as well as by inefficient protein depletion. Upon screening various auxin receptors and degrons, we identified a significantly improved AID system that we expect to be widely applicable. We found that this system shows minimal basal degradation and enables rapid, auxin-inducible depletion of endogenous human transmembrane, cytoplasmic and nuclear proteins in 1 h with robust functional phenotypes (Li et al., 2019).

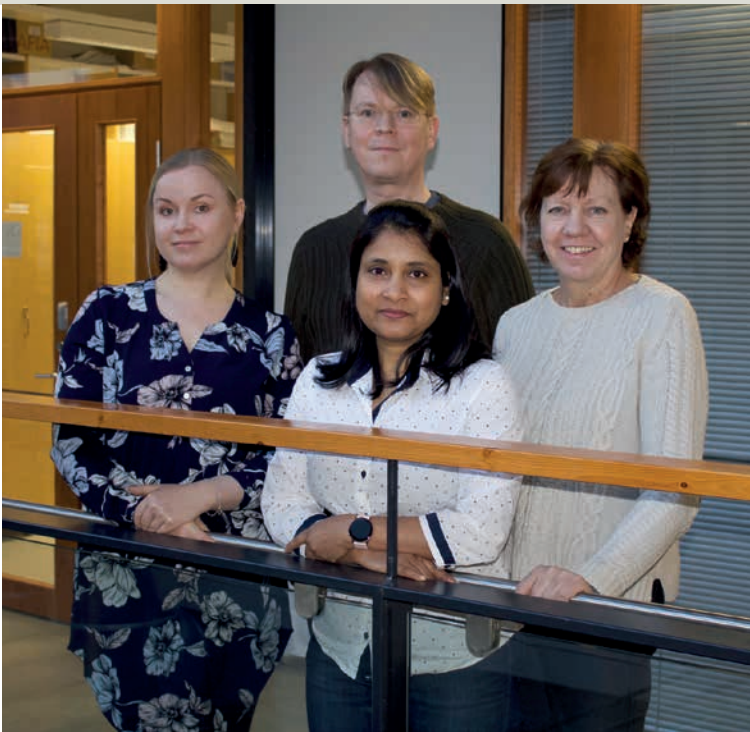
Distribution of triglycerides between lipid droplets and the endoplasmic reticulum regulated by seipin

One of our long-standing interests has been the function of the endoplasmic reticulum (ER) protein seipin. Loss-of-function mutations in this protein cause the most severe lipodystrophy in humans, and our earlier work shows that seipin localizes to ER-lipid droplet (LD) contacts. By acutely removing seipin from ER-LD contacts using the AID principle (see above), we found that seipin facilitates the continuous transfer of triglycerides from the ER, i.e. their site of synthesis, to LDs. This helps to ensure the simultaneous growth of the entire cellular LD pool. Instead, when seipin is acutely lost, the biophysical process of ripening starts to dominate in triglyceride partitioning. This leads to inhomogeneous LD growth, because triglycerides are transferred from smaller LDs with high internal pressure to larger LDs through droplet-ER bilayer contacts. Together, these findings reveal that the ER and LDs form a joint system for triglyceride partitioning and that seipin-regulated triglyceride flux is critical for the sustained growth of small LDs (Salo et al., 2019).



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METABOLISM

Main research activities

We study the molecular mechanisms of insulin resistance in human skeletal muscle, using primary human muscle cells as a research model.

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

As part of the FUSION Tissue Study, we have obtained skeletal muscle biopsies from well-characterized individuals covering all stages of glucose tolerance (normal, impaired fasting glucose, impaired glucose tolerance, as well as newly diagnosed type 2 diabetes), and have performed mRNA sequencing to obtain complete skeletal muscle transcriptome (Scott et al., 2016). We analyzed gene expression and DNA methylation data in 265 human skeletal muscle biopsies from the FUSION Tissue Study. We found hundreds of genes and DNA methylation sites that were associated with fasting insulin, waist, and body mass index, as well as thousands of DNA methylation sites that were associated with gene expression. Using Mendelian randomization and mediation techniques, we found evidence of several causal relationships where methylation is predicted to causally influence expression. We also found that increased *RXRA* expression in skeletal muscle may decrease lean tissue mass (Taylor et al., 2019).

As part of a large research collaboration, in which the FUSION study also participated, we reported exome-sequencing analyses of more than 20 000 individuals with type 2 diabetes (T2D) and over 20 000 non-diabetic control participants from several different ancestries. We identified rare variants, of which the strongest T2D gene-level signals explain at most 25% of the heritability of the strongest common single-variant signals (Flannick et al., 2019).

Publications 2019

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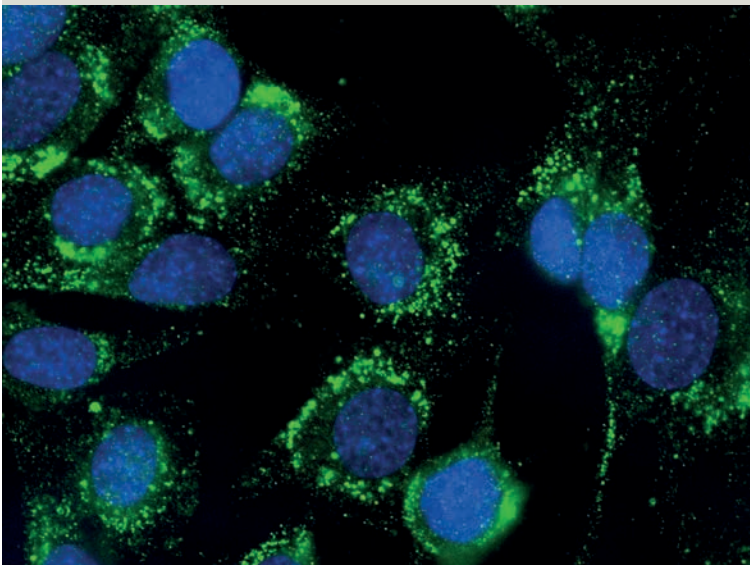


Figure. Immunocytochemistry of ER signaling protein, XBP1u forming aggresome like induced structures (ALIS) (Green) upon proteasome inhibition and nuclei (Blue) in striatal neuronal cell line.

NEURONAL SIGNALING

Main research activities

We study trophic factors in neurodegenerative diseases, such as Huntington's (HD) and Parkinson's disease (PD). We focus on the roles of endoplasmic reticulum (ER) stress and protein degradation pathways, including the ubiquitin proteasome and autophagy systems. We employ various biochemical, proteomic, molecular biology, and cell biology methods for our studies, such as primary neuron cultures, and genetically modified mice. The majority of the group is housed in Medium, Faculty of Medicine of the University of Helsinki, and the group is actively engaged in research at Minerva. During 2019, the group worked on three main areas of research.

1. Dynamic roles of USP14 in neurons and in the HD model.

Protein homeostasis (proteostasis) is known to be dysfunctional in several human diseases. In neurodegenerative disorders such as HD, there is an accumulation of mutant or misfolded proteins impairing cell functions and viability. A deeper understanding of mechanisms governing proteasome degradation and autophagy processes could have therapeutic consequences for protein aggregation diseases. USP14 is a deubiquitinating enzyme associated with the proteasome important for protein degradation. We have recently employed proteomic analyses and cell cultures to depict a role of USP14 in the control of proteostasis pathways in models of HD. USP14 was shown to interact with the molecular chaperone, HSC70 and had a dynamic role in proteostasis by controlling the proteasome, ER stress signaling, and the formation of GABARAP-positive autophagosomes in neuronal cells (Srinivasan et al., 2019). Our results showed that mutant Htt expressing striatal neuronal cells as a model for HD have a defect in autophagy at the GABARAP-sensitive stage, and that this can be influenced by the USP14 and HSC70 proteins. Modulation of the USP14-

HSC70 axis using different compounds could be a target for future drug development in neurodegenerative diseases including HD

2. PPAR γ /PGC-1 α signaling in GABAergic neurons, and use of Helsinki Biobank samples

Peroxisome proliferator-activated receptor-gamma (PPAR γ) is a nuclear receptor involved in the control of cell metabolism and inflammation in different tissues. Drugs acting on PPAR γ are used for the treatment of human metabolic disorders, such as type 2 diabetes, but they may also have beneficial effects in brain disorders (Patrone et al., 2014). We have previously studied transgenic mice with over-expression of PGC-1 α , a transcriptional coactivator for PPAR γ , in neurons (Mudo et al., 2012; Mäkelä et al., 2016). Transcriptome and proteomic analyses showed an increase in specific GABA-A receptors in the PGC-1 α transgenic mice, which was confirmed by stimulation of the PPAR γ receptors using specific ligands (Ms). Biochemical and neurophysiological studies revealed changes in the hippocampus of the transgenic mice, reflected also in altered behavior (manuscript). Dysfunctional GABAergic signaling is a consistent finding in neuropsychiatric diseases including schizophrenia (SZ). We will hence cross-breed our transgenic mice with those carrying risk genes for SZ in order to reveal possible novel therapies for the disorder. Along with this, we are employing a novel method to detect antibody profiles in biological material (Sadam et al., 2018), by analyzing serum samples of SZ patients obtained from the Helsinki Biobank. This project is an EU-funded collaboration called SZ_ TEST Horizon2010 to facilitate early molecular diagnostics and novel treatments of SZ.

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

p75NTR is a receptor for neurotrophins, including the nerve growth factor (NGF) and pro-NGF, and is expressed in both the brain and peripheral tissues. We have described a signaling pathway for p75NTR in the regulation of cholesterol and lipid uptake in cells (Pham et al., 2016). This pathway is based upon activation of the transcription factor, Sterol regulator element binding protein-2 (SREBP2), with an increase in low-density lipoprotein receptors (LDLRs). The expression of the neurotrophins was also elevated in mouse models of fatty liver. During the last year, we observed that caspase-2 plays a crucial role in SREBP and LDLR regulation. Gene profiling using RNAseq further revealed a network of lipid genes that are altered in p75NTR KO mice compared with controls (Pham et al., 2019). The serum levels of cholesterol and triglycerides were also substantially reduced in the p75NTR KO (unpublished), showing a physiological role of p75NTR in dyslipidemias that may be of clinical significance. In addition, p75NTR KO revealed distinct changes in gene expression in the brain that warrant further studies.

Publications 2019

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