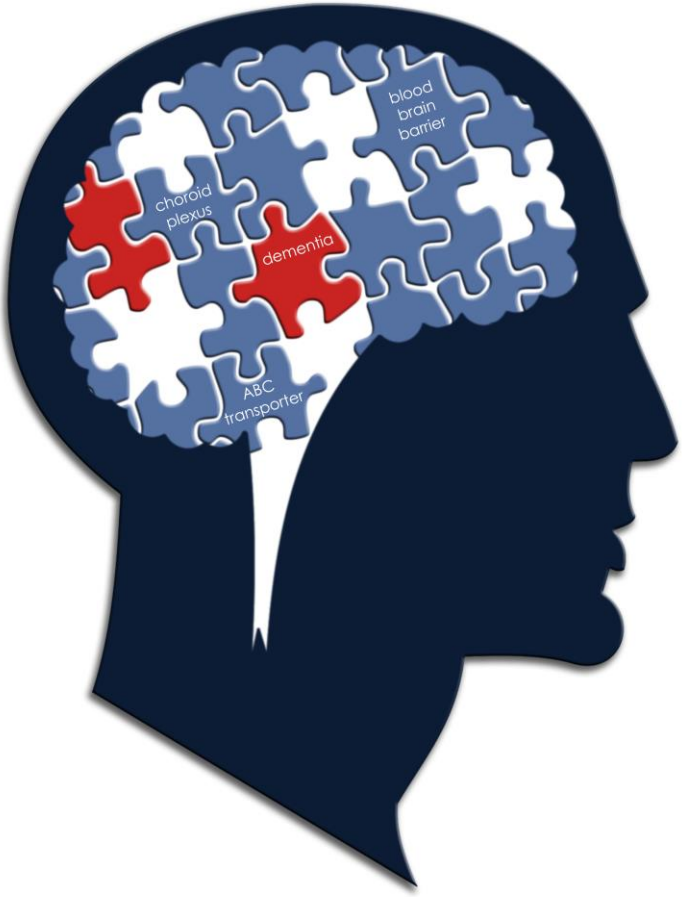
Forskningsrådet Oslo universitetssykehus UiO : Universitetet i Oslo

TransportDEMENTIA3

General Information

PROGRAM

SVOLVÆR
SEPTEMBER 1ST – 5TH 2017



PAHNKELAB.EU

Source: www.visitnorway.com

WELCOME

Dear Ladies and Gentlemen. Dear friends.

The TransportDEMENTIA meeting series has already reached the third venue.

The first meeting in Oslo in December 2015 set the start for this exceptional event series. The second meeting in September 2016 was a roundtrip on the Hurtigruten boat 'Nordkapp' from Tromsø to Kirkenes with perfect weather and beautiful *Aurora borealis* every evening and it actually topped the Oslo meeting by far. In 2017 we decided to visit the beautiful Lofoten Islands and hope that you will also enjoy this stay.

The scientific output of the meeting series - that was planned as a cross-specialization meeting - was so far beyond what we expected and led to a number of collaborations, applications and publications between the attendees.

The meeting series is 50% co-funded by Norges Forskningsrådet (The Research Council of Norway) in 2015-2017 and we are very happy that the previous meetings made you all interested to come again to Norway even though this time there was a substantial share to pay.

This time we also thought to interest pathologists and clinicians in our research and combined the meeting with the Scandinavian Neuropathological Society (SNS) meeting.

Enjoy your stay and the scientific discussions.

Jens Pahnke


Source: www.visitnorway.com

GETTING TO KNOW NORWAY

Since this is not only a scientific but also a cultural event, here are some facts about Svolvær, the biggest town of the Lofoten. The participants will have the opportunity to get to know this town and its surroundings.

LOFOTEN

Lofoten is known for excellent fishing, spectacular nature attractions such as the **northern lights** and the **midnight sun**, and small villages off the beaten track. Kayak between the islands, go fishing for the catch of your life, or look for sea eagles soaring in the sky.

The Lofoten Islands are draped across the turbulent waters of the Norwegian Sea, far above the Arctic Circle. This rare wilderness outpost offers an untrammeled landscape of majestic mountains, deep fjords, squawking seabird colonies and long, surf-swept beaches.

If you are seeking **unforgettable nature experiences**, Lofoten will definitely not let you down. Due to the area's diverse landscape, you can go hiking, skiing, fishing, ocean rafting or scuba diving. Lofoten is also one of Norway's best sites for surfing, and one of the world's northernmost.


Source: www.scandichotels.no

Lofoten has a strong connection to the Viking Age, and at Lofoten Viking Museum you can experience the Viking Age as it really was. At Borg, archaeologists have discovered the largest Viking longhouse ever found from this era. The building is 83 meters long and has been reconstructed as a living museum.

Due to the warm **Gulf Stream**, Lofoten has a much milder climate than other parts of the world at the same latitude. Between late May and mid-July you can experience the midnight sun, whilst the northern lights can be viewed from September to mid-April.

Fishing has been, and still is, to a degree, the reason why people have lived here and the region is known for its many small fishing villages. Here, you can stay in a Rorbu – an old fishermen's cabin – and eat stockfish, made from spawning cod. The stockfish is often the base product in many of the food dishes served in local restaurants. Today, there are several options for getting to Lofoten and around.

The rapidly changing weather and magnificent light conditions have inspired artists and drawn them to this area for several decades, which is evident in the many art galleries and photo exhibitions.

(Source: *visitnorway.com*)


Source: www.scandichotels.no

SVOLVÆR

We know little about Svolvær from the Stone Age and Viking times. The first written record about the farm Svolvær dates back to the middle of 16th century, where it was mentioned as the king's property.

Today, **Svolvær** has about 4590 inhabitants and it is considered the capital of the Lofoten. It is the local center of the Vågan municipality, which has a little less than 10000 inhabitants and is one of the biggest fishing municipalities in Norway. The district court of the Lofoten is also located in Svolvær.

The group of mountains surrounding Svolvær is called Fløyfjellet. **Svolvægeita** ("the Svolvær goat", 569m) is the most prominent one of the mountains because of its two horns on the top. These horns are about 20m high but only 2m apart from each other and climbers reaching the top often jump between the horns. Climbing the very top is difficult, but there is a hiking trail up until just below the horns (see "Expeditions").

Source: www.scandichotels.de

HOTEL "SCANDIC SVOLVÆR"

From 1st to 5th of September (Friday to Tuesday) participants will stay at the Scandic Hotel in Svolvær, located on a small island just by the city center.

Address:	Lamholmen 8305 Svolvær Norway
Phone:	+47 76 07 22 22
E-mail:	svolvaer@scandichotels.com
Web:	www.scandichotels.com/hotels/norway/svolvaer/scandic-svolvaer
Check-in:	14:00
Check-out:	12:00
Breakfast:	Monday to Friday 6:30-9:30 Saturday and Sunday 8:00-11:00


Source: www.lofotr.no

EXPEDITIONS WITH EXTRA BOOKING AND SURCHARGE

SATURDAY SEPTEMBER, 2ND

After lunch (13:00-16:00) **RIB Safari to Trollfjorden**

Take an adventurous ride in a RIB (rigid-inflatable boat)! We will have the chance to watch sea eagles when they're being fed and enjoy the spectacular landscape of the Lofoten including the famous Trollfjorden.

After dinner (21:00-1:00) **Northern Lights Tour**

Hunt the Lady Aurora! Together with experienced guides we will search for Northern Lights and with a little bit of luck we may find them.

SUNDAY SEPTEMBER, 3RD

After lunch (13:00-16:00) **Svolvægeita Hike**

Hike the local mountain of Svolvær! The steep hike to Svolvægeita (369m) takes approx. 30-45min (one way). Ambitious hikers can continue to Fløya (590m), which takes another 30-45min (one way). The total tour should be completed within 2-3h. We will organize a bus (departure hotel: 13:00; departure Svolvær Kirkegård: 15:30).

TRANSPORTATION

Flights

Every participant received a flight ticket and a reference number some weeks ago. If you did not get the reference number, contact Kristin Paarmann.

Please keep in mind to **check your baggage to the final destination** (Svolvær airport, SVJ) if possible.

Bus transport between Svolvær airport and hotel

We organized collective bus transport between Svolvær airport and Scandic hotel Svolvær for all participants who arrive on 1st of September and leave on 5th of September 2017. Participants were assigned to bus transports according to the departure of their flights (see page 16). Bus transport from hotel to airport takes approx. 15 minutes. If you would like to change your departure time, please contact us.

Participants who arrive and depart on other dates have to organize their own transportation, e.g. by taxi.

CONTACT

If you have any problems regarding the conference, do not hesitate to contact Kristin Paarmann.

E-Mail: TransportDementia@gmail.com
kristin.paarmann@medisin.uio.no

Phone: +47 230 71478 (office)
+49 1578 1986 883 (mobile)
+47 4012 0063 (mobile)

Please keep in mind that the official **currency of Norway** is the Norwegian krone (NOK). While most places accept credit cards, it may be useful to bring some cash as well.

FRIDAY, SEPTEMBER 1ST

Arrival Svolvær

20:00-22:00 Scientific Dinner Buffet

SATURDAY, SEPTEMBER 2ND

Breakfast

9:00-9:10 **Jens Pahnke** (Oslo)
Introduction SNS

9:10-9:40 **Holger Moch** (Zurich)
The WHO 2016 classification and new molecular findings in renal cancer

9:40-10:10 **Tibor Hortobagyi** (Debrecen/London)
Nucleo-cytoplasmic transport dysfunction and protein aggregation in neurodegeneration

10:10-10:40 **Bjarne Winther Kristensen** (Odense)
Use of novel approaches to improve glioma diagnostics

Coffee Break (15 min)

10:55-11:15 **Maria Gardberg** (Turku)
Fishing for translocations using RNA-based NGS

11:15-11:35 **Pitt Niehusmann** (Oslo)
Epilepsy-associated neoplasms in the context of the current 2016 WHO classification for CNS-tumors

11:35-11:55 **Hrvoje Miletic** (Bergen)
Mechanisms of invasion and cell communication in glioblastoma

11:55-12:15 **Olli Tynninen** (Helsinki)
Molecular diagnostics of pediatric brain tumors

Lunch Break

Optional Activity: RIB Safari to Trollfjorden

Scandinavian Neuropathological Society (SNS)

13:50-14:10	***Thomas Brännström** (Umeå)* *Mutant SOD1 aggregates from human ventral horn transmit templated aggregation and fatal ALS-like disease*
14:10-14:30	***Sverre H. Torp** (Trondheim)* *EGFR/c-erbB1 in human meningiomas*
14:30-14:50	***Stefanie Brendecke, Henning Leske** (Oslo)* *Neuropathological case reports*
14:50-15:20	***General assembly** for all members*
15:20-16:00	***Board meeting***

16:00-16:20 **Dietmar Thal** (Leuven)
Neuropathological and experimental approaches for analyzing the blood-brain barrier integrity in Alzheimer's disease

16:20-16:35 **Jens Pahnke** (Oslo)
Introduction Transport Dementia 3

16:35-17:20 **Iliya Lefterov** (Pittsburgh)
Multi-omics profiling to identify APOE-allele dependent lipid and gene expression patterns in Alzheimer's disease

Coffee Break (15 min)

17:35-18:05 **Rada Koldamova** (Pittsburgh)
APOE isoform-specific effect on Aβ clearance by microglia

18:05-18:35	**Claus Pietrzik** (Mainz) Generation and clearance of Aβ peptides
	Coffee Break (15 min)
18:50-19:20	**Anika Hartz** (Lexington, Kentucky) Two Strategies to Protect and Restore P-gp in Alzheimer's Disease
19:20-20:05	**Mihaela Gherghiceanu** (Bucharest) The role of electron microscopy in the investigation of blood brain barrier
20:15	Scientific Dinner Buffet
	Optional Activity: Northern Lights Tour

SUNDAY, SEPTEMBER 3RD

	Breakfast
9:00-9:45	**Zoltan Takats** (London) Imaging and in-vivo analysis of tissues by ambient mass spectrometry - a new toolbox for histopathology
9:45-10:15	**Jean-François Ghersi-Egea** (Lyon) The blood-CSF barrier in brain protection, degenerative disease and aging: Does-it matter?
	Coffee Break (15min)
10:30-11:00	**Joana Palha** (Braga) New challenges for the choroid plexus during aging and in Alzheimer's disease
11:00-11:30	**Fabien Gosselet** (Lens) Use of in vitro models to decipher Blood-brain barrier physiology and its roles in neurodegenerative diseases

Coffee Break (15 min)

11:45-12:15 **Maria Deli** (Szeged)
Pericytes and blood-brain function

Lunch Break

Optional Activity: Svolvægeita Hike

16:15-16:45 **Viktoria Muchitsch** (Seibersdorf)
PET Imaging of efflux transporter function at the blood-brain barrier

16:45-17:15 **Maciej Lalowski** (Helsinki)
Utilizing -omics approaches to tackle protein dynamics in the brain: example of CLN1 child epilepsy

Coffee Break (15 min)

17:30-18:00 **Tuula Nyman** (Oslo)
Proteomics in biomedical research

18:00-18:30 **Matias Zurbriggen** (Düsseldorf)
Optogenetic tools for the control and understanding of cellular processes in animal and plant systems

Coffee Break (15 min)

18:45-19:15 **Martin Fuhrmann** (Bonn)
Cellular and synaptic correlates of learning and memory

19:15-19:45 **Sven Schmitt/Katja Stefan** (Bonn)
Modulators of ABCC1: A summary of three decades of research

20:00-22:00 Scientific Dinner Buffet

MONDAY, SEPTEMBER 4TH

Breakfast

9:00-9:45 **Ludger Wessjohann** (Halle)
Chemoinformatics and Protein-3D-Modelling as Support Tools for Medicinal Chemistry on ABC-Transporters and BBB-active Compounds

9:45-10:15 **Timothy Sharbel** (Saskatoon)
Why sex and agriculture conflict: Evolutionary omics approaches to developing asexual (apomictic) seed production

10:15-10:45 **Peter Verhaert** (Beerse)
Making biobanked clinical material accessible for top-down (neuro)peptide biomarker discovery. MS Histochemistry for peptide localization and characterization in formalin fixed paraffin embedded tissue.

Coffee Break (20 min)

11:05-11:35 **Margarida Correia-Neves** (Braga)
Cognitive decline and immunosenescence: a link thought the CD4+ effector memory T cells.

11:35-12:05 **Igor Meglinski** (Oulu)
Transcranial Optical Vascular Imaging – Perspectives in Brain Diagnostics

Lunch Break

14:45-15:45 ***Leibniz Project Meeting*: St. John's wort as Alzheimer's treatment – New ways of identification, exploitation and use of natural resources**

14:45 Paride Rizzo (Gatersleben)
15:00 Katrin Franke (Halle)
15:15 Luisa Möhle (Oslo), Surya Prakash Rai (Oslo)
15:30 Discussion

Coffee Break (10 min)

15:55-16:40 ***DFG Project Meeting*: The role of the blood-brain barrier and Alzheimer's disease: Interaction of LRP1 and ABC transporter function**

15:55 Kristin Paarmann (Oslo)
16:10 Steffen Storck (Mainz)
16:25 Discussion

Coffee Break (10 min)

16:50-17:35 ***DFG Project Meeting*: PET imaging to assess blood brain function in Alzheimer's disease**

16:50 Markus Krohn (Oslo)
17:05 Oliver Langer (Vienna), Thomas Wanek (Seibersdorf)
17:20 Discussion

Coffee Break (10 min)

17:45-18:45 ***JPND Project Meeting*: Propagation behaviour of peripheral amyloid-β towards brain structures: effects of the blood-brain barrier**

17:45 Mirjam Brackhan (Oslo)
18:00 Henrik Biverstål (Huddinge)
18:15 Giulio Calza (Meilahti)
18:30 Discussion

Coffee Break (10 min)

18:55-19:40 ***JPND Project Meeting*: Identification of genes that commonly modulate the severity of neurodegenerative diseases**

Jörg Gsponer (Vancouver), via Skype
18:55 James D. Mills (Amsterdam)
19:10 Markus Krohn (Oslo)
19:25 Discussion

20:00-22:00 Scientific Dinner Banquet

Breakfast

Bus departure from hotel

Bus I For flight departing 11:05	Anika Hartz Claus Oliver Langer Claus Pietrzik Dietmar R. Thal Holger Moch James D. Mills Katrin Franke Maria Deli Paride Rizzo Steffen Storck Thomas Wanek Tibor Hortobagyi Viktoria Muchitsch Zlotan Takats
Bus II For flight departing 11:45	Erica Stenvall Fabien Gosselet Guilio Calza Henning Leske Henrik Biverstål Igor Meglinski Jean-François Ghersi-Egea Jens Pahnke Kjell Ove Eriksson-Rosenberg Kristin Paarmann Ludger Wessjohann Luisa Möhle Maciej Lalowski Markus Krohn Mihaela Gherghiceanu Mirjam Brackhan Pitt Niehusmann Sabine Leske Stefanie Brendecke Surya Prakash Rai Thomas Brüning Tuula Anneli Nyman
Bus III For flight departing 13:25	Joana Palha Margrida Correia Neves Martin Fuhrmann Peter Verhaert

Jens Pahnke

President of the Scandinavian Neuropathological Society (SNS)

Translational Neurodegeneration and Neuropathology Lab

University of Oslo, Oslo, Norway

Jens Pahnke, MD, PhD is Professor for Neuropathology at the University of Oslo (UiO) and head of the Department of Neuropathology at the Oslo University Hospital (OUS) since 2014. He is also affiliated to the University of Lübeck (UzL), the Leibniz Institute for Plant Biochemistry (IPB) in Halle/Germany and the German University in Cairo (GUC), and he is the current president of the Scandinavian Neuropathological Society (SNS).

Previously he has been working at the Universities of Greifswald, Rostock, Magdeburg and Zürich. He graduated from the University of Greifswald in 2000 as medical doctor (MD) and molecular biologist (MSc/Diploma).
The research lab focusses on the function of the blood-brain barrier for the clearance of the brain.
Recent projects investigate *i)* the infectious nature of neurodegenerative diseases: the JPND PROP-AD project wants to disprove the hypothesis that amyloid-related disease are prion-like diseases, *ii)* the Aβ-sequence EEA project aims to assess sequence and aggregation propensity of fragments of Aβ peptides for the treatment of AD, *iii)* use of herbal extracts from *Hypericum perforatum* and *Sideritis scardica* for the treatment of AD. More projects are described on the webpage of our lab.
Additionally, we are very interested to investigate new methods, e.g. Mass Spectrometry Imaging, for research and diagnostics.

Holger Moch

Vice-President of the German Pathological Society/President of the European Pathological Society

University Hospital Zurich, Department of Pathology, Switzerland

Title: The WHO 2016 classification and new molecular findings in renal cancer

September 2, 2017
9:10-9:40

Holger Moch, MD is Professor of Pathology at the University Zurich and Chairman of the Institute for Pathology and Molecular Pathology, University Hospital Zurich, Switzerland since 2004. Holger Moch is board certified in Pathology and in Molecular Pathology. He graduated from Humboldt University Berlin (Charité), Germany and received his MD in 1988. He was a research fellow in the Division of Molecular Cytometry, Department of Laboratory Medicine, University of California San Francisco; USA and a visiting fellow at Harvard Medical School, Department of Pathology, Massachusetts General Hospital Boston, Massachusetts, USA. His work is described in more than 400 peer-reviewed papers and he edited the 2016 WHO "blue book" World Health Organization Classification of Tumours of the Urinary System and Male Genital Organs. He is a member of the German National Academy of Science Leopoldina and the Swiss Academy of Medical Sciences. Holger Moch is member of the Executive boards of the European Society of Pathology (ESP) and the German Society for Pathology (DGP).

In the present talk, an overview about the pattern of somatic mutations in kidney tumours will be given. The von Hippel-Lindau (VHL) tumour suppressor gene is mutated as an early event in almost all cases of clear cell renal cell carcinoma (ccRCC). Recent advances in terms of understanding how dysregulation of the many hypoxia inducible factor a (HIFa)-dependent and -independent functions of the VHL tumour suppressor protein (pVHL) will be discussed. Current evidence showing extensive inter- and intra-tumoural genetic diversity has given rise to the

idea that ccRCC should actually be considered as a series of molecularly-related, yet distinct, diseases defined by the pattern of combinatorial genetic alterations present within the cells of the tumour. The talk will also highlight the range of genetic alterations that occur recurrently in other RCC subtypes. In the current 2016 WHO classification, this molecular background of a renal tumour has become, in addition to histopathology, a major criterion for tumour classification. The talk will also discuss morphology and genetics of novel renal epithelial cancers included in the new WHO classification.

Tibor Hortobagyi

Department of Basic and Clinical Neuroscience, King's College London, London, UK

Department of Neuropathology, University of Debrecen, Debrecen, Hungary

Title: Nucleo-cytoplasmic transport dysfunction and protein aggregation in neurodegeneration

September 2, 2017
9:40-10:10

Nucleocytoplasmic transport is one of the crucial processes in the cells and its errors result in mislocalization and aberrant accumulation of cargos with consequent often deadly loss of cellular homeostasis. Cytoplasmic mislocalization, aggregation, and nuclear clearance of several RNA binding proteins such as TDP-43 is one of the hallmark pathological features of ALS and FTLD and have also been described in Alzheimer disease, in dementia with Lewy bodies and in a variety of other neurodegenerative conditions.

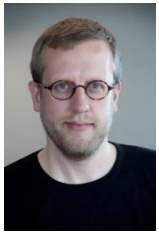
In a study led by Boris Rogelj at King's College London we observed a drastic reduction of nuclear transport factor CSE1L (cell apoptosis susceptibility, also known as CAS) in FTLD-TDP cases, and the reduction of CSE1L in cultured cells leading to cytoplasmic accumulation of TDP-43. We have shown that TDP-43 is imported into the nucleus via the KPNB1 pathway and that reduction in KPNB1 or CSE1L can lead to cytoplasmic accumulation of TDP-43. Importantly, CSE1L was strongly reduced in the brains of FTLD patients.

The case for nuclear transport involvement in TDP-43 proteinopathies has been further substantiated by recent reports showing impairment of nucleocytoplasmic transport associated with the *C9orf72* mutation. On the other hand, several studies have shown that TDP-43 levels impact on the nuclear transport, suggesting a potential feed-back regulation mechanism. Overall, the regulation of the nuclear transport machinery by TDP-43 raises the question whether initial modest TDP-43 mislocalization and aggregation can additionally impair nuclear transport and form a positive feedback loop, causing more TDP-43 mislocalization and aggregation.

Bjarne Winther Kristensen

Department of Pathology, Odense University Hospital, Odense, Denmark

Title: Use of novel approaches to improve glioma diagnostics



September 2, 2017
10:10-10:40

Brain tumor diagnostics is known to be challenging and some areas of brain tumor diagnostics have a high inter-observer variation. Moreover heterogeneity and inflammatory cells can cause difficulties when evaluation immunohistochemical markers. We introduced next generation sequencing and genome-wide methylation profiling in the daily diagnostic setting in order to improve glioma diagnostics. In addition we used a multiplexing approach to prevent pitfalls in immunohistochemical biomarker analysis. The talk will focus of practical issues in a daily pathology setting and cover initial experience and data associated with these approaches.

Maria Gardberg

Department of Pathology, Turku University Hospital, Turku, Finland

Title: Fishing for translocations using RNA-based NGS

September 2, 2017
10:55-11:15

In recent years, many tumor entities have emerged or been re-defined based on a tumor-specific genetic profile. This has increased application of next generation sequencing (NGS) based methods in clinical pathology. One group of tumor-defining genetic alterations is chromosomal translocations which can lead to fusion gene formation. Translocations are typical/defining for several tumor entities among CNS tumors and sarcomas. RNA based NGS can be used to detect the fusion transcript, RNA-level product of a fusion gene. In sarcoma diagnostics, several fluorescent in situ-hybridizations are typically needed in the diagnostic algorithm, which is time-consuming and laborious. In situ hybridization is useful when both partners of the fusion gene are known but in case of an unknown or new fusion gene they are not the method of choice. During the last year, we have used a novel approach to detect many possible gene translocations simultaneously. This talk will present our practical experiences in looking for a multitude of translocations simultaneously, using RNA-based NGS from formalin fixed paraffin embedded CNS and sarcoma tumor samples.

Pitt Niehusmann

University Hospital Oslo (OUS), Oslo, Norway

Title: Epilepsy-associated neoplasms in the context of the current 2016 WHO classification for CNS-tumors

September 2, 2017
11:15-11:35

Recent progress in molecular genetic approaches and corresponding results have substantially improved our knowledge of the pathogenesis and derived therapies of brain tumors. Therefore, the world health organization (WHO) has prompted the responsible expert panels to revise the current classification system of brain neoplasms and to perform a paradigm shift by introducing a molecular genetic diagnostic level for distinct tumor entities and thereby adding a novel level of complexity into brain tumor diagnostics in addition to classical histopathology and immunohistochemistry.

The revised 4th edition of the so-called 'blue book' was introduced in May 2016 and has already fundamentally impacted diagnosis and treatment of many frequent brain neoplasms. Nevertheless, low-grade neuroepithelial tumors that manifest clinically often with early-onset focal seizures and are mostly diagnosed in children and young adults (often designated as long-term-epilepsy-associated neuroepithelial tumors, LEAT) so far lack respective revised and standardized clinic-pathological and molecular genetic classification schemes. However, it is recognized in the 2016 WHO classification that LEATs lack IDH1/2 mutations as well as 1p/19q co-deletions. BRAF V600E mutations represent the most frequent reported genetic alteration in gangliogliomas, while Tyrosine kinase activating FGRR1 mutations are the prevailing type in dysembryoplastic neuroepithelial tumors (DNT). MYB/MYBL1 alterations seem to be characteristic for angiocentric gliomas.

Further studies are needed to develop a useful clinic-pathological LEAT classification on the basis of current WHO system by integrating histological, immunohistochemical and molecular genetic diagnostics.

Hrvoje Miletic

Department of Pathology, Haukeland University Hospital, Norway

Department of Biomedicine, University of Bergen, Norway

KG Jebsen Brain Tumor Research Center, University of Bergen, Norway

Title: Mechanisms of invasion and cell communication in glioblastoma

September 2, 2017
11:35-11:55

Glioblastoma are highly invasive brain tumors with single cells migrating long distances away from the main tumor mass. This diffuse spread of tumor cells poses major challenges in treatment of the disease. Radical neurosurgery is often not possible and even when applied not curable. Adjuvant therapies such as chemotherapy and radiation induce resistance in surviving tumor cells, which are responsible for tumor recurrence.

Many drivers of GBM invasion have been identified so far and my research group has contributed to this knowledge. We have identified EGFR as a main driver of invasion in PDX models of glioblastoma. However, EGFR-targeted therapies have so far failed in clinical trials. An important question still remains whether the blood-brain barrier impedes efficient delivery of drugs into invasive tumor areas or whether the drugs as such are inefficient.

Microtubes, which are tubular cytoplasmic extensions of glioblastoma cells, have recently been discovered as new important communication structures. An extensive microtube network is often found at the border in invasive tumor areas. We are currently investigating the influence of TGF-beta on microtube formation and how the microtube mediated communication network can contribute to therapy resistance.

Olli Tynninen

Department of Pathology, University of Helsinki

HUSLAB, Helsinki University Hospital, Helsinki, Finland

Title: Molecular diagnostics of pediatric brain tumors

I am working as surgical neuropathologist in Helsinki University Hospital. My main research interest is molecular diagnostics of brain tumours.

September 2, 2017
11:55-12:15

Thomas Brännström

Department of Medical Biosciences, Umeå University, Umeå, Sweden

Title: Mutant SOD1 aggregates from human ventral horn transmit templated aggregation and fatal ALS-like disease

September 2, 2017
13:50-14:10

Mutations in SOD1 are a common known cause of ALS. Both ALS patients and transgenic mice develop aggregates of hSOD1 in motor neurons. In symptomatic mice two different types of aggregates (denoted A and B) can arise (Bergh et al. *Proc Natl Acad Sci U S A,* 2015, 112:4489-94). Inoculation of minute amounts of these into spinal cords of asymptomatic hSOD1 transgenic mice initiates spreading, exponentially growing templated hSOD1 aggregation concomitantly with premature fatal motor neuron disease (Ekhtiari Bindhendi et al. *J. Clin. Invest.* 2016:126:2249-53).

Here we explored whether prion-competent mutant hSOD1 aggregates also exist in human ALS using an binary epitope mapping assay (Bergh et al, as above) enhanced with 7 extra antibodies. Aggregate seeds were prepared from spinal cords from a patient and transgenic mice carrying the hSOD1G127Gfs*7 truncation mutation (first described by Andersen et al, *Brain,* 1997, 120:1723-37) by centrifugation through density cushions. The core structure of hSOD1G127Gfs*7 aggregates was strain A-like. Inoculation of the seeds into lumbar spinal cord of hSOD1-expressing mice induced spreading strain A aggregation and fulminant ALS-like disease, demonstrating for the first time the presence of prion-competent hSOD1 aggregates in human ALS. The patterns seen by the binary epitope mapping assay suggest that the fibril cores of $hSOD1^{G85R}$ strain A aggregates and $hSOD1^{G127X}$ aggregates could be identical. The potency of the seeds was extremely high, and initiated disease under conditions plausible to exist in human motor areas.

Our results suggest that templated spread of hSOD1 aggregation could be the primary pathogenic mechanism, not only in hSOD1 transgenic models, but also in human ALS.

Sverre H. Torp

Dept. of Pathology, St. Olavs Hospital, Trondheim, Norway

Dept. of Clinical and Molecular Medicine, NTNU, Trondheim, Norway

Title: EGFR/c-erbB1 in human meningiomas

September 2, 2017
14:10-14:30

Meningiomas are the most common intracranial tumour in humans. In general, they are regarded as benign. However, even benign meningiomas have recurrence rates of about 7-25 %. The current WHO classification is not optimal to identify these tumours, and search for reliable biomarkers is therefore highly desired. Potential candidates are growth factor receptors as they are commonly involved in the tumourigenesis of these tumours. Much attention has hereby been paid to epidermal growth factor receptor (EGFR). However, studies are ambiguous as far as expression pattern and clinical significance are concerned. In a large series of meningiomas we investigated the expression of this receptor by immunohistochemistry and searched for any relation / associations with malignancy grade and risk of recurrence. In this talk results and clinical relevance will be presented.

Stefanie Brendecke

University Hospital Oslo (OUS),
Oslo, Norway

Neuropathological case reports
September 2, 2017
14:30-14:50

I did my medical studies at the Albert-Ludwigs-University in Freiburg, Germany, from 2003-2010, acquiring my M.D. in the field of clinical neurology/radiology studying vascular MRI flow imaging in stroke patients.

From 2011 to 2015, I worked as an intern specializing in the field of diagnostic neuropathology at the Institute for Neuropathology at the University Hospital in Freiburg. Here my research work was focused on the role of myeloid immune cells within and surrounding the CNS, meaning mainly microglia, CNS perivascular, meningeal and choroid plexus macrophages and dendritic cells a well as monocytes. Working with one of the most common mouse models used for human multiple sclerosis, experimental autoimmune encephalomyelitis (EAE), I investigated myeloid cell interactions during the initiation, propagation and maintenance of CNS autoimmune inflammation as well as the function of various immune mediators, such as Interferon beta.

Currently, I am an intern in neuropathology/pathology at the University Hospital of Oslo, Norway. My research at the Translational Neurodegenerative and Neuropathology Lab in Oslo once again centers on neuroinflammation with the aim to understand how ABC transporters influence pathology in autoimmune CNS inflammatory disease.

Henning Leske

University Hospital Oslo (OUS),
Oslo, Norway

Neuropathological case reports
September 2, 2017
14:30-14:50

Glioblastoma is the most frequent primary brain tumor in the elderly with a dismal prognosis of approximately 15 month. To date there are no curative treatment options available for this diffuse glioma. One potential reason is that the biology of these tumors, with their ability to diffusely infiltrate the surrounding brain parenchyma, is not fully understood.

I have started a study in which we analyze different patterns of MGMT methylation of glioblastoma tissue and correlate them with their clinical outcome. My aim of research is to better understand the mechanisms that lead to the development and the treatment resistance of this primary brain tumor.

I studied human medicine (2003-2009) at the Ludwig-Maximilians-University in Munich, Germany. Thereafter, I started my specialization as a neuropathologist at the Department of Neuropathology at the University Hospital in Zurich, with one year of education in the Department of Surgical Pathology. In Zurich I was performing research on prion diseases and different prion mutations. After finishing my specialization of neuropathology (2016) I moved to Oslo. Here I am working at the Rikshospitalet in the Department of Neuropathology.

Dietmar Thal

KU-Leuven, Department of Neurosciences – Laboratory of Neuropathology, Belgium

UZ-Leuven, Department of Pathology, Belgium

Title: Neuropathological and experimental approaches for analyzing the blood-brain barrier integrity in Alzheimer's disease

September 2, 2017
16:00-16:20

Alzheimer's disease (AD) is pathologically characterized by amyloid plaques and neurofibrillary tangles. Amyloid plaques are protein aggregates mainly composed of the amyloid β-peptide (Aβ). Active and passive immunization strategies targeting Aβ have been developed to reduce the cerebral amyloid burden. Passive vaccination is carried out by the peripheral administration of Aβ-targeting antibodies. In so doing, these antibodies and/or Aβ must pass the blood-brain barrier. To clarify the question which types of Aβ-aggregates can be targeted with peripherally given antibodies we treated APP-transgenic mice (APP23 mice) with antibodies against an N-terminal epitope of Aβ (β1). In the brain of treated as well as untreated mice we found at the age of 11 months fully mature Aβ aggregates containing non-modified as well as modified Aβ species. In the serum of the treated animals we found antibody-bound Aβ which was not present in PBS-treated animals. However, modified $A\beta_{N3pE}$ and $A\beta_{pS8}$ were not seen in the serum although being present in the cerebral amyloid deposits. On the other hand, antibody-bound cerebral Aβ aggregates containing modified $A\beta_{pS8}$ were observed not only in treated but also in PBS-treated mice showing that the blood-brain barrier in animals with Aβ pathology is leaky for antibodies as in AD patients. However, in the antibody-treated animals we found that the antibody given for treatment blocked further binding of the same antibody whereas other antibodies were still be able to detect Aβ plaques in these animals.

In conclusion, these experiments indicate that amyloid pathology can cause blood-brain barrier dysfunction that can be used for therapeutic approaches. However, in our experiments it appeared to be that only non-mature Aβ was subject of clearance from brain into the serum whereas the presence of modified $A\beta_{N3pE}$ and $A\beta_{pS8}$ seemed to preclude such a clearance in the APP23 mouse model.

Iliya Lefterov

University of Pittsburgh,
Pittsburgh, Pennsylvania, USA

Title: Multi-omics profiling to identify APOE-allele dependent lipid and gene expression patterns in Alzheimer's disease

September 2, 2017
16:35-17:20

Inheritance of APOEε4 allele is a major genetic risk factor for Alzheimer's disease. Important functions of brain lipids indicate possible role of dysregulated lipid metabolism in neurodegeneration.

Shot-Gun lipidomics and mRNA-seq were used to determine the effects of APOE on brain transcriptome and lipidome in inferior parietal lobule of APOEε3/3, APOEε3/4 and APOEε4/4 AD patients. Pathway analysis and multi-omics approach were applied using differentially expressed genes and lipid sub-species to identify correlations within and between transcriptomics and lipidomics data.

In APOEε4/4 samples, dysregulated expression of genes involved in the control of apoptosis, phospholipid metabolism, and mitochondrial functions are characteristic at the late stage of AD in correlation with changes in cardiolipin, phosphatidylinositol bisphosphate, and phosphatidylethanolamine species.

The results of this study demonstrate correlations between brain lipidomes and transcriptomes and indicate dysregulation in cardiolipin and phosphatidylethanolamine content, as well as genes involved in apoptosis, autophagy, and mitophagy as important for APOEε4 mediated differences in AD pathology.

Rada Koldamova

University of Pittsburgh, Pittsburgh, Pennsylvania, USA

Title: APOE isoform-specific effect on Aβ clearance by microglia

September 2, 2017
17:35-18:05

The inheritance of APOE4 allele is a major genetic risk factor for late-onset Alzheimer's disease (AD) but the mechanisms underlying this association remain elusive. APOE can differentially modulate amyloid β (Aβ) accumulation and clearance through blood brain barrier, or by astrocytic and microglial degradation.

Using primary astrocytes, we isolated and characterized native APOE3- and APOE4 lipoprotein particles. Lipidomics analysis of these native lipoproteins determined that anionic phospholipids, phosphatidylserine and cardiolipin, important for phagocytosis, are increased in APOE3 compared to APOE4-containing lipoproteins. Using *in vitro* assays, we established that native APOE3-containing lipoproteins are more effective than APOE4 in promoting Aβ phagocytosis by microglia. Intracranial injection of Aβ in complex with these native particles affected the transcriptome of sorted microglial cells in APOE-isoform depended manner. Interestingly, *Trem2*, *Aif1/IBA-1, Mertk, Bin1, Picalm* were up-regulated in mice injected with Aβ+APOE3 whereas *Clu*, *Sorl1* and several members of *Ms4a* family had a higher expression in mice injected with Aβ+APOE4. Finally, hippocampal infusion of native APOE3 but not APOE4 particles significantly ameliorated the deleterious effect of oligomeric Aβ on cognitive performance in WT mice.

We demonstrate that APOE exerts an isoform-specific effect on microglia mediated Aβ phagocytosis, subsequently affecting cognitive performance and phenotype.

Claus Pietrzik

Institute of Pathobiochemistry,
University Medical Center of
the Johannes Gutenberg
University, Mainz, Germany

Title: Generation and clearance of Aβ peptides

September 2, 2017
18:05-18:35

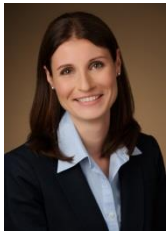
According to the neurovascular hypothesis, impairment of the low-density lipoprotein receptor-related protein-1 (LRP1) in brain capillaries of the blood-brain barrier (BBB) contributes to neurotoxic amyloid-beta (Aβ) brain accumulation and drives Alzheimer's disease (AD) pathology. However, conflicting findings on LRP1's involvement in Aβ transport and its expression in brain end othelium have questioned the role of LRP1 at the BBB. With a novel Slco1c1-CreERT2 mouse, we generated the first brain endothelial-specific LRP1 knockout mouse to accurately evaluate LRP1-mediated Aβ BBB-clearance in vivo. Additionally our laboratory focusses on the generation of N-terminal truncated Aβ-peptides which cannot be generated by BACE1. We have identified meprin β as an enzyme upregulated in brain of AD patients which is capable to generate 2-40 and 2-42 peptides in vitro. These peptides are very abundant in AD patients and may be one reason for the failure of BACE inhibitors in clinical trials. In general we are focusing on novel generation pathways of Aβ peptides and emphasizing the importance of systemic Aβ elimination via the BBB.

Anika Hartz

Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY, USA

Department of Pharmacology and Nutritional Sciences, University of Kentucky, Lexington, KY, USA

Title: Two Strategies to Protect and Restore P-gp in Alzheimer's Disease



September 2, 2017
18:50-19:20

Background: Failure to clear Aβ from the brain is partly responsible for Aβ brain accumulation in Alzheimer's disease (AD). A critical protein for clearing Aβ across the blood-brain barrier is the efflux transporter P-glycoprotein (P-gp). In AD, P-gp is reduced, which contributes to impaired Aβ brain clearance. However, the mechanism responsible for P-gp reduction is poorly understood and there are no strategies available to protect or restore P-gp. Our work focuses on two independent strategies: 1) Protecting P-gp by inhibiting its Aβ-induced degradation through the ubiquitin-proteasome system and 2) restoring P-gp levels by activating the nuclear receptor PXR.

Methods: *Strategy 1*: hAPP mice were dosed *in vivo* with a ubiquitination inhibitor, a microtubule inhibitor, or a proteasome inhibitor. After 2 weeks, brain capillaries were isolated to determine P-gp expression and function; Aβ levels were measured in plasma and brain. *Strategy 2*: In a long-term *in vivo* study, starting at age 3 months, hAPP mice received a purified diet containing the PXR activator PCN; wild-type and hAPP control mice received purified diet alone. Mice underwent behavioral testing every 6 months, after which brain capillaries were isolated and plasma and brain samples were collected for molecular analysis.

Results: *Strategy 1*: Inhibiting ubiquitination, cellular trafficking, or the proteasome prevented Aβ-induced P-gp degradation and significantly reduced Aβ brain levels in hAPP mice. *Strategy 2*: Within 6 months of treatment, P-gp activity levels in PCN-treated

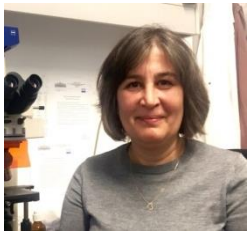
hAPP mice were comparable to those in wild-type mice. Behavioral tests to assess cognitive impairment are ongoing.

Summary: Our data may provide therapeutic avenues within the blood-brain barrier to 1) limit Aβ-induced P-gp degradation and to 2) restore P-gp function in AD. These two independent strategies have the potential to improve Aβ brain clearance, delay or prevent cognitive impairment, and may provide novel treatment options for AD therapy.

Mihaela Gherghiceanu

Victor Babes National Institute of Pathology, Bucharest, Romania

Title: The role of electron microscopy in the investigation of blood-brain barrier



September 2, 2017
19:20-20:05

I am associate Professor for Cellular and Molecular Biology and lead the diagnostic and research Ultrastructural Pathology laboratory at the Victor Babeș National Institute of Pathology Bucharest. I am the vice-president of Romanian Electron Microscopy Society and expert in ultrastructural diagnostic. My research is focused on interstitial cells and their interactions with resident cells with emphasis on telocytes involvement in stem cell niches structure in various organs, nervous system included. Using new ultrastructural investigation techniques such as electron tomography and FIB-SEM imaging we investigate atypical junctions and extracellular vesicles involvement in heterocellular communication.

One of the explored brain structures in ageing and dementia is the blood-brain barrier (BBB), a complex cellular gate which regulates tightly the transport of molecules into and from the central nervous system. Alteration of this barrier is now increasingly documented in brain vascular disease; neurodegenerative disorders and ageing but "leaky" BBB are less often directly demonstrated by morphological investigation. Cumulative damage of mitochondria due to ROS accumulation accounts for the mitochondrial theory of aging. Electron microscopy (EM) of BBB clearly shows abundant mitochondria with different morphology in endothelial, pericytes and glial cells. EM assessment of BBB in aged laboratory animals might offer a clue about mitochondria content and morphology and their contact sites with neighbouring organelles in different BBB cell types.

Zoltan Takats

Faculty of Medicine, Department of Surgery and Cancer, Imperial College London, London, United Kingdom

Title: Imaging and in-vivo analysis of tissues by ambient mass spectrometry - a new toolbox for histopathology

September 3, 2017
9:00-9:45

The recent development of ambient mass spectrometric methods enabled the rapid chemical imaging of tissue specimens as well as in-vivo classification of tissue features of interest. The presentation will describe Desorption Electrospray Ionization (DESI)- MS and Laser Desorption Ionization (LDI) -MS approaches developed for the MS imaging (MSI) analysis of tissue specimens regarding instrumentation, workflow and the nature of information provided by these methods. Surgical mass spectrometric methods commonly known as Rapid Evaporative Ionization Mass Spectrometry will also be described. These methods are based on the utilization of surgical tissue ablation modalities (electrosurgery, RF ablation, laser surgery, ultrasonic tissue ablation) as mass spectrometric ion sources for the real-time histological tracking of surgical dissection. The combination of the imaging and in-vivo approaches will be put forward as a general solution for fast histopathological assessment.

Jean-François Ghersi-Egea

Blood-brain interfaces exploratory platform BIP and FLUID Team, Lyon Neurosciences Research Center, INSERM U1028 CRNS UMR 5292, Université Claude Bernard Lyon-1, Lyon, France

Title: The blood-CSF barrier in brain protection, degenerative disease and aging: Does-it matter?

September 3, 2017
9:45-10:15

A large number of studies have focused on the role of the blood-brain barrier, located at the endothelium of the cerebral microvessels, in protecting the brain from deleterious blood-borne or brain-produced substances. The barrier between the blood and the ventricular CSF, formed by the choroid plexuses, can also have a substantial influence on the cerebral bioavailability of numerous toxic compounds, endogenous metabolites and peptides. It is also an important site of neuro-immune interaction. Thus the choroid plexuses, in combination with the blood-brain barrier, could contribute to prevent degenerative and inflammatory diseases by controlling the cerebral fluid homeostasis. Using animal, cellular and ex vivo models developed by the Blood-brain Interfaces exploratory Platform (BIP), we provide evidence that the choroidal epithelium efficiently fulfills a protective function against pro-oxidant species, forms an enzymatic barrier, and fulfills efflux transport functions that collectively protect the brain fluid environment. These different functions are mature early during development and are maintained throughout life, which suggests their significance for the preservation of brain functioning during aging.

Joana Palha

School of Medicine, University of Minho, Braga, Portugal

Title: New challenges for the choroid plexus during aging and in Alzheimer's disease

September 3, 2017
10:30-11:00

ABOUT THE TALK: The choroid plexus is formed by a monolayer of epithelial cells that lies in a highly vascularized stroma of connective tissue. It floats in the brain ventricles. Two key features, its role as the main producer of cerebrospinal fluid, and the fact that the capillaries that irrigate the choroid plexus are fenestrated; makes it ideally positioned for the communication in and out of the brain. Specifically, transporters and receptors in both the apical and in the basolateral sides of the choroid plexus epithelial cell can control the homeostasis of what is transported into and out of the brain, and the composition of the cerebrospinal fluid.

In the context of aging and Alzheimer's disease, we and others have showed that alterations of the choroid plexus function may contribute to Alzheimer's disease. To highlight is the evidence of altered clearance of the Alzheimer's disease amyloid beta peptide, of altered signaling mediated by immune mediators, and impaired circadian rhythms. Ongoing research is characterizing the human choroid plexus transcriptome during aging and in Alzheimer's disease, which will be presented and discussed at the meeting.

ABOUT THE RESEARCH: The research on the choroid plexus we are developing is integrated in the Neuroscience research domain of the Life and Health Sciences Research Institute (ICVS) (http://www.icvs.uminho.pt/) at the School of Medicine, at the University of Minho (Braga, Portugal). The research at the ICVS is organized around projects that gather researchers from various

backgrounds, with a strong clinical emphasis. Among our main interests is the search for predictors of healthy cognitive aging, which may provide clues on how the processes that lead to neurodegenerative diseases. As such, we are following longitudinally a cohort of healthy individuals, older than 55 years of age, recruited from the community, through the health centers. We are also following individuals with established disease, particularly multiple sclerosis and Alzheimer's disease. For all the research in humans, we count with excellent conditions provided by clinical academic center (https://www.ccabraga.org/).

Given the anatomical localization and the functions already attributed to the choroid plexus, we consider that the basic research developed in rodent models have potential to reveal novels molecules with diagnostic, prognostic and prevention value, as we recently showed in the case of lipocalin 2 in multiple sclerosis. Altogether, our team is interested in understanding diseases of the central nervous system, for which we develop basic, translational and clinical research.

Fabien Gosselet

Laboratoire de la barrière hémato-encéphalique (LBHE), University Artois, Lens, France

Title: Use of in vitro models to decipher Blood-brain barrier physiology and its roles in neurodegenerative diseases

September 3, 2017
11:00-11:30

Fabien Gosselet is head of the Blood-brain Barrier (BBB) Laboratory located in Lens, France. The 30 members of his Lab use house-made in vitro BBB models not only for investigating BBB physiology and its role in neurodegenerative diseases (Alzheimer's and Parkinson diseases, stroke, brain tumor metastasis, …) but also for improving drug delivery and for providing data in pharmaco-toxicological field and to pharmaceutical companies.

Since its creation in 2000, the BBB-lab actively contributed to several FP6 (AcuteTox) and FP7 (Predict IV, Eustroke) programs and is currently involved in three H2020 consortia (BtRAIN, In3, Nanostem) and two European projects (DiaSyn and SnowBall). In 2014, the BBB-lab developed and patented a human in vitro BBB models consisting to co-cultivate human umbilical cord blood stem cells with brain pericytes. This model is now transferred into 15 other European Labs.

Maria Deli

Institute of Biophysics,
Biological Research Centre,
Szeged, Hungary

Title: Pericytes and blood-brain function

September 3, 2017
11:45-12:15

Pericytes, mural cells of small blood vessels, are highly abundant in cerebral capillaries, which form the blood-brain barrier (BBB). There is increasing evidence that pericytes are crucial for both the development and maintenance of the BBB. In many CNS pathologies, like stroke and Alzheimer's disease pericyte dysfunction contributes to BBB damage and neuronal loss. We have pioneered static and dynamic blood-brain barrier co-culture models using not only two cell types, brain endothelial cells and glial cells, but also a third one, brain microvascular pericytes. These complex models revealed that brain pericytes are able to strengthen the barrier properties of brain endothelial cells, which are indispensable for small molecule transport studies. Co-culture with pericytes increases the expression of important transporters and enzymes too, thus contributing to better BBB function of the in vitro models.

Research interest: Maria Deli is a scientific advisor and head of the Biological Barriers Research Group of the BRC. The team examines solid and vesicular nanoparticles for targeted drug delivery to the nervous system. The safe and reversible opening of intercellular junctions by lipids and peptides to increase drug penetration is also investigated. Another major research topic of the group is BBB injury/dysfunction in pathologies, including Alzheimer's disease with the aim to identify protective molecules. In a joint interdisciplinary project with the Biomolecular Electronics research group new dynamic human co-culture models of the BBB and BCSFB are developed in the frame of the H2020 BtRAIN network.

Viktoria Muchitsch

Center for Health & Bioresources, AIT Austrian Institute of Technology GmbH, Seibersdorf, Austria

Title: PET Imaging of efflux transporter function at the blood-brain barrier

September 3, 2017
16:15-16:45

Introduction: P-glycoprotein (Pgp), a membrane transporter expressed at the blood-brain barrier (BBB), may contribute to clearance of beta-amyloid (Aβ) from the brain [1, 2]. Positron emission tomography (PET) with the Pgp substrate (*R*)-[^{11}C]verapamil ([^{11}C]VPM) has shown that cerebral Pgp function is reduced in Alzheimer's disease (AD) patients and during healthy ageing [3, 4]. Transgenic mouse models are commonly used in AD research. It is currently not known if [^{11}C]VPM PET possesses adequate sensitivity to detect a moderate reduction in Pgp function at the BBB as it occurs in AD mouse models. In the present pilot study, we employed a novel partial Pgp inhibition protocol using tariquidar [4] to assess Pgp function with [^{11}C]VPM PET in one commonly employed AD mouse model (APPPS1 mice) and in age-matched wild-type mice.

Methods: Female C57BL/6N wild-type and APPPS1 mice aged 50 days or 200 days underwent dynamic [^{11}C]VPM PET scans after pre-treatment with vehicle or the Pgp inhibitor tariquidar at a dose of 4 mg/kg leading to partial Pgp inhibition at the BBB (n = 3 - 6). At the end of the PET scan a venous blood sample was collected by retro-orbital puncture. Brain uptake of [^{11}C]VPM was expressed as the brain-to-plasma ratio of radioactivity in the last PET frame ($K_{p,brain}$). Plasma was analyzed by radio-TLC for radiolabeled metabolites of [^{11}C]VPM. Immunohistochemical (IHC) staining was performed to visualize the spatial distribution of Pgp in the brain.

Results: K_p,brain values of [^{11}C]VPM were not significantly different between vehicle-treated wild-type and APPPS1 mice of both age groups. Administration of tariquidar led to increases in

K_p,brain values, which differed among groups with the following rank order: wild-type 50 days: +9 ± 16%, APPPS1 50 days: +19 ± 18%, wild-type 200 days: +30 ± 14%, APPPS1 200 days: +37 ± 22%. After tariquidar administration, K_p,brain values were significantly higher in wild-type mice aged 200 days than 50 days (p = 0.01, Student's t-test). In addition, there was a trend for higher K_p,brain values in APPPS1 mice aged 50 days than in wild-type mice aged 50 days (p = 0.09). No differences in the percentage of radiolabeled metabolites of [^{11}C]VPM in plasma were observed between wild-type and APPPS1 mice. IHC analysis of brain slices confirmed reduced Pgp expression in brain capillaries of APPPS1 mice as compared with wild-type mice (both aged 200 days).

Conclusion: Our pilot data confirm previous findings that [^{11}C]VPM PET without inhibitor administration lacks the sensitivity to detect moderate changes in Pgp function at the BBB [4]. We obtained first evidence that [^{11}C]VPM PET in combination with partial Pgp inhibition with tariquidar can be used to detect age- and AD-related reductions in Pgp function at the BBB of mice. Our data need to be confirmed in larger sample sizes. The employed PET protocol may prove useful in future studies evaluating different therapeutic approaches to restore Pgp function at the BBB.

Funding: Lower Austria Corporation for Research and Education (NFB) project LS14-008, Austrian Science Fund (FWF) project I 1609-B24 and Deutsche Forschungsgemeinschaft (DFG) project DFG PA930/9-1.

References: **1.** PMID: 21148344; **2.** PMID: 16239972; **3.** PMID: 24842892; **4.** PMID: 28401570

Maciej Lalowski

Medicum, Meilahti Clinical Proteomics Core Facility, Meilahti, Finland

Title: Utilizing -omics approaches to tackle protein dynamics in the brain: example of CLN1 child epilepsy

September 3, 2017
16:45-17:15

Introduction/Rationale: Neuronal ceroid lipofuscinoses (NCL) are a group of lysosomal storage diseases (LSD) mainly affecting the brain and retina [1]. Thirteen NCL genes have been identified so far and an equivalent number of NCL forms are currently considered in NCL nosography. Five NCL genes encode lysosomal proteins, whereas the remaining gene products localize to other cell compartments. CLN1 disease (OMIM #256730) refers to a NCL form associated with a childhood onset phenotype, and a rare adult onset one. Common biomarker of both phenotypes is the reduced enzyme activity of PPT1, the gene product of *CLN1*, whose mutations are disease-related. PPT1 is a palmitoyl-thioesterase highly expressed in neuronal cells. It is a soluble, hydrolytic lysosomal enzyme, involved in the degradation of S-acylated proteins by removing the palmitate residues. In neurons, PPT1 expression is also linked to specific functions in the synaptic compartment.

Results: In the recent study, we analyzed proteome alterations in the brain of a *Ppt1* knockout mouse model of CLN1 disease and its age-matched counterparts at different stages. For this purpose, we utilized MALDI Mass Spectrometry Imaging and LCM- based label free tandem mass spectrometry to visualize and quantify the changes in protein expression. The identified changes in protein levels were further validated by quantitative interactomics [2], functional bioinformatics and network approaches, immunohistochemistry on brain tissues and literature querying to pinpoint functional modules which can be targeted therapeutically [3].

The aim of the current study was to recognize molecular signatures and functional modules connected with overexpressed *CLN1* in a human neuronal cellular system [4]. We utilized SH-SY5Y neuroblastoma cells (differentiated into a neuronal-like phenotype [5]), to overexpress wt*CLN1* and a selection of disease related mutations previously detected in CLN1-affected children. The differentiated cell lines underwent whole transcriptomic profiling by RNA-seq, to identify differentially expressed genes (DEGs) which are functionally related to the overexpression of wild-type or mutated PPT1. Following bioinformatic investigations, we focused on DEGs involved in palmitoylation of neuronal proteins as well as related cellular functions. Interestingly, genes coding for palmitoylated proteins assigned to neuronal functions, such as axonal growth, and to the synaptic compartment were the most significantly expressed. Moreover, to identify potential therapeutic targets for CLN1 disease, we aimed to demonstrate possible links with other NCL genes, particularly *CLN4* and *CLN10*, sharing common pathological traits with *PPT1* [6].

References: 1. PMID: 21990111; **2.** PMID: 25865307; **3.** PMID: 26707855; **4.** Pezzini, F.*, et al.* The networks of genes encoding palmitoylated proteins in axonal and synaptic compartments are affected in PPT1 overexpressing neuronal cells. *Front Mol Neurosci* (2017), under review. **5.** PMID: 27422411; **6.** PMID: 22959893

Tuula Nyman

Head of Proteomics,
Department of Immunology,
University of Oslo and Oslo
University Hospital, Norway

Title: Proteomics in biomedical research

September 3, 2017
17:30-18:00

Proteomics is 'the large-study of proteins'. It is critical to obtain proteome-level data to understand cellular signalling events in detail (1). With proteomics, we can collect data on protein expression levels, their post-translational modifications, interactions, as well as on their secretion.

A key technique in modern proteomics is high-resolution mass spectrometry (MS). The sample preparation techniques, MS instruments and data analysis tools for proteomics have developed during the last decade immensely, making it possible to identify and characterize thousands of proteins in one experiment. Never before has it been possible to globally and dynamically characterize cellular responses to different stimuli in such a detail than what modern MS-based proteomics methods allow (2). It has been speculated that proteomics will play an important role in future precision medicine (3), but for that to happen the seamless integration and close collaboration of basic science and clinical researchers with practicing clinicians is needed. The Proteomics Unit in Department of Immunology (University of Oslo and Oslo University Hospital) is centrally located by the hospital making these collaborations possible, and I will show selected examples on the proteome studies we have done with different sample types.

Innate immunity has a central role in immune-mediated inflammatory diseases including Crohn's disease, psoriasis, and gout. Inflammasomes are protein complexes formed during activation of the innate immune system and they are critical to both local and systemic inflammation. My own research is

focused on innate immunity, and we have extensively used different proteomics workflows to have a global view of cellular signaling events related to the activation of innate immune response to different activation stimuli in human macrophages. The main focus of my research during the last years has been to characterize the global protein secretion activated during innate immune response. These studies have shown that unconventional, vesicle-mediated protein secretion is a critical component in innate immune response. Our studies have also shown that both canonical NLPR3 inflammasome as well as the non-canonical caspase-4/5 inflammasome activate vesicle-mediated protein secretion from human macrophages (reviewed in 1).

References: 1. PMID: 28406322; **2.** PMID: 27629641; **3.** PMID: 28248239

Matias Zurbriggen

Institute of Synthetic Biology and CEPLAS, Heinrich-Heine-Universität Düsseldorf, Germany

Title: Optogenetic tools for the control and understanding of cellular processes in animal and plant systems

September 3, 2017
18:00-18:30

The engineering of neurons with light-regulated ion channels has enabled the non-invasive study of neuronal networks *in vivo* at unprecedented spatio-temporal resolution. This experimental breakthrough has revolutionized neurosciences, with hundreds of applications contributing key insights into nervous system function having taken root within only few years. The success of optogenetics in neurobiology is followed by the more generalized used of light as stimulus to remote control a wide range of cellular processes, from gene expression up to cell viability and function.

Our synthetic biology research focuses on engineering bacterial and plant photoreceptors sensitive to different wavelengths of the white light spectrum (UV-B, blue, green, orange, red/far-red) into synthetic photoswitches rewired to control molecular processes with high precision, quantitative and high spatio-temporal resolution, in a non-invasive way and with minimized toxicity. We implement these molecular tools into microbial, mammalian and plant cells, and *in vivo* in animals and plants for selectively manipulating signaling networks and metabolic pathways. This synthetic biology approach opens up unforeseen perspectives in fundamental and applied research, as exemplified hereby in the study of signalling pathways, biomedical field, crop design as well as for the production of high value biopharmaceuticals.

Martin Fuhrmann

Neuroimmunology & Imaging, German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany

Title: Cellular and synaptic correlates of learning and memory

September 3, 2017
18:45-19:15

Alzheimer's disease (AD) is characterized by cognitive decline and neuronal network dysfunction, which have been attributed to synapse and neuron loss. However, whether instantaneous changes in synaptic connectivity and rewiring of neuronal networks are also impaired during learning and memory processes remains an open question. Therefore, we investigated changes in the composition of the hippocampal neuronal network on the cellular and synaptic level during learning and memory comparing wild-type and mouse modes of AD. We found memory encoding neurons in both healthy and diseased mice indicating intact memory formation. However, the composition of the hippocampal neuronal network was changed during memory retrieval. Here, aberrantly active neurons superimposed the memory encoding neurons presumably affecting successful memory retrieval. On the synaptic level, we found that inhibitory neurons in the hippocampus receive decreased cholinergic input from the medial septum under AD-like conditions affecting associative learning. Excitatory post-synapses were lost in response to memory formation a process that was absent in a mouse model of AD. Decreased input via Schaffer collaterals from CA3 may represent a mechanism for this phenomenon. Summarizing, we identified cellular and synaptic impairments in response to memory acquisition and retrieval that contribute to memory impairment under AD-like conditions.

Research Interest: Alzheimer's disease is characterized by the extracellular deposition of Aβ and the intracellular aggregation

and formation of fibrils consisting of the hyperphosphorylated protein tau. Additionally, synapse and neuron loss are hallmarks of AD, which correlate well with cognitive decline. These and other changes presumably affect the integrity of individual neurons, micronetworks and ultimately lead to neuronal network dysfunction that underlies learning and memory impairment. One possible explanation for neuronal network dysfunction represents an imbalance between excitation and inhibition, which destabilizes the network and contributes to aberrant neuronal activity. Indeed, encephalogram (EEG) recordings in AD patients revealed phases of abnormal epileptiform activity. However, the cellular and sub-cellular mechanisms that contribute to an imbalance between excitation and inhibition and how this might influence learning and memory impairment remain unexplored. In fact we are just starting to understand how learning and memory works in healthy brains on cellular and sub-cellular levels. Models of neurodegenerative diseases like AD may help to understand learning and memory processes if we use them as a defect example. Based on this background research in my lab focuses on the synaptic and neuronal network processes involved in learning and memory and how are they impaired in AD? In order to address these questions we use state of the art *in vivo* imaging techniques and combine them with electrophysiological, histological and biochemical techniques in transgenic mice and rats. Moreover, we use optogenetics and pharmacogenetics to specifically activate or inactivated neuronal subsets.

Katja Stefan

Pharmaceutical Institute, University of Bonn, Germany

Title: Modulators of ABCC1: A summary of three decades of research

September 3, 2017
19:15-19:45

ABC transport proteins are crucial in the distribution and elimination of endo- and xenobiotics, using the energy of ATP hydrolysis. In cancer research, three major ABC transport proteins have been identified to be upregulated in different cancer types causing the phenomenon called multidrug resistance (MDR): ABCB1 (P-gp), ABCC1 (MRP1), and ABCG2 (BCRP). This phenomenon is characterized by an altered distribution of structurally diverse antineoplastic agents resulting in multiple resistant cancer cells. Our work group focuses on the elucidation of the transport mechanism itself and the mode of action regarding drug binding and transport employing different *in vitro* assays.

I studied chemistry with material science at the University Bonn-Rhein-Sieg, where I graduated 2009. I continued my scientific career as a master student at the work group of Prof. Wiese in the field of ABC transporters. After graduation, I started my PhD thesis with the focus on elucidation of the mode of action of ABC transport proteins and the development as well as establishment of new methods to characterize ABC transporters in general.

Sven Schmitt

Pharmaceutical Institute, University of Bonn, Germany

Title: Modulators of ABCC1: A summary of three decades of research

September 3, 2017
19:15-19:45

In oncology, ABC transport proteins have been identified as important targets since these are considered to be mediators of the phenomenon of multidrug resistance (MDR). Here, cancer cells are resistant against structurally unrelated antineoplastic agents due to their extrusion out of cells caused by the upregulation of ABC transporters. Three major ABC transport proteins have been found to cause MDR resulting in reduced intracellular retention of cytostatics: ABCB1 (P-gp), ABCC1 (MRP1), and ABCG2 (BCRP). Our work group focuses on the development of new, potent and selective inhibitors of these transporters and the elucidation of the mode of action of ABC transport proteins in general.

In our most recent research we were able to find modulators of ABCC1 (MRP1) that could enhance the transport velocity of this transporter. While some modulators had only an activating property, others were dual modulators with activating and inhibiting feature, dependent on the used concentration. The literature of the last decades stated only few compounds with this ability. Hence, the focus of our current research lies on the elucidation of the modulating quality of the compounds and their possible usage in other fields of ABC transport proteins-related diseases, like Alzheimer's disease (AD).

The topic of this presentation will be a summary of 30 years of science with respect to modulators of ABCC1, used cell lines and *in vitro* assays. This includes inhibitors and activators that can be used to down- or upregulate ABCC1 function as possible tools to study altered ABCC1 function in cells. Finally, the aspect

of transport activation will be highlighted as possible treatment of AD.

I studied pharmacy at the University of Bonn between 2006 and 2010 and became a pharmacist one year later. In my master thesis I worked with a marine fungus with the focus on the elucidation of its metabolism and isolation of natural or mutasynthesis-modified compounds. Since my master graduation in 2012, I worked as a PhD student in the field of ABC transporters with focus on ABCC1. The synthesis of new, potent and selective as well as broad-spectrum inhibitors was in focus of my thesis as well as the functional analysis of the modulating property of the compounds employing different assay types.

Ludger Wessjohann

Leibniz Institute of Plant Biochemistry (IPB), Bioorganic Chemistry, Halle (Saale), Germany

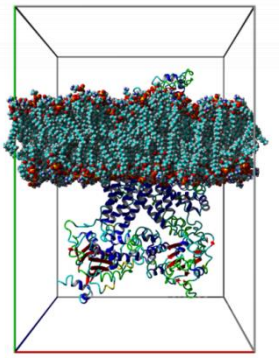
Title: Chemoinformatics and Protein-3D-Modelling as Support Tools for Medicinal Chemistry on ABC-Transporters and BBB-active Compounds

September 4, 2017
9:00-9:45

The identification and pharmacological optimization of neuro-active compounds usually is more costly in terms of time and money, if compared to other drug developments, e.g. of antibiotics. Assays with sufficient quality or quantity of data points are often missing or even unavailable and one has to rely exclusively on animal experiments. I.e. obtaining the biodata to be related to compound structural data (QSAR) is slow, costly, or of low statistical relevance. Thus it is highly desirable, to have alternative tools that help to minimize wet lab efforts. Last time I introduced our reverse metabolomics strategy to facilitate identification of bioactive components avoiding dereplication procedures. The progress in this area with respect to bioactive compounds from the common St. John's wort (*Hypericum perforatum* L.) will be presented by Katrin Franke in a separate lecture.

Another tool, useful even prior to the analytical measurements, is computational analysis involving virtual screening and chemoinformatic processing of potential candidates. As a basis, however, sufficient data of a potential target and potential candidate compounds is required.

Following a general introduction on the demands and possibilities of virtual screening and homology modelling, including blood-brain-barrier penetration models and some practical examples, I will present our latest data on modelling of the ABC-C1 transporter – see figure for a model within a cell membrane -- and its interaction with selected natural and synthetic compounds.



Acknowledgements: The Leibniz Association, DAAD and the EU with Land Sachsen-Anhalt (EFRE supported project PhytoAD) are acknowledged for financial support.

References: "Virtual screening - tools for a faster selection of new drug leads" Stephanie Tennstedt, Juliane Fischer, Wolfgang Brandt and Ludger Wessjohann, Medicinal Chemistry in Drug Discovery - Review Book, **2013**: 219-236 (Ed. Dubravko Jelić), ISBN: 978-81-7895-560-5.

Upcoming book chapters, expected late 2017 in "Practical Medicinal Chemistry with Macrocycles", Wiley, ISBN 878-1-119-09256-8.

Profile: Professor Wessjohann studied chemistry in Hamburg (Germany), Southampton (UK), and Oslo (N, Prof. Skattebøl). He earned his doctorate in 1990 with Prof. de Meijere in Hamburg. After a short period as lecturer in Brazil, he became a postdoctoral Feodor-Lynen fellow of the Alexander von Humboldt foundation with Prof. Paul Wender at Stanford University (USA) working on the total synthesis of Taxol®. After an assistant professorship in Munich (LMU, 1992-1998) he was appointed to the Chair of Bioorganic Chemistry at the Vrije

Universiteit Amsterdam (NL), working on organometallic chemistry and biocatalysis. Since 2001 he is director of the dept. of bioorganic chemistry at the Leibniz Institute of Plant Biochemistry (IPB) in Halle (Germany), and in parallel holds the chair of natural product chemistry of the Martin Luther-University Halle-Wittenberg. Since 2010 he is the Managing Director of the IPB (www.ipb-halle.de).

Prof. Wessjohann focuses on the discovery, synthesis and application of natural products and derivatives. He has some 350 publications, 25 patent applications, and is co-founder of five companies. He is member of many boards and commissions, and received several scholarships, prizes and honors, e.g. Microsoft IT Founders Award, honorary membership of the Argentinean Soc. of Synth. Org. Chem and in 2016 the "Leibniz drug of the year" award for Tubugis, small anticancer peptides with picomolar activity. He is a foreign member of the Brazilian Academy of Science and speaker of the "Science Campus Plant Based Bioeconomy" in Halle and the Leibniz Research Alliance "Bioactive Compounds and Biotechnology".

Timothy Sharbel

Global Institute for Food Security (GIFS), University of Saskatoon, Saskatoon, Canada

Title: Why sex and agriculture conflict: Evolutionary omics approaches to developing asexual (apomictic) seed production

September 4, 2017
9:45-10:15

An organism's choice to reproduce with or without sex has long puzzled evolutionary biologists. Apomixis, a natural form of reproduction in plants whereby seeds are produced asexually, has evolved repeatedly from sexual ancestors in many taxa. Apomixis is of interest on a number of levels, ranging from population genetics to evolution, but also from an applied perspective, as it represents a disruptive technology which could significantly change agricultural practices (e.g. fixing heterosis in hybrid crops). The switch from sex to apomixis is hypothesized to result from deregulation of developmental pathways leading to sexual seed development, and the trigger for deregulation involves the global genomic effects of hybridization and polyploidy.

We study apomixis in wild plant populations, and use evolutionary theory to guide our experimental approaches. High-throughput methods are employed to understand population-level phenotypic (seed production) and genetic (polyploidy, genetic structure) variability. These data are then used to design targeted experiments, whereby candidate genes for apomixis are identified using tissue-specific "omics" methods in particular genotypes. These candidates are then used (1) in transformation experiments to attempt apomixis induction in sexual plants, and (2) in population-level studies to understand the origin and evolution of apomixis with respect to sexuality in natural populations.

Peter Verhaert

ProteoFormiX, Beerse, Belgium

M4i Maastricht University, Maastricht, Netherlands

Title: Making biobanked clinical material accessible for top-down (neuro)peptide biomarker discovery.
MS Histochemistry for peptide localization and characterization in formalin fixed paraffin embedded tissue.

September 4, 2017
10:15-10:45

We have established a workflow enabling top-down analysis of biologically relevant secretory (neuro)peptides, which works on paraffin embedded tissue after standardized formalin fixation.

Successful mass spectrometry imaging of neuropeptides is achieved, which could previously only be detected in either fresh or freshly frozen neurosecretory tissue. Proof-of-concept was realized on a well-studied model system, i.e., the insect (*Periplaneta americana*) neuroendocrine retrocerebral complex.

We then continued to study actual human clinical biopsies. The latter included a variety of different carcinoids, well characterized carcinomas (breast, colon,) and selected ('healthy') control tissues (lymph tissue, unaffected intestinal mucosa, and kidney). The technique developed is fully top-down, without trypsin or any other enzyme for protein identification purposes. Whereas the data obtained from high resolution mass spectrometers have superior quality in comparison to less performant systems, the protocol principally is independent of the actual mass spectrometer employed. The method works best with MALDI as ion source, although it can be optimized for SIMS systems as well. The approach has an evident bias towards small representatives of the proteome/secretome. We are now evaluating whether also typical biogenic amine neurotransmitters or neuromodulators can be imaged.

We will show the very first human neuropeptide images obtained by mass spectrometry histochemistry on FFPE (formalin fixed paraffin embedded) human pituitary.

Margarida Correia-Neves

Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Campus Gualtar, Braga, Portugal

Title: Cognitive decline and immunosenescence: a link thought the CD4+ effector memory T cells.

September 4, 2017
11:05-11:35

Introduction: Longevity is increasing worldwide. Although with marked inter-individual differences, ageing is associated with a gradual decline in cognitive functions. Thus, the identification of factors that might delay cognition decline, promoting a healthy aging, is an increasingly relevant challenge. In addition to the cognitive alterations, the immune system also progressively changes with age. Recent data on the interplay between cognition and the immune system led to the current vision that the brain rather than being an immune privileged organ, enjoys the privilege of being regulated by the immune system. The immune system has been shown to play modulatory functions in several brain functions, namely cognition.

Objective: Immunosenescence and cognitive decline are common markers of the aging process. Taking into consideration the heterogeneity observed in cognitive decline along aging and the now recognized link between lymphocytes and cognition, we explored the association between alterations in lymphocytic populations and cognitive performance parameters.

Methods: In a cohort of cognitively healthy adults (n=114), previously characterized by diverse neurocognitive/psychological performance patterns, detailed peripheral blood immunophenotyping of both the innate and adaptive immune systems was performed by flow cytometry.

Results: Better cognitive performance was associated with lower numbers of effector memory CD4+ T cells and higher numbers of naive CD8+ T cells and B cells. Furthermore, effector memory

CD4+ T cells were found to be predictors of general and executive function and memory, even when factors known to influence cognitive performance in older individuals (e.g., age, gender, education and mood) were taken into account.

Conclusions: This is the first study in humans associating specific phenotypes of the immune system with distinct cognitive performance in healthy aging.

Igor Meglinski

Opto-Electronics and Measurement Techniques, University of Oulu, Finland

Title: Transcranial Optical Vascular Imaging – Perspectives in Brain Diagnostics

September 4, 2017
11:35-12:05

In vivo imaging of cerebral vasculature is highly vital for clinicians and medical researchers alike. For a number of years non-invasive optical-based imaging of brain vascular network by using standard fluorescence probes has been considered as impossible. We developed a robust non-invasive optical-based imaging approach that allows visualize major cerebral vessels at the high temporal and spatial resolution. The developed technique is simple to use, utilizes dynamic light scattering and standard fluorescent dyes, inexpensive imaging and computation procedures. The ability to clearly visualize middle cerebral artery and other major vessels of brain vascular network, as well as the measurements of dynamics of blood flow are presented. The developed imaging approach has a great potential in neuroimaging and can significantly expand the capabilities of preclinical functional studies of brain and notably contribute for analysis of cerebral blood circulation in disorder models.

Paride Rizzo

Apomixis research group, Leibniz Institute for Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany

Title: Morphology meet transcriptomics in the flower of *Hypericum perforatum*

Leibniz project meeting
September 4, 2017
14:45-15:45

The speaker: Paride Rizzo, a post-doctoral researcher with Professor Sharbel, is specialized in the developmental biology of apomictic species like *Boechera spp.* and *Hypericum perforatum*. The core of his research is to compare the sexual and apomictic seed developmental pathways using transcriptomics with the goal of explaining the molecular machinery responsible for the trigger of apomixis. This research concept has led him to interesting observations that constitute the basis of a new project focused on dark gland formation in the frame of the SAW-Projekt „Johanniskraut gegen Alzheimer".

The project: The multiple applications of *Hypericum perforatum* (Saint John's wort) range from the treatment of depression (Zhai et al. 2015; Ramalhete et al. 2016) to the diagnosis and treatment of specific types of cancer (Abhisheck et al. 2011). Recent research even suggests a possible application of *Hypericum* in the treatment of neurodegenerative diseases (Hofrichter et al., 2013).

The dark and translucent glands of *Hypericum* are important accumulation sites of several metabolites (e.g. hypericin and hyperforin) that are of pharmacological interest (Garg et al. 2012, Dudek-Peric et al. 2015).

The characterization work carried in our lab during the last years has lead us to the discovery of different glands formation patterns in the reproductive tissues of *Hypericum* that have been used for the transcriptome analysis of dark glands formation with the aim of identifying genes putatively involved in dark glands organogenesis and in the biosynthesis of pharmaceutically interesting metabolites, especially hypericin.

Katrin Franke

CNS active Natural Products,
Leibniz Institute of Plant
Biochemistry, Halle, Germany

Title: St. John's wort against Alzheimer's disease: Metabolomic approaches towards the identification of bioactive compounds

Leibniz project meeting
September 4, 2017
14:45-15:45

The genus *Hypericum* comprises more than 450 species widely occurring in temperate regions and tropical highlands. Many species are used locally as traditional medicine against a variety of diseases. The Common St. John's wort (*Hypericum perforatum* L.) is a well-known medicinal herb employed for the treatment of mild to moderate depressions. Recent investigations suggest in addition a positive influence on Alzheimer's disease, mediated among others by activation of the Aβ exporting transporter ABCC1.

Altogether more than 1000 natural products are described to occur in the genus *Hypericum*, several hundred in *H. perforatum*. Prominent secondary metabolites of St. John's wort include flavonoids, naphthodianthrones such as hypericin, and polyprenylated phloroglucinols such as hyperforin. These metabolites account for a number of pharmacological activities, however, their relation to anti-Alzheimer properties is unknown.

We developed untargeted metabolomic approaches aiming at the identification of bioactive metabolites in complex mixtures. In this project, comparative metabolite profiles of *H. perforatum* accessions with different genetic background will be investigated to use the intraspecific variance for determination of anti-Alzheimer constituents. Several analytical methods have to be combined to detect a high number of metabolites with different physical and chemical properties simultaneously. Metabolite profiles obtained by nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS) were

evaluated by multivariate data analysis and can be furthermore compared with various bioassay results using statistical methods to detect correlations between chemical constituents and their biological activity.

Metabolite profiling of various *Hypericum* species combined to multivariate data analysis was by our group successfully applied to compare and evaluate commercial *Hypericum* preparations, to investigate the secondary metabolite diversity within the genus and to select species or samples for the discovery of unknown natural products. As an example, detailed investigations of *Hypericum* species selected by this method resulted in the isolation and structure elucidation of approximately 30 novel natural products including compounds with promising anthelmintic or anti-HIV activities.

Research background: Katrin Franke studied Biology with focus on Plant Physiology at the Martin-Luther-University Halle-Wittenberg, Germany. After a postdoc at the Institute of Pharmaceutical Biology of MLU she joined the Natural Products Group at the Department of Bioorganic Chemistry, Leibniz Institute of Plant Biochemistry. Since more than 15 years her research focus is on isolation and structure elucidation of bioactive plant secondary metabolites, in recent years with an emphasis on the investigation of traditional medicinal plants containing CNS active metabolites. The current "*Hypericum*"-project is funded by the Leibniz association (SAW-2015-IPB-2) and combines the expertise of an interdisciplinary network to identify the metabolites connected to anti-Alzheimer properties

by new metabolomics and correlation methods, to investigate their formation in the plant with respect to the physiological, biochemical and genetic background, and to use the acquired knowledge to breed better cultivars and prepare more efficient, defined extracts. Within an EFRE funded project (PhytoAD) the basic knowledge on the effects of natural neuroactive compounds will be expanded for the treatment of age-related cognitive disorders.

Luisa Möhle

Translational Neurodegeneration Research & Neuropathology Lab, University of Oslo, Norway

Leibniz project meeting
September 4, 2017
14:45-15:45

I studied biology with molecular immunology as major at the University of Freiburg im Breisgau, Germany from 2007 to 2012. In 2012, I moved to Magdeburg for my diploma thesis at the Institute of Medical Microbiology and Hospital Hygiene. After my graduation, I continued to work in Ildiko Dunay's group and finished my PhD thesis "The role of Ly6C^{hi} monocytes in brain homeostasis and neurodegeneration" in 2016. In the same year, I joined the Translational Neurodegeneration and Neuropathology Lab in Oslo.

As I come from an immunological background, my previous research focused on neuroimmunological questions and the interactions between the peripheral immune system and the central nervous system. After moving to Oslo, I am now working on developing biochemical assays to study the function of ABC transporters and to evaluate the manipulation of these transporters by plant extracts and chemical compounds.

Surya Prakash Rai

Translational Neurodegeneration Research & Neuropathology Lab, University of Oslo, Norway

Leibniz project meeting
September 4, 2017
14:45-15:45

Alzheimer's disease (AD) is a debilitating progressive neurodegenerative disorders affecting significantly old age population across the globe. AD is characterized by gradual, yet progressive decline in cognitive function. Memory decline is usually an early observed symptom. Additionally, *post mortem* analysis of AD patients reveals remarkable pathological changes in the brain. These neuropathological changes – Aβ containing amyloid plaques (A), neurofibrillary tangles (B), and neuritic amyloid plaques (C) - commonly referred to as "ABC score". Evidences also suggest that there is a correlation between cognitive dysfunction and amyloid plaques in early stage of disease pathology in AD patients but there is little evidence that supports contribution of continued amyloid plaques in late stage AD cognitive decline.

Different mouse models have been developed to study pathology and cognitive decline in AD. Visual-based working memory is exploited extensively to study memory in rodent model. Morris water maze (MWM) is the most widely employed spatial memory task that consists of a tank filled with water: a submerged platform of suitable size in a particular place within a water filled tank. Animals are trained to find a submerged platform based on spatial cues. Animals under appropriate physical set up will learn and remember the location of the platform. Hence, as the trial proceeds, animals find the platform using spatial cues.

AD-related cognitive deficits have been shown in different mouse models using MWM. In one APP/PS1 AD mouse model,

cognitive dysfunction starts as early as seven month in MWM test. This finding is not consistent with the neuropathological findings in which Aβ containing amyloid plaques already starts appearing in brain of APP/PS1 model as early as 45 days of age. Hence, we aim to set up a working protocol for MWM that can show difference in learning curve.

Our lab already showed that MWM of wild type and AD mouse have different learning curves. Since we moved our lab from Germany to Norway we again need to re-establish the protocols for MWM evaluation. As MWM is highly sensitive and requires significant time and effort to establish but once it is established, it is one of the most reliable tests to assess spatial working memory. We hope that showing differences in learning curves between groups, a new avenue for testing different drugs including different phytoextracts will be opened. Also, our previous publications show that specific plants such as *Hypericum perforatum* and *Sideritus species* modulate the learning curve of AD mice already at early disease stages. Hence, once we establish our MWM protocol, we aim to test different subfractions of *Hypericum perforatum, Sideritis species* and also Nepalese medical plants to hunt for the active moiety responsible for anti-AD activity.

Kristin Paarmann

Translational Neurodegeneration and Neuropathology Lab, University of Oslo, Oslo, Norway

DFG project meeting
September 4, 2017
15:55-16:40

This DFG project is performed in cooperation with the Pietrzik Group (University of Mainz) and investigates the interaction between ABC transporters ABCB1 and ABCC1 as well as LRP1, a large endocytic receptor belonging to the LDL receptor family, at the blood-brain barrier (BBB) in healthy and Alzheimer's disease (AD) mice. It is speculated that ABCB1, ABCC1 and LRP1, interact with each other to regulate the import and export function of the BBB. Moreover, it is hypothesized that these Aβ transporters co-operate to transport Aβ out of the brain parenchyma. Therefore, it can be assumed that a dysfunctional interaction leads to accelerated Aβ plaque numbers etc. Investigations with newly generated mouse models that have an inducible LRP1 knockout and constitutive ABC transporter knockouts (immunohistochemistry, transwell assays, behavioral studies etc.) will show whether and how transports via ABC transporters and LRP1 are connected.

Kristin Paarmann completed her studies in Biology in 2011 at the University of Rostock. Since 2010 she is working in the Pahnke Lab. Between 2011 and 2015 her research focused on inflammatory processes in AD at the University of Magdeburg. Beside her basic research she was the coordinator for patient studies with off-site blood sampling, questionnaire design etc. Currently, she is investigating the function of ABC transporters ABCB1 and ABCC1 regarding their interactions with LRP1, genetic backgrounds in mouse models and plant extracts.

Steffen Storck

University Medical Center of the Johannes Gutenberg-University of Mainz, Institute of Pathobiochemistry, Department Molecular Neurodegeneration, Mainz, Germany

Title: Deciphering the molecular mechanisms of amyloid-beta transcytosis

DFG project meeting
September 4, 2017
15:55-16:40

Decreased clearance of metabolites across brain capillaries that form the blood-brain barrier (BBB) has been suggested to contribute neurotoxic accumulation of the amyloid-beta (Aβ) protein in Alzheimer's disease. To date, several receptors (LRP1, LRP2, FcRn, etc.) and transporters (P-glycoprotein, Abcg4, etc.) have been described to be involved in BBB clearance of Aβ. However, mechanical studies on the exact transport mechanisms are scarce. Recently, we engineered mice with selective knockout of the low-density receptor-related protein 1, LRP1, in the brain endothelium. In this mouse model we could show that endothelial LRP1 is a major receptor for rapid BBB clearance of Aβ. However, the exact molecular mechanisms of endothelial Aβ transcytosis remain elusive. Recent studies have shown that transcytosis of Aβ is initiated by clathrin-mediated endocytosis of the Aβ/LRP1 complex by binding to PICALM. This complex is also transiently associated with the two endosomal trafficking proteins Rab5, found in early endosomes, and Rab11, which regulates transport of vesicles across the cell to the luminal membrane. But how is Aβ released at the luminal side of the endothelium? In an in vitro BBB model using primary endothelial cells or isolated intact capillaries we have collected data that LRP1 and ABC transporters like P-glycoprotein may act in concert in transporting Aβ through the endothelium. Our data suggest the interplay of transport machinery that traditionally has been regarded as being separate transport mechanisms and complement our understanding of Aβ transport at the BBB.

Markus Krohn

Translational Neurodegeneration and Neuropathology Lab, University of Oslo, Oslo, Norway

I did my studies in Biology 1997-2002 at the University of Greifswald, Germany. As Ph.D. student I started working about ABC transporter functionality and their implications for Alzheimer's disease. After moving to the Neurodegeneration Research Lab at the University of Rostock, Germany, I finished my PhD thesis entitled "The role of ABC transporters in Alzheimer's disease". During the following years I have been working at the German Center for Neurodegenerative diseases, the University of Magdeburg and since 2015 at the Translational Neurodegeneration and neuropathology Lab in Oslo, Norway, where I am now leading a subgroup within the Pahnke Lab.

My research is aimed towards the improvement of mouse models, the role of the choroid plexus in neurodegeneration, understanding the physiologic function of ABC transporters (apart from extrusion of xenobiotics) and their manipulation. As one approach to tackle those questions I have been developing ABCC1 and ABCA7 transporter humanized mice in cooperation with GenOway. In both strains the human CDS in flanked with loxP sites and replaces the mouse gene. This setup allows cell specific knockout of the ABC transporter of interest from any age *in vivo*! In doing so, it is possible to find out in which type of cells which ABC transporter is of importance for normal brain homeostasis. We can also assess where its functionality is most essential with regard to, for example, amyloid-beta clearance in Alzheimer's disease mouse models. The expression of the human protein will help to close the inter-species gap in translational projects and thus make the mice interesting tools

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for other fields of research like drug development and cancer research as well.

Other projects I am propelling or involved in are: characterization and re-design of a third-party humanized ABCB1 mouse line that is not-functional; the search for ABCC1 activating compounds and development of a robust screening assay; refinement of the Morris water maze towards very early detection of memory deficits; a collaborative project with the Austrian Institute of Technology to assess changes of ABC transporter activity at the blood brain-barrier *in vivo* using PET; functionalization of ABC transporters; evaluation of DESI-MS imaging as a research and diagnostic tool in a routine pathology setting.

Oliver Langer

Health & Environment Department, AIT Austrian Institute of Technology GmbH, Seibersdorf, Austria and Department of Clinical Pharmacology, Medical University of Vienna, Austria

Title: PET Imaging of efflux transporter function at the blood-brain barrier

DFG project meeting
September 4, 2017
16:50-17:35

Summary of talk: Adenosine triphosphate-binding cassette (ABC) transporters expressed at the blood-brain barrier (BBB) and at the blood-cerebrospinal fluid barriers, such as P-glycoprotein (ABCB1), breast cancer resistance protein (ABCG2) and multidrug resistance-associated proteins 4 and 1 (ABCC4 and ABCC1), control the exposure of brain parenchyma to exogenous and endogenous compounds and have been implicated in the pathophysiology of neurological disease, such as drug resistant epilepsy and Alzheimer's disease. Positron emission tomography (PET) in combination with radiolabelled transporter substrates can be used to non-invasively assess the function of cerebral ABC transporters in animals and humans. In the present talk I will give an overview of currently available PET protocols to measure efflux transporter function in the brain and some applications in animal disease models.

Short CV: Oliver Langer studied pharmacy at the University of Vienna, where he graduated with a Master's degree in 1995. He then obtained a PhD degree at the Karolinska Institute in Stockholm, Sweden in 2000, where he specialized in the development of radiotracers for the imaging of neurotransmitter systems with positron emission tomography (PET). Since 2002 he has been employed at the Department of Clinical Pharmacology at the Medical University of Vienna, where he became Associate Professor ("Privatdozent") in Radiopharmaceutical Chemistry in 2006. In 2006, he became Senior Scientist at Austrian Institute of Technology in Seibersdorf, which is Austria's largest non-university research organization. In

his research, he uses preclinical and clinical PET to address different questions related to drug disposition and pharmacodynamics with a particular emphasis on studying drug transporters.

Thomas Wanek

Health & Environment Department, AIT Austrian Institute of Technology GmbH, Seibersdorf, Austria

DFG project meeting
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16:50-17:35

Thomas Wanek obtained a PhD degree in 2013 at the Medical University of Vienna specializing on preclinical PET imaging of ABC transporter function. He currently holds the position of a Thematic coordinator for Molecular Imaging at the AIT Austrian Institute of Technology, Austria largest non-university research organization. His research focuses on drug DMPK studies using PET imaging and the interaction of drugs with ABC and SLC transporters located at the blood-brain barrier and other tissue barriers in the body. Additionally he is interested in tumor hypoxia imaging using novel imaging approaches. He is currently responsible for all preclinical imaging research activities at the AIT and project administration of the Molecular Imaging group.

PET Imaging of ABCG2 function in animal models of Alzheimer's disease

A major hallmark of Alzheimer's disease (AD) is the accumulation of senile plaques containing beta-amyloid (Aβ) in the brain. Several lines of evidence suggest that reduced Aβ clearance from the brain underlies Aβ accumulation. Adenosine triphosphate-binding cassette (ABC) transporters that are expressed in endothelial cells of the blood-brain barrier (BBB) may play an important role in excreting Aβ from brain into the blood.

A number of studies suggest that ABC transporter function at the BBB may be impaired in AD patients as compared with age-matched control subjects. P-glycoprotein (ABCB1) cooperates

closely with another ABC transporter at the BBB, breast cancer resistance protein (ABCG2). Given this cooperative action between ABCB1 and ABCG2 and previous data suggesting that ABCG2 may be up-regulated at the BBB of AD patients as possible compensatory mechanism for ABCB1 down-regulation, the role of ABCG2 in AD is currently of high interest.

In the present talk we will present an overview about the *in vivo* function of ABCG2 at the BBB of an AD mouse model and age-matched wild-type control animals using the non-invasive nuclear imaging method positron emission tomography (PET).

Mirjam Brackhan

Translational Neurodegeneration and Neuropathology Lab, University of Oslo, Oslo, Norway

Title: Propagation behaviour of peripheral amyloid-β towards the brain

JPND project meeting
September 4, 2017
17:45-18:45

Over the last few years, the question whether Alzheimer's Disease (AD) is a transmissible disease involving misfolding and seeded aggregation mechanisms as known from prion diseases has been of great interest. The recent finding of Jucker M. et al. that peripheral injection of amyloidogenic brain material from a transgenic mouse model of AD resulted in immunopositive material in the brains of injected pre-depositing mice of the same model suggests propagation of amyloid-β (Aβ) from the periphery to the brain. In order to explore this possible risk of pathogenic Aβ spread in greater detail, we investigate by means of mass spectrometric techniques whether and by which routes isotope-labelled, brain-derived Aβ reaches the brain from the periphery. Special emphasis lies thereby on recently discovered clearance mechanisms at the blood-brain barrier, more specifically ATP-binding cassette (ABC) transporters and low-density lipoprotein receptor–related protein-1 (LRP1).

For this purpose, we feed APPPS1 transgenic mice, a model of cerebral β-amyloidosis, for up to 100 d with a diet containing the $^{13}C_6$-substituted version of lysine in order to generate isotope-labelled amyloidogenic brain material. This material is utilized to inoculate APPPS1 transgenic mice with and without genetic modification of the blood-brain barrier (humanised ABC transporters, cell-specific ABC transporter or LRP1 knockout) intraperitoneally either by bolus injection or continuous infusion via osmotic pumps, starting at an age of 50 d. Peripheral organs and the brain are harvested at 60, 100, 150, and 200 d of age, followed by extraction and immunoprecipitation of Aβ from the

samples and subsequent quantification of isotope-labelled and native Aβ by LC-MS/MS and MALDI-TOF/TOF. This innovative method enables us to detect the spatiotemporal distribution and thus the propagation routes of peripherally inoculated Aβ. Mass spectrometric techniques are complemented by classical biochemical and immunohistological techniques in order to investigate putative pathologic consequences of Aβ propagation from the periphery to the brain. We assume that the results will greatly contribute to the assessment whether there is a common risk for the population of administered amyloids advancing towards the brain.

Henrik Biverstål

Karolinska Institutet, Dept NVS, Center for Alzheimer Research, Division for Neurogeriatrics, Huddinge, Sweden

Title: Improved overexpression of Amyloid-β peptide by solubility enhancer NT* from spider silk

JPND project meeting
September 4, 2017
17:45-18:45

Alzheimer's disease is an incurable neurodegenerative disorder linked to misfolding and amyloid formation of the amyloid β-peptide (Aβ). What causes Alzheimer's disease is not fully understood, but it is believed that soluble intermediate oligomers of Aβ peptides play a crucial role in synaptic dysfunction and neurodegeneration. Most of the data available of *in vitro* aggregation studies of Aβ have been conducted with synthetic preparations of Aβ. Synthetic Aβ preparations have although several drawbacks such as batch to batch variations, intrinsic impurities in synthetic preparations and is also relative expensive, especially for isotope labelling. Herein, we present a new protocol for a quick, inexpensive method to produce $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ with superior yield compared to previous described protocols. This system is also very suitable for isotope labelled Aβ and to introduce mutations in the native sequence.

Spiders can produce silk proteins at huge concentrations by sequestering their aggregation-prone regions in micellar structures, where the very soluble N-terminal domain (NT) forms the shell. A consecutive monomer of the NT has just recently been developed in our lab to facilitate high expression levels and prevent aggregation of aggregation prone proteins (NT*). By fusing NT* to Aβ with a TEV cleavage site between, expression levels were enhanced and aggregation prevented during purification. Several protocols have been established to facilitate recombinant production of $A\beta_{40}$ and $A\beta_{42}$ but all of them have in common low yields when grown in M9 minimal media, usually 1/5th to 1/10th of yields from LB media. Yields of

NT*Aβ fusion protein grown in M9 media is comparable to the yields from LB media. We have now started to clone constructs of other amyloidogenic peptides such as IAPP (amylin) HD46Q (huntingtin) and a-synuclein together with NT* to establish a system for overexpression of aggregation prone peptides and proteins.

Giulio Calza

Medicum, Meilahti Clinical Proteomics Core Facility, Meilahti, Finland

Title: Update on the PROP-AD project: Combining transgenic mouse models with systems proteomics to study the effect of the blood-brain barrier in Alzheimer's disease for amyloid propagation.

JPND project meeting
September 4, 2017
17:45-18:45

Introduction/Rationale: Amyloidoses comprise a group of proteopathies in the central nervous system and peripheral organs, characterized by specific aggregation behavior leading to ß-sheeted aggregates of proteins, including amyloid-ß (Aβ) in the form of senile plaques and vascular amyloid angiopathy in Alzheimer's disease (AD). Here, we present an update on the joint PROP-AD project (funded by NFR (Norway), MOS (Israel), BMBF (Germany), SRC (Sweden), AKA (Finland)). This project aims at clarifying the spatial and temporal distribution of native and peripherally injected ^{13}C labelled amyloid-β (Aβ) in the brain.

Results: In the first round of experiments we employed a transgenic mouse model of AD (tgAPPPS1), carrying the Swedish mutation of Amyloid-β precursor protein (KM670/671NL) and a mutation in presenilin 1 (L166P), with documented robust Aβ pathology in the brain [1] and the APP-KO mouse model as negative control.

In Mass Spectrometry Imaging experiments, utilizing the modified protocol of Carlred *et al*. [2] and employing custom-generated bioinformatics pipeline, we assigned the identities (by tandem mass spectrometry LIFT MS/MS) of different Aβ fragments present in senile plaques directly from the tissue. Furthermore, by employing magnetic beads immunoprecipitation (IP) with specific anti-Aβ antibodies (4G8 and 6E10 mAb) we detected fragments corresponding to Aβ 1-40/42/43 and other truncated species specifically in the APPPS1 model, characterizing for the first time the plaque content from a proteomic standpoint [3].

We are currently developing MS-based methods for *i*) high sensitive detection of amyloidogenic peptides in the brain utilizing known amounts of Aβ spiked into APP-KO mouse brain homogenates, *ii*) nHPLC-MS platforms for the quantitation of Aβ species, and *iii*) new protocols involving tryptic digestion to address the aggregation propensity of Aβ peptides and enabling complete digestion of the full-length Aβ peptides, coupled with the Aβ tryptic fragments identification by bioinformatic pipelines.

The results of these preliminary studies will be presented.

References: 1. PMID: 16906128; **2.** PMID: 27115712; **3.** Calza G, Nyberg E, Soliymani R, *et al.* PROP-AD project: Combining transgenic mouse models with MSI and systems proteomics to study the effect of the blood-brain barrier in Alzheimer's disease for amyloid propagation. In: *Ourcon IV Conference - Ustron (Poland)*. (2016).

James D. Mills

Academic Medical Center,
University of Amsterdam,
Netherlands

JPND project meeting
September 4, 2017
18:55-19:40

I completed my PhD in biochemistry and molecular genetics in 2016 at the University of New South Wales in Sydney. On completion of my PhD I took up a post-doctoral position in the Aronica laboratory based at the Academic Medical Center in Amsterdam. Our laboratory is involved in various different research lines, including neurodegenerative diseases (NDs), neurooncology and neurodevelopmental disorders. My personal research interest is the non-coding transcriptome, which consists of a diverse array of RNA molecules including, microRNAs (miRNAs), long non-coding RNAs (lncRNA) and circular RNAs (circRNAs). I am interested in elucidating how this varied class of molecules interact with DNA, proteins and other RNA molecules to influence organism phenotype particularly during brain development and neurodegeneration.

As a member of the JPND project, 'Identification of genes that modulate the severity of neurodegenerative diseases', our laboratory is responsible for providing human post-mortem brain tissue from various NDs for RNA-Sequencing (RNA-Seq). Comparison of RNA-Seq data from spinocerebellar ataxia type 3 (SCA3) and Parkinson's disease (PD) brain tissue with age and region matched post-mortem controls revealed that while there was minimal overlap of differentially expressed genes from each ND, many of the underlying biological pathways were conserved. In both SCA3 and PD there was a marked down-regulation of genes related to the function of the extracellular matrix (ECM). These results suggest that disruption of the ECM might be a fundamental mechanism in the development and progression of NDs. These results await further confirmation in other NDs, along with validation in the analogous rodent models.

Jörg Gsponer

Michael Smith Laboratories,
University of British Columbia
Vancouver, Canada

How to find common regulators and disease genes? The computational integration of diverse and often large data sets in a corner stone of systems medicine and systems biology. My lab develops and applies computational tools for the systems-wide analysis of regulatory processes in eukaryotic cells and their alteration in disease states. One area in the focus of our research is proteostasis where we are aiming to (i) map and characterize the constituents of the proteostasis network and (ii) reveal how deficits in this network modulate the onset and progression of neurodegenerative disorders.

JPND project meeting
September 4, 2017
18:55-19:40
Via Skype

Erica Stenvall

University Hospital of Umeå,
Umeå, Sweden

I studied at Umeå University, Sweden where I received my Masters degree in Biomedical science followed by a Medical Degree in 2012. I currently work as a resident physician in neuropathology at the University Hospital of Umeå.

Ove Eriksson

Biochemistry/Developmental Biology, Faculty of Medicine, University of Helsinki, Helsinki, Finland

Introduction/rationale: Metformin is the first line drug for the treatment of type 2 diabetes currently prescribed to more than 100 million people. Metformin is currently under study also for potential use in neurodegeneration and cancer. Several molecular mechanisms have been invoked to explain the antidiabetic effect of metformin, but it is unclear which mechanisms operate at therapeutic concentrations. Progress in these issues has been hampered by the lack of methods to assess the concentration and distribution of metformin within healthy and diseased tissues (1,2). Here, we have employed MALDI-MSI to elaborate conditions for loading the perfused rat liver with a pharmacological concentration of metformin, followed by an assessment of metabolic parameters during pulsed gluconeogenesis with particular attention on the role of the mitochondrial glycerolphosphate dehydrogenase (3).

Methods: Wistar rat livers were perfused with Hanks-balanced salt solution supplemented with relevant nutrients and with metformin in concentrations ranging from 1 µM to 1 mM. The perfusions medium was collected after passing through liver for analysis of metabolites. Fresh-frozen 12 µm rat liver sections were mounted on custom made nanostructured TiO2 coated ITO slides (Tethis S.p.a, Milan, Italy). Sections were coated with a solution of alpha-cyano 4-hydroxycinammic acid utilizing iMatrixSpray (Tardo Gmbh, Subingen Switzerland) to achieve ~60 µg/cm^2 on the tissue, and imaged in reflectron-positive, LIFT MS/MS and Single Reaction Monitoring (SRM) modes, on a Bruker© UltrafleXtreme MALDI-TOF/TOF Mass Spectrometer with a

50-200 µm lateral raster. Hematoxylin- Eosin Y stained images were co-registered with MSI data using SCiLS Lab software.

Results: Metformin-treated liver sections showed a distinct peak at *m/z* 130.16 Da, which was unambiguously assigned to metformin by LIFT MS/MS and SRM, utilizing identities of various drug fragments reported in the literature. Metformin was detected evenly distributed in parenchyma irrespective of medium concentration and length of perfusion, with sensitivity at the order of a few µM, and hence below the established active clinical concentration of the drug. Kinetic analysis indicated that uptake of metformin from the perfusion medium to the liver parenchyma occurred with an estimated Km and Vmax at the order of 0.3 mM and 0.2 micromol/(gxmin), respectively. Metabolic parameters during gluconeogenesis after a short-time exposure to metformin did not support an involvement of the mitochondrial glycerolphosphate dehydrogenase in the antidiabetic effect of the compound.

Conclusion/novel aspect: These results show that MALDI-MSI can be applied to determine the spatiotemporal tissue concentration and distribution of metformin in the µM concentration range in mammalian tissues. Our results highlight the usefulness of MALDI-MSI as a tool in helping to define the molecular mechanism(s) underlying the beneficial effects of metformin in T2D, neurodegeneration and cancer.

References: 1. PMID: 27076069; **2.** PMID: 27076070; **3.** PMID: 24847880

NOTES