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HBPP Workshop 2018
Adelaide, 8-9 May
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2018 HBPP WORKSHOP
MAY 8-9, 2018
SOUTH AUSTRALIAN HEALTH AND MEDICAL RESEARCH INSTITUTE (SAHMRI), ADELAIDE

PROGRAMME

Tuesday 8 May 2018

8.30 Registration

9.15 Welcome on behalf of HBPP
Oliver Schubert, University of Adelaide, Australia

9.30 A vision for HUPO's Human Protein Project (HPP)
Mark Baker, HPP Co-Chair, Macquarie University, Sydney, Australia

Session 1: Psychiatric disorders 1 (Chair: Scott Clark)

10.00 A neuroproteomics-centered approach to understand schizophrenia
Daniel Martins-de-Souza, University of Campinas, Brazil

10.20 Profiling autoantibody repertoires in neurodegenerative and psychiatric disorders
Peter Nilsson, KTH-Royal Institute of Technology, Stockholm, Sweden

10.40 A Proteomics approach to childhood maltreatment
Johannes Zang, Ruhr-University Bochum, Germany

11.00 ProBDNF is a biomarker and therapeutic target for major depression
Xin-Fou Zhou, University of South Australia, Adelaide

11.20 *Coffee Break*

Session 2: The Neuronal Life Cycle and Neuronal Stress (Chair: Peter Nilsson)

12.00 Quantitative N-terminomics and phosphoproteomic analysis of primary cortical neurons reveal distinct signalling networks governing neuronal death in excitotoxicity
Heung-Chin Cheng, University of Melbourne, Australia

12.20 Quantitative proteomics analysis highlights the dynamic changes in signaling events associated with glutamate-induced neuronal excitotoxicity
Ashfaqul Hoque, University of Melbourne, Australia

12.40 Global ubiquitinome profiling implicates Nedd4 as a key determinant of cell identity through regulation of cell-type specific translation
Quenten Schwarz, University of South Australia, Adelaide, Australia

13.00 Tour of SAHMRI
Peter Pannach, Facility Manager, SAHMRI

13.30 *Lunch break*
Coffee throughout afternoon

Session 3: Novel proteomic technologies for diagnostic and therapeutic biomarker development (Chair: Peter Hoffmann)

14.20 A new workflow for CSF proteome analysis in biomarker research
Katrin Marcus, Ruhr-University Bochum, Germany

14.40 Targeting the cellular glycan surface for nanoparticle based uptake and imaging in the central nervous system
Lindsay Parker, Macquarie University, Sydney, Australia

Session 4: Brain tumours, traumatic brain injury, neurological disorders (Chair: Katrin Marcus)

15.00 Identification of neoepitopes of relevance to immunotherapy of paediatric gliomas
Kirty Pandey, Monash University, Melbourne, Australia

15.20 Evidence of motor neuron disease pathology in the chronic stages post an experimental diffuse traumatic brain injury
Alina Arulsamy, University of Adelaide, Australia

15.40 Ocean of tears: Analysis of the tear proteome in the context of polyneuropathy
Caroline May, Ruhr-University Bochum, Germany

16.00 Identification of Novel Cerebrospinal Fluid Biomarker Candidates for diagnosis of Dementia with Lewy Bodies: A Proteomic Approach
Charlotte Teunissen, VU University, Medical Centre, Amsterdam, Netherlands

18.00 Dinner and Pub, "Peter Rabbit", 234-244 Hindley St, Adelaide

Wednesday 9 May 2018

8.45 Registration

9.30 Creating translational infrastructure: BioMedCity Adelaide and Health Industries SA
Marco Baccanti, CEO, Health Industries South Australia

Session 5: Affinity Proteomics

10.00 The Human Protein Atlas® - an update
Peter Nilsson, KTH-Royal Institute of Technology, Stockholm, Sweden

Session 6: Psychiatric disorders 2 (Chair: Caroline May)

10.20 Multimodal approaches towards personalized psychiatry
Bernhard Baune, University of Adelaide, Australia

10.40 Genome-wide gene expression signature of depression
Liliana Ciobanu, University of Adelaide, Australia

11.00 Transcriptomic and proteomic characterization of lithium response in bipolar disorder
Oliver Schubert, University of Adelaide, Australia

11.20 *Coffee break*

Session 7: Novel bioinformatics approaches (Chair: Bernhard Baune)

12.00 Prediction Modelling in Psychosis: A Comparison of Simple Bayesian and Support Vector Machine Techniques
Scott Clark, University of Adelaide, Australia

12.20 The polygenic score approach to –omics data
Azmeraw Amare, University of South Australia, Adelaide

12.40 Machine Learning for the Modelling of Complex Biological Systems and Emergent Clinical Properties
Micah Cearns, University of Adelaide, Australia

13.00 *Lunch break*

Session 8: MALDI imaging for biomedical research in neuropsychiatric disorders (Chair: Liliana Ciobanu)

14.00 MALDI Imaging: an overview of technologies and opportunities
Peter Hoffmann, University of South Australia, Adelaide

14.20 Protein and N-glycan MALDI imaging mass spectrometry in spinal cords of neuropathic pain induced murine models
Sanam Mustafa, University of Adelaide, Australia

14.40 MALDI Imaging in neuropsychiatric disorders
Oliver Schubert, University of Adelaide, Australia

15.00 **Session 9: How do we increase the research impact of neuroproteomics? HBPP Panel Discussion**

Peter Nilsson, KTH-Royal Institute of Technology, Stockholm, Sweden
Katrin Marcus, Ruhr-University Bochum, Germany
Oliver Schubert, University of Adelaide, Australia

16.00 Close

HBPP 2018 is supported by:

Abstracts

Session 1: Psychiatric disorders 1 (Chair: Scott Clark)

A neuroproteomics-centered approach to understand schizophrenia

Daniel Martins-de-Souza

Laboratory of Neuroproteomics, Institute of Biology, University of Campinas, Campinas, São Paulo, Brazil

Background: Schizophrenia affects over 20 million people worldwide through a wide range of symptoms. As an incurable disorder, the disease management is normally based in antipsychotics, which may present severe side effects and does not work properly to half of the patients. This is mainly because we lack in understanding the molecular basis of the disease, impairing the development of new and more effective medication.

Methods: Here, we employed a neuroproteomics-centered approach to unravel molecular the underpinnings of schizophrenia as well as reveal protein biomarkers associated to antipsychotic effectiveness. For that, we used mass spectrometry-based proteomics to studying brain tissue collected *postmortem* from patients and mentally healthy controls, *in vitro* pre-clinical models such as cell lines and iPSC-derived cerebral organoids, besides blood plasma collected *in vivo* from patients before and after antipsychotic medication (risperidone, olanzapine and quetiapine).

Results: Brain tissue proteomics from seven different brain regions led us to investigate *in vitro* the role of the energy metabolism in cultured oligodendrocytes treated with MK-801 and antipsychotics, to learn more about biochemical processes that may be involved in schizophrenia. iPSC-derived cerebral organoids, neurons and astrocytes were also investigated in terms of proteome dysregulations associated to the disease. Additionally, we revealed blood plasma proteins, which are potential candidates to clinical implementation in the personalized choice of the correct antipsychotic to each patient.

Conclusion: Neuroproteomics may be a powerful asset in the investigation of complex human disorders as schizophrenia, contributing to knowledge that may lead to more effective medication and potential biomarkers for clinical use.

Profiling autoantibody repertoires in neurodegenerative and psychiatric disorders

Peter Nilsson[1]
KTH-Royal Institute of Technology, Stockholm, Sweden

A Proteomics approach to childhood maltreatment

Johannes C.S. Zang[1], **Caroline May**[2], **Robert Kumsta**[1], **Katrin Marcus**[2]
1 Ruhr University Bochum, Department of genetic psychology, Bochum, Germany
2 Ruhr University Bochum, Medizinisches Proteome Center (MPC), Bochum, Germany

Background: Early trauma experience has been linked to a wide range of psychological and physical health problems in adulthood. These observations raised the question of how the effects of early adverse rearing conditions are sustained, or "biologically embedded". A growing body of research integrates the study of epigenetic effects into epidemiological studies. However, most investigations rely exclusively on quantitative levels of DNA methylation, without consideration of downstream processes. The presented project aims to integrate proteomics into the investigation of the long-term effects of early adversity.
Methods: Utilizing LC-MS/MS, we investigated the proteome of 118 monocyte samples derived from 30 participants with a history of traumatic experience and a matching control group before and after exposure to social stress
Results: Of 3846 protein groups, 3519 were identified by a minimum of 2 unique peptides. 1162 protein groups that were present in at least 80% of the trauma and/or the control group were accepted for further analysis. Overall, 109 protein groups showed a significantly different expression between trauma and control group before stress exposure while following stress exposure there were 135 protein groups differentially regulated between groups and might play a role in the mediation of trauma-induced health risk.
Conclusion: So far, current results demonstrate a functional impact of early trauma experience on the protein level of monocytes and highlight the feasibility of studying protein expression following stress exposure in the context of bio-psychological trauma research.

ProBDNF is a biomarker and therapeutic target for major depression

Xin-Fu Zhou[1]

[1]*University of South Australia, Adelaide, Australia*

Background: Depression is a major disease that causes heavy health care burden due to the loss of capacity to work in a large population. Mature brain derived neurotrophic factor (BDNF) is recognized as a biomarker for major depression as reflected by reduced serum level in patients. In recent years, accumulating evidence show the precursor of BDNF plays an opposing role to that of mature BDNF in neuronal functions. We hypothesize that proBNDF may also be a biomarker for major depression and play a role in the pathogenesis of the disease.

Methods: Forty drug-free women patients diagnosed with major depression and 50 healthy female controls were enrolled in our study. Peripheral blood was sampled from all the subjects. With the blood samples, we assessed the relationship between BDNF and major depression from following aspects: the levels of BDNF, proBDNF and their receptors in the sera and lymphocytes. Furthermore, ProBDNF and its receptors were also investigated in rat model of depression, and potential roles of proBDNF in the development of depression was also examined.

Results: It was found that: (a) the protein and serum levels of proBDNF, sortilin and p75NTR were higher in major depressive patients than in healthy controls while mature BDNF and TrkB levels were lower; (b) The levels of mature BDNF and TrkB had negative correlations with the major depression severity, and the levels of proBDNF, p75NTR and sortilin were positively correlated with the scores of HRSD-21; (c) ProBDNF and its receptor p75 are also upregulated in animals with chronic stress and depression-like behaviors; (d) Neutralization of proBDNF in rats with depression could reverse the depression like symptoms.

Conclusion: proBDNF and its receptors are likely biomarkers for major depression and play detrimental roles in the development of depression. Suppression of proBDNF or its receptor may be a novel approach for developing drugs for major depression.

Session 2: The Neuronal Life Cycle and Neuronal Stress (Chair: Peter Nilsson)

Quantitative N-terminomics and phosphoproteomic analysis of primary cortical neurons reveal distinct signalling networks governing neuronal death in excitotoxicity

Ashfagul Hoque[1, 2], Sadia Ameen[1], Giuseppe, D. Ciccotosto[1], Dominic C.H. Ng[3], Nicholas A. Williamson[1], Ching-Seng Ang[1] and **Heung-Chin Cheng**[1]

[1]*University of Melbourne and* [2]*St. Vincent's Institute for Medical Research, Melbourne, Australia, and* [3]*University of Queensland, Brisbane, Australia*

Background: Excitotoxicity, initiated by over-stimulation of ionotropic glutamate receptors (iGluRs), is a major pathological process directing neuronal death in stroke and neurodegenerative diseases. The over-stimulated iGluRs allow massive influx of calcium ions into the affected neurons, leading to over-activation of neurotoxic enzymes such as calpains and neuronal nitric oxide synthase (nNOS), which over-produces NO to induce oxidative damages. The calpain-proteolysed proteins and the NO-induced oxidative damages in turn modulate the activities protein kinases and phosphatases to perturb the expression and phosphorylation of specific neuronal proteins. Presumably, these perturbed proteins form signalling networks that direct neuronal death in excitotoxicity.

Methods: We used the quantitative "Terminal Amine Isotopic Labelling of Substrates" (TAILS) and stable isotope dimethyl labelling phosphoproteomics methods to define the neurotoxic signalling networks. Specifically, we aim to identify the calpain substrates and the neuronal proteins of which the phosphorylation levels are perturbed in primary neurons undergoing excitotoxic cell death.

Results: We identified the cleavage sites in ~300 neuronal proteins proteolytically processed by proteases activated in excitotoxicity. Some of them were defined as calpain substrates. Additionally, we identified ~150 neuronal proteins exhibited dynamic changes in abundance and/or phosphorylation in excitotoxicity. Bioinformatic analysis revealed that some of these

perturbed proteins form distinct signalling networks. Using biochemical approaches, we found that some components of the networks induce neuronal death by aberrant regulation of key protein kinases critical to neuronal survival.
Conclusion: Results of our proteomic analyses form the conceptual framework for future investigation to define the molecular mechanism governing neuronal death in diseases.

Quantitative proteomics analysis highlights the dynamic changes in signaling events associated with glutamate-induced neuronal excitotoxicity

Ashfaqul Hoque[1,2,3], Nicholas A. Williamson[2], Ching-Seng Ang[2], Jonathan S. Oakhill[3], and Heung-Chin Cheng[1,2]
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[2]*Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Parkville, Victoria, Australia*
[3]*Metabolic Signalling Laboratory, St Vincent's Institute of Medical Research, Fitzroy, Victoria, Australia*

Background: Excitotoxicity, caused by over-stimulation or dysregulation of ionotropic glutamate receptors (iGluRs) such as NMDA, AMPA and kainate receptors, is a major pathological process directing neuronal death in both acute and chronic neurological disorders. However, the underlying molecular mechnanism(s) still needs further investigation.
Methods: We utilised stable-isotope dimethyl labelling-based quantitative proteomics and phosphoproteomics approaches to define neuronal proteins undergoing significant changes in abundance and/or phosphorylation levels at different time points (5 min to 4 h) after glutamate over-stimulation in cultured primary cortical neurons. To define downstream effectors of GluN2B-containing NMDA receptors, neurons were co-treated with glutamate and ifenprodil before proteomics analysis. Ingenuity pathway analysis (IPA) software was used for pathway and network analysis. Representative

proteomics data were validated using immunoblots and heavy-labelled synthetic phosphopeptides.

Results: Over 150 neuronal proteins showed significant dynamic temporal changes in abundance and/or phosphorylation levels at different time points (5 min to 4 h) in excitotoxicity. Bioinformatic analyses predict that many of them participate in signalling networks in which they interplay with calpains and the protein kinases Erk1/2 and Akt to perturb the morphology and function of neurons and to direct neuronal death. From the phosphoproteomics data, signalling dynamics of several protein kinases including Akt, Gsk3, Cdk5, CK2, SGK1 and Erk1/2 are identified and implicated in the control of neuronal survival. We also identified >40 neuronal protein molecules as potential downstream signals from neurotoxic GluN2B-containing extra-synaptic NMDA receptors.

Conclusion: Our predicted signalling networks and signalling dynamics of neuronal protein kinases form the conceptual framework for future investigation to define the spatial and temporal organisation of cell signalling pathways governing neuronal death in excitotoxicity.

Global ubiquitinome profiling implicates Nedd4 as a key determinant of cell identity through regulation of cell-type specific translation

Peter McCarthy, Iman Lohresab**,** Sophie Wiszniak, Roger Daly, **Quenten Schwarz**[1]

[1]*University of South Australia, Adelaide, Australia*

Background: The neuronal precursor cell expressed and developmentally downregulated protein, Nedd4, is one of the most abundantly expressed E3 ubiquitin ligases in mammalian neurons and neural crest cells. Nedd4 plays critical roles in neuronal and neural crest cell development and has been implicated in many brain pathologies. However, the molecular mechanisms through which Nedd4 regulates developmental processes and brain function remain largely unknown.

Methods: Here we combine ubiquitin remnant enrichment with total proteome analyses and total transcriptome analysis to provide the first insight into the role of Nedd4 in shaping the ubiquitination landscape of thee cell types.
Results: Our analyses reveal a novel role for Nedd4 in cell-type specific regulation of protein translation machinery with profound effects on neuronal / neural crest stem cell identity and function. Loss of Nedd4 led to a global reduction in specific ubiquitin lysine linkages, akin to that recently reported for Rsp5, the yeast homolog of Nedd4. Finally, we report 276 candidate Nedd4 targets, including 150 novel substrates.
Conclusion: Taken together, our data provide the first insight into how Nedd4-mediated ubiquitination exerts considerable influence over key cellular processes that control neuronal and neural crest cell development.

Session 3: Novel proteomic technologies for diagnostic and therapeutic biomarker development (Chair: Peter Hoffmann)

A new workflow for CSF proteome analysis in biomarker research

Katalin Barkovits[1], Lukas M. Schilde[1], Steffen Kösters[1], Simone Steinbach[1], Andreas Linden[1], Sara Galozzi[1], Nadine Stoepel[1], Sandra Pacharra[1], Brit Mollenhauer[2], Maike Ahrens[1], Karin Schork[1], Michael Turewicz[1], Julian Uszkoreit[1], Martin Eisenacher[1], Caroline May[1], **Katrin Marcus[1]**

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Cerebrospinal fluid (CSF) is in direct contact with the brain and serves as a valuable specimen to examine diseases of the central nervous system through analyzing its ingredients like metabolites, cells and proteins. Numerous studies have been published over the years suggesting proteins present in cerebrospinal fluid as potential biomarkers for diverse neurological diseases. Yet, only a limited number could be validated in other study groups. We examined the effect of protein variability in CSF on the generation of reliable proteomic data and reproducible detection of protein biomarker candidates. Additionally, we evaluated a promising strategy for mass spectrometric analysis of CSF protein: instead of conventional bottom-up data-dependent acquisition (DDA) we used a data-independent acquisition (DIA) approach. DIA overcomes several limitations of DDA like stochastic and irreproducible precursor ion selection since it combines the benefits of DDA and targeted methods like selected reaction monitoring (SRM). For the first time, we established a DIA method for in-depth proteome analysis of CSF. Compared to a conventional DDA method, our DIA approach both increased the number of identified protein groups over 50 %, decreased the coefficient of variation to 6 % (11 % with a DDA), increased the

reproducibility of measurements to 90 % (77 % with DDA) and increased the number of identified brain specific proteins to 60 (only 11 with DDA). Summarized, our optimized DIA method substantially outperforms DDA and could develop into a powerful tool for biomarker discovery in CSF.

Targeting the cellular glycan surface for nanoparticle based uptake and imaging in the central nervous system

Lindsay M Parker[1*], Arun Everest-Dass[1,2], Nicole M Cordina[1], Sameera Iqbal[1], Christopher Ashwood[1], Philipp Reineck[3], Mina Ghanimi-Fard[1], Nicolle H. Packer[1,2]

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Although central nervous system (CNS) cells are well known to functionally communicate via electrical and chemical signals, cell-surface glycans mediate the initial contact between cells and exogenous proteins. Glycans, carbohydrates bound to cellular membrane proteins, are prominently expressed on CNS cell-surface receptors (such as GPCRs), where they can be absolutely critical for receptor trafficking and function, as is the case for the angiotensin II AT1a receptor that regulates blood pressure. Membrane transporter expression and activity (such as crucial solute carrier family proteins that transport serotonin, dopamine, noradrenaline, GABA and glycine) are also highly affected by glycan expression. Cellular glycan expression is dynamic and is closely associated with the innate immune response as well as neuroinflammation driven by microglia. A unique recent concept in biomarker discovery has been to determine

cell type-specific profiles through the characterisation of glycans. By characterising CNS cells in this way we could tailor nanotechnology and drug delivery vehicles towards individual CNS cell types with lectins, which can recognise highly specific glycan receptor structures. The *in vivo* application of lectins stimulates endocytosis of synaptic and/or somatic membrane glycolipids and subsequent trafficking to intracellular organelles such as endosomes and the endoplasmic reticulum. We are working to understand the cell-surface dynamics of CNS cells during inflammation using LC-MS to detect and quantify glycans and MS/MS to confirm the linkage structures of glycans on the surface of different CNS cells. Using this glycan profiling data, we have engineered lectin coated luminescent nanodiamonds and are testing their ability to recognise specific CNS cell types for imaging and drug delivery.

Session 4: Brain tumours, traumatic brain injury, neurological disorders (Chair: Katrin Marcus)

Identification of neoepitopes of relevance to immunotherapy of paediatric gliomas

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Background: Diffuse intrinsic pontine glioma (DIPG) is the most common and highly prevalent form of brainstem glioma in young children with no effective treatment. In 70% of DIPG cases, a somatic mutation of histone H3.3 resulting in a lysine 27 to methionine substitution (H3.3K27M) occurs. This mutation results in generation neo antigenic peptides that have potential for immunotherapy and diagnostics. The aim of this study is to identify these peptides and evaluate their immunogenicity across different immunogenetic backgrounds.

Methods: HEK293 wildtype and HEK293 (H3.3K27M) mutant cell lines were lysed and immunoaffinity purification was used to purify antigenic peptides bound to Human Leukocyte Antigen (HLA) class I molecules. The identity of the peptides was determined using liquid chromatography followed by tandem mass spectrometry (LC-MS/MS).

Results: A total of 4607 peptides were detected in the HEK293 (H3.3K27M) mutant cell line, with 1076 peptides bound specifically to HLA-A*02:01 and 3531 peptides bound to the remaining HLA class I molecules. Several of these peptides were derived from Histone 3.3, with some containing the mutated methionine residue at position 27 (i.e. bona fide neoepitopes).

Conclusion: We have assessed the prominence of neoepitopes derived from a driver mutation in DIPG. In addition to detecting known peptides we have identified a number of novel neoeptiopes. Further

studies using primary DIPG cell lines and biopsy material will conform their presentation. This information will inform immunotherapeutic approaches for treatment of pontine gliomas.

Evidence of motor neuron disease pathology in the chronic stages post an experimental diffuse traumatic brain injury

Alina Arulsamy[1], Frances Corrigan[2], Lyndsey Collins-Praino[1]

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Background: Traumatic brain injury (TBI) is a known risk factor for the development of motor neuron disease such as amyotrophic lateral sclerosis (ALS). Despite this, their neuropathological relationship remains unknown. The current study investigated the motor related neurological changes in the spinal cord and motor cortex post-TBI.

Methods: Spinal cord tissue consisting of cervical, middle thoracic and lumbar regions as well as the motor cortex of Sprague Dawley rats (n= 5-7/group) were sampled at 6 months post diffuse moderate-severe TBI. Following protein extractions, semi-quantitative western blot analysis were performed on the samples, investigating the neuroinflammatory, oxidative stress and neuronal myelination markers as well as ALS pathological marker (TDP43) post-TBI.

Results: Markers of neuroinflammation and neuronal myelination showed no significant changes in any of the spinal cord regions and motor cortex post-TBI when compared to sham animals. However, this study found a trend of increase in TDP43 marker ($p=0.0654$) in the lumbar region of moderate-severe TBI animals when compared to shams. No significant TDP43 changes were seen in the cervical, middle thoracic or motor cortex post-TBI at 6 months.

Conclusion: Our results suggest the possible emergence of ALS pathology at chronic stages post moderate-severe diffuse TBI which may begin from the lumbar region of the spinal cord, despite neuroinflammation resolution. This coincides with clinical studies showing the pathology of a subset of ALS cases progressing from the lumbar to bulbar regions. Thus, this study believes that moderate-

severe TBI may predispose individuals to the development of motor neuron diseases such as ALS.

Ocean of tears: Analysis of the tear proteome in the context of polyneuropathy

Annika Guntermann.[1], Simone Steinbach[1], Christoph Maier[2], Marc Schargus [3], Stephanie Joachim [3], Katrin Marcus[1], **Caroline May[1]**
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[3] *University Eye Hospital, Ruhr-Universität Bochum, Bochum, Germany*

Background: Polyneuropathy affects the myelin sheath and axons of peripheral nerves. Specific symptoms can start with paresthesia and tingling in the feet or hands, or burning pain as well as numbness. So far, only a symptomatic therapy is possible, because the pathogenesis behind the neurological damage is largely unknown. Previous studies described that the small nerve fibers within the skin show a similar degeneration to corneal nerve fibers. The cornea is covered by a thin film of tear fluid. Since tears are easily accessible, protein analysis of alterations in tear fluid composition might help to identify crucial pathways causing polyneuropathy.
Methods: To evaluate this hypothesis, we explored tear proteomic changes resulting from polyneuropathy itself or accompanying illnesses in comparison with a healthy group. Therefore, we collected tear fluid from 300 individuals by using Schirmer test I. The study group comprised 109 patients with diabetes mellitus, with or without retinopathy and macular edema, 44 with cancer, 38 with cluster headache, 24 with polyneuropathy of unknown origin, 20 with CIDP, 19 with thyroid disease, 15 with other neuropathies as well as 84 healthy controls.
Results and Conclusion: Up to now, we could show that the protein concentration does not significantly correlate with the tear flow rate. Instead, there was a high inter- and intra-group specific variability. In detail, tear secretion and protein concentration of polyneuropathy patients do not significantly differ from healthy controls or other

diseases. 170 samples were further analyzed via a DIA mass spectrometric approach. The group specific statistical data analysis is still ongoing.

Proteomics identification of novel cerebrospinal fluid biomarker candidates of dementia with Lewy bodies

Inger van Steenoven[1], Marleen J.A. Koel-Simmelink[2], Leonie J.M. Vergouw[3], Thang V. Pham[4], Wiesje M. van der Flier[1,5], Frank Jan de Jong[3,4], Connie R. Jimenez[4], Afina W. Lemstra[1] and **Charlotte E. Teunissen[2]**

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WvdF

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Background: Recognizing dementia with Lewy bodies (DLB) remains challenging due to highly variable clinical presentation and overlap with Alzheimer's disease (AD) and Parkinson's disease. As a consequence, DLB is often misdiagnosed and diagnosis is made on average 3 years after symptom-onset. Cerebrospinal fluid (CSF) biomarkers could improve the early diagnostic accuracy, monitor disease progression and treatment response. However, no specific biomarkers for DLB are available. We set out a proteomics study to identify novel CSF biomarker candidates for DLB.

Methods: We included 20 probable DLB patients(age 65±5yr; 15%F; Mini-mental State examination (MMSE) 23±4) and 20 age and gender matched cognitively normal controls(age 65±5yr; 15%F; MMSE 28±1) with CSF available from the Amsterdam dementia cohort. In-depth

proteomics workflow involving immune-depletion of high-abundant proteins, mono-dimensional SDS-PAGE in conjunction with nanoLC-MS/MS-based proteomics, database searching (MaxQuant) and label-free protein quantification was applied. Candidate biomarkers were selected based on the following criteria: (1)fold change > 1.2 and p-value <0.05 p<0.05; (2)mean spectral count in all subjects in one of the groups >2; and (3)number of identified peptide sequences covering ≥20% of the protein. Validation was performed using the same proteomics workflow in an independent cohort consisting of 17 DLB patients(age 67±7yr; 24%F; MMSE 24±4) and 13 cognitively normal controls(age 66±8yr; 30%F; MMSE 28±2).

Results: In the discovery cohort 57 out of 2100 identified proteins were differentially expressed in DLB patients compared to controls (p<0.05). 35 proteins were down-regulated and 22 proteins were up-regulated in DLB. 18 proteins fulfilled the selection criteria for candidate biomarkers. In the validation cohort, 68 out of 2289 identified proteins were differentially expressed (p<0.05). 34 proteins were down-regulated and 34 proteins were up-regulated in DLB. 38 proteins fulfilled the selection criteria. Three highly significant overlapping candidate biomarkers were found. All three proteins were down-regulated in DLB and are involved in synaptic plasticity. Logistic regression analysis showed that a biomarker panel consisting of these three proteins could discriminate DLB from controls (AUC: 0.81[95%CI: 0.65-0.97].

Conclusions: Our proteomics analysis of CSF identified and validated several novel potential candidate biomarkers for DLB. The identified proteins could aid in early diagnosis of DLB.

Session 5: Affinity Proteomics

The Human Protein Atlas® - an update

Peter Nilsson[1]

[1]*KTH-Royal Institute of Technology, Stockholm, Sweden*

Session 6: Psychiatric disorders 2 (Chair: Caroline May)

Multimodal Approaches towards Personalized Psychiatry

Bernhard Baune[1]
[1]*Discipline of Psychiatry, Adelaide Medical School, University of Adelaide*

Abstract: Psychiatry as a clinical and scientific field is moving towards individualising diagnostic and treatment processes. Often terms such as Precision Psychiatry / Medicine and Personalised Psychiatry are used interchangeably. In this presentation, the concept of Personalized Psychiatry as a multimodal approach that A) integrates biological systems with clinical markers and B) that includes prevention, prediction, and participation is introduced. It is explored how these key principles could be applied to people who are suffering from depression or bipolar disorder.

Biological parameters such as genetic variation and blood-based transcriptomic and proteomic markers are broadly investigated for their potential to aid diagnostic stratification and treatment decisions in mood disorders. Further, for a more accurate prediction of illness course and treatment outcomes, a multimodal modelling approach is required that considers both clinical and biological markers and that takes the longitudinal perspective into account. Translating promising clinical and biological markers of disease progression and treatment outcomes into clinical algorithms that can be empirically tested may facilitate therapeutic interventions that target individual patient needs, aiding to avoid unnecessary and potentially harmful interventions in people suffering from severe mental illness.

Genome-wide gene expression signature of depression

Liliana G. Ciobanu[1], Perminder S. Sachdev[2], Julian N.Trollor[2,3], Simone Reppermund[2, 3], Anbupalam Thalamuthu [2], Karen A. Mather[2,6], Sarah Cohen-Woods[4], David Stacey[5], Catherine Toben[1], K. Oliver Schubert[1,7], Bernhard T. Baune[1]
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Background: The molecular factors involved in the pathophysiology of major depressive disorder (MDD) remain poorly understood. One approach to examine the molecular basis of MDD is co-expression network analysis, which facilitates the examination of complex interactions between expression levels of individual genes and how they influence biological pathways affected in MDD.
Methods: We applied an unsupervised gene-network based approach to a prospective experimental design using microarray genome-wide gene expression from the peripheral whole blood of older adults. We utilised the Sydney Memory and Ageing Study (sMAS, N=521) and the Older Australian Twins Study (OATS, N=186) as discovery and replication cohorts, respectively. We constructed networks using Weighted Gene Co-expression Network Analysis (WGCNA), and correlated identified modules with four subtypes of depression: single episode, current, recurrent, and lifetime MDD.
Results: Four modules of highly co-expressed genes were associated with recurrent MDD in our discovery (sMAS) cohort, with no significant findings for a single episode, current or lifetime MDD. Functional characterisation of these modules revealed a complex interplay between dysregulated protein processing in the endoplasmic reticulum (ER), and innate and adaptive immune response signalling, with possible involvement of pathogen-related pathways. We did not replicate findings at the network level in an independent cohort (OATS), however, 9 individual genes with similar co-expression and dysregulation patterns were found associated with recurrent MDD in both cohorts.
Conclusion: Our findings support other reports on dysregulated immune response and protein processing in the ER in MDD and provide novel insights into the pathophysiology of depression.

Transcriptomic and proteomic characterization of lithium response in bipolar disorder

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Background: Lithium is a first line treatment for acute mania and for relapse prevention in bipolar disorder (BD), and possesses unique properties compared to other mood-stabilizing medications. However, only 30% of BD patients respond optimally to this drug. To date, the cellular processes involved in therapeutic lithium treatment response are poorly understood, and there are no biomarkers that could guide treatment selection.

Methods: We applied a gene-network based approach using microarray genome-wide gene expression from peripheral whole blood of BD patients treated with lithium (n=50) and compared responders with non-responders. We constructed networks using Weighted Gene Co-expression Network Analysis (WGCNA), and correlated identified modules with patient scores on the ALDA scale, a validated tool for quantitation of lithium response in BD. Gene-expression findings were followed up on the proteome level, in the same patient cohort, using a targeted DIA approach.

Results: One module of 46 highly co-expressed genes was associated with favourable lithium response. Pathway analysis indicated that genes involved in the mitochondrial electron transport chain are down-regulated in lithium responders, but not in non-responders. Preliminary results of the proteomic follow up will be presented.

Conclusion: Our findings support previous reports on the centrality of mitochondrial mechanisms in the pharmacology of lithium in BD.

Session 7: Novel bioinformatics approaches (Chair: Bernhard Baune)

Prediction Modelling in Psychosis: A Comparison of Simple Bayesian and Support Vector Machine Techniques

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Background: Outcomes for people suffering psychosis are difficult to predict. Subthreshold psychotic experiences are not uncommon, however transition to full psychotic illness is very low at less than 1%. Even in those defined at clinical high risk (CHR) of psychosis the long-term rate of transition is only 30%. Function both in the CHR group and following the first psychotic episode (FEP) is highly variable and not necessarily dependant on positive psychotic symptom burden. Multimodal models combining historical, clinical, cognitive and biological markers are able to improve the accuracy of outcome prediction. We compare the use of the odds ratio form of Bayes' rule (OR) and support vector machine (SVM) techniques used to build multimodal models.

Methods: Examples constructed from clinical CHR, and post FEP cohorts using the OR and an SVM will be contrasted.

Results: Simulation suggests that OR models combining historical, clinical, cognitive, imaging and electrophysiological data have the potential to more accurately stratify risk and personalise assessment and intervention across the stages of psychotic illness. In clinical cohorts the addition of biomarker data improves the accuracy of structured clinical assessments. For instance, using the OR to combine history, clinical and fatty acid markers in one cohort of CHR participants predicted transition to psychosis with high accuracy

(ROC=0.919; sensitivity=72.73%; specificity=96.43%). In a large cohort of 258 UHR patients, a SVM model achieved an accuracy of 83% in comparison to 76.7% for OR.
Conclusions: Bayesian models using the OR technique are flexible, clinically intuitive, and relatively simple to construct. In comparison SVM may be more accurate but require all data at the time of calculation to make predictions. Due to their simplicity, OR models show promise for implementation in complex clinical environments.

The polygenic score approach to –omics data: Application in the pharmacogenomics of mood disorders

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Background: Given a substantial advancement in omics technologies, personalised medicine has become the 21st model of health care. While Omics technologies generate a huge amount of data, molecular profiling and an integration of knowledge from omics pillars (genomics, microbiome, epigenomes, proteome, and metabolome) with clinical data is challenge.
Methods: Here, we presented a polygenic approach in the pharmacogenomics of mood disorders and highlighted the potentials of poly-omics as a strategy to comprehensively compile omics data and develop algorithms (tools) to predict complex traits. The polygenic score method is a quantitative measure of genetic load and quantifies the aggregate effect of genome wide genetic variants. An application of PGS has been successful in psychiatric and other complex traits.
Results: Recently, we investigated the polygenic determinants of response to; a) SSRIs in patients with MDD and b) lithium treatment in patients with BPD. Using a polygenic score, we revelaed an inverse assocation between polygenic loading for schizophrenia (SCZ), major depressive disorder (MDD) and depressive symptoms and response

to lithium in patients with BPD. Moreover, we demonstrated the association of the PGSs for personality traits (openness, conscientiousness and neuroticism) and response to SSRIs in patients with MDD.
Conclusion: Poly-omics model with similar analogy to polygenic score, based on matrix weights for each omics component (and the environmental), may be applied in complex omics data.

Machine Learning for the Modelling of Complex Biological Systems and Emergent Clinical Properties
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Abstract: A complex system is a system containing a large number of interacting elements whose collective activity is non-linear, meaning that the emergent properties from the system cannot be explained via the summation of elements within the system. In the context of psychiatric research, an emergent property of interest could be a diagnostic phenotype such as major depression or schizophrenia, a trajectory of illness such as relapse within a specified time frame, or a specific drug response profile. Thus, these emergent properties form the focal point of investigation for clinician's investigation, diagnosis, and subsequent treatment. Given the complex system structure of psychiatric disorders, a problem arises. Classical statistical methods tend to focus on reduced components of a system, such as the relationship between an individual biomarker and its linear relationship with an emergent clinical phenotype. In this presentation, it is proposed that certain machine learning models that are more analogous to complex system structure, e.g., can model non-linear relationships through the quantification of multiple high-level interaction terms, may provide increased insight and translatable clinical utility in the field of proteomics. A range of models and their application in the field of proteomics and other omics disciplines will be explored.

Session 8: MALDI imaging for biomedical research in neuropsychiatric disorders (Chair: Liliana Ciobanu)

MALDI Imaging: an overview of technologies and opportunities

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Protein and *N*-glycan MALDI imaging mass spectrometry in spinal cords of neuropathic pain induced murine models

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Background: Spinal cord as a connection between brain and peripheral nervous system is vital for studying neural transmission, particularly in pain-related research. Chronic neuropathic pain affects a vast majority of the population. Outcomes are hard to determine due to the subjective nature of pain. The molecular mechanisms underlining the development and maintenance of pain is not completely understood.

A recent novel imaging technique that could be used to elucidate the signalling mechanism of pain is matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS). MALDI-IMS of biomolecules provides label-free spatial analysis over conventional histological analysis, providing unbiased visualization of the distribution of several analytes of interest.

Methods: In this study, MALDI-IMS was used to identify differentially expressed proteins and glycans from formalin-fixed paraffin embedded (FFPE) mouse spinal cord tissue from chronic constriction injury and sham animals. The imaging was carried out at 20µm FFPE sections through *in-situ* enzyme digestion followed by matrix application. Consecutive sections were used to identify the detected

m/z signals through liquid-chromatography and tandem mass-spectrometry (LC-MS).
Results: MALDI-MSI revealed several proteins to be significantly different spatially and semi-quantitatively between allodynic versus control mice. For example, the proteins 2-oxoglutarate dehydrogenase and Sodium/potassium-transporting ATPase were significantly altered. Similarly, *N*-glycan anlaysis revealed the glycan carrying the bisecting GlcNAc elevated in allodynic mice spinal cords. Interestingly these differences were observed in the dorsal, a key somatosensory nucleus of the spinal cord.
Conclusion: These data are the first example of the promising application of MALDI-IMS in the exploration of the aetiology of chronic pain.

MALDI Imaging in neuropsychiatric disorders
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Background: Matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) is a mass spectrometry technique used for the analysis of macromolecules on an intact tissue of interest, thereby allowing the assessment of molecular signatures in health and disease in the anatomical context.
Methods: We reviewed MALDI-MSI studies on neurodegenerative and psychiatric disorders, and explored whether the technique could accelerate the translation of proteomic information into improved understanding and ultimately better therapeutic applications.
Results: Over the last decade, biomedical research into neurodegenerative and psychiatric disorders has gradually begun to integrate MALDI-MSI into studies aiming at the targeted detection of proteins, lipids, neuropeptides, and small molecule therapeutics in human and animal CNS tissue.

Conclusion: MALDI-MSI is still at an early stage in the neurosciences, and that its full potential remains to be realized for neurodegenerative and psychiatric disorders. Nevertheless, a small number of research groups have made considerable inroads into the investigation of disorders of the brain using MALDI-MSI, particularly in the fields of PD and AD