

## NEUROLIPIDOMICS 2010

Annual meeting and research symposium

### session 1

**Dr. Christa Studzinski**

*Characterizing a novel protein-lipid-protein complex in Parkinson's Disease*

**Dr. Hongbin Xu**

*Cyclic fluctuations in glycerophosphocoline metabolism over a 24h period in the murine temporal cortex*

**Matthew Cooke & Crystal Hanley**

*Spatial and temporal modeling of the entry of lipid second messengers into neural stem cells over the course of specification: Step One*

**Camille Juzwik & Carolina Cieniak**

*Validating the impact of Cree medicine on the glycerophosphocholine lipidome in peripheral diabetic neuropathy*

### session 2

**Dr. Emdadul Haque**

*Understanding the role of abnormal mitochondrial lipid species in Parkinson's disease*

**Dr. Leigh Anne Swayne**

*Mapping and validating the late-stage Alzheimer disease glycerophospholipidome*

**Nico Valenzuela**

*Visualization of lipid-protein interactions*

**Ruthanna Okorosobo & Andrew Syrett**

*Profiling the Impact of Opa1 Null-Mutation on the Mitochondrial Glycerophospholipidome using High Performance Liquid Chromatography Electrospray Ionization Mass Spectrometry (LC-ESI-MS)*

### session 3

**Dr. Michael Kennedy**

*Identification and validation of the cellular targets involved in glycerophosphocholine lipid signalling*

**Dr. Corey Yanofsky**

*Evidential statistical analysis for neurolipidomics*

**Sarah Gelbard**

*Modeling lipidomic visualization in representational theory*

### keynote presentation

**Dr. Alex Brown**

Vanderbilt University School of Medicine

### invited guest speaker

**Dr. Gregory A. Grabowski**

Cincinnati Children's Hospital Medical Center

### poster session

Alexandre P. Blanchard, Jenna Boulanger, Brett Hawley, Weimin Hou, Stephanie Fowler, Ashleigh McLean, Maxime W.C. Rousseaux, Andrew Syrett

Monday November 22, 2010

8:30 a.m. to 7:00 p.m.

Canadian Museum of Nature  
240 McLeod Street, Ottawa Ontario

## **KEYNOTE AND INVITED GUEST SPEAKERS**

### **A CHEMISTRY THAT ‘CLICKS’ WITH LIPIDS.**

H. Alex Brown, PhD  
Vanderbilt University School of Medicine

Phosphatidic acid species are essential for biosynthesis of cellular glycerophospholipids and functions as a modulator of various cell signaling enzymes. Tracking the plethora of sources of phosphatidic acid has long been a goal, but even with advances in mass spectrometry the identification of substrate-product relations for such a complex and rapidly metabolized intermediate has been elusive. Alkyne-modified phospholipids can be unambiguously identified and differentiated from native species in complex mixtures by formation of dicobalthexacarbonyl complexes. This reaction is specific for alkynes and is unaffected by other glycerophospholipid-related moieties. Enrichment of cells with alkyne-derivatized fatty acids or glycerophospholipids followed by solid-phase sequestration and release is a promising new method for unequivocally monitoring individual glycerophospholipids following incorporation into cells. This robust method for tracking modified lipids, is similar in application to ‘click’ chemistry, in that it provides a platform for metabolomic and molecular tracking of pathways. This technology is being used to interrogate substrate-product relationships in lipid signaling networks, particularly to define the differential origins of phosphatidic acid. The chemistry can be applied to a broad spectrum of biological molecules beyond lipids as well.

### **LIPIDOMICS OF GAUCHER DISEASE: ORGAN AND MUTATION SPECIFICITY**

Greg Grabowski, MD  
Cincinnati Children’s Hospital Medical Center.

### CHARACTERIZING A NOVEL PROTEIN-LIPID-PROTEIN COMPLEX IN PARKINSON'S DISEASE

Christa M Studzinski<sup>1</sup>, Tammy Langman<sup>1</sup>, Daniel Figeys<sup>2</sup>, Stefany AL Bennett<sup>2,3</sup>, Anurag Tandon<sup>1</sup>

<sup>1</sup>Centre for Research in Neurodegenerative Diseases, University of Toronto, Toronto, ON, M5S 3H2, Canada; <sup>2</sup>Ottawa Institute of Systems Biology, Department of Biochemistry, Microbiology, and Immunology; <sup>3</sup>Neural Regeneration Laboratory, Faculty of Medicine, University of Ottawa, Ottawa, ON K1H 8M5, Canada

Parkinson's disease (PD) is a common neurodegenerative disorder affecting 1% of the population by age 65. Current therapies for PD are purely symptomatic and do not address the underlying pathology, which includes the abnormal accumulation of  $\alpha$ -synuclein ( $\alpha$ -syn) in the brain. Although  $\alpha$ -syn is predominantly found in presynaptic nerve terminals, its function remains unknown. In vivo,  $\alpha$ -syn is found in both the cytosolic pool and in a membrane-bound form however little is known about the regulation of  $\alpha$ -syn's cellular localization. Recently, our group has shown that a specific lipid (1-O-hexadecyl-2-acetyl-sn-glycerol-3-phosphocholine or C16:0 PAF) in combination with delipidated cytosol could increase  $\alpha$ -syn's binding to the membrane. These findings suggest the presence of a protein-lipid- $\alpha$ -syn complex that is influencing the cellular location of  $\alpha$ -syn. In the present study, we attempt to replicate and expand on our previous findings. In contrast to our previous findings, the increase in  $\alpha$ -syn membrane binding appears to be mostly dependent on the presence of delipidated cytosol, although C16:0 PAF may also be contributing. Additional studies will attempt to determine what the role of lipid signalling plays in  $\alpha$ -synuclein's cellular localization.

\*This project was supported by the CIHR Institute of Aging and a Canadian Institutes of Health Research (CIHR) Training Program in Neurodegenerative Lipidomics Postdoctoral Fellowship to CMS.

### CYCLIC FLUCTUATIONS IN GLYCEROPHOSPHOCHOLINE METABOLISM OVER A 24 H PERIOD IN THE MURINE TEMPORAL CORTEX\*

Hongbin Xu<sup>1,2</sup>, Alexandre P. Blanchard<sup>1,2</sup>, Katy Morin<sup>2</sup>, Leigh Anne Swayne<sup>1,2</sup>, Daniel Figeys<sup>1,2</sup>, Steffany A.L. Bennett<sup>1,2</sup>

<sup>1</sup>CIHR Training Program in Neurodegenerative Lipidomics, <sup>2</sup>Neural Regeneration Laboratory and Ottawa Institute of Systems Biology, Dept of Biochemistry, Microbiology, and Immunology, University of Ottawa, Ottawa, <sup>3</sup>Centre for Research in Neurodegenerative Disease, University of Toronto, ON, Canada

About half of human brain dry weight is accounted for by lipids. Yet their roles in normal brain function and disease development are not extensively explored. In fact, one class of the major building blocks of brain cell membrane, glycerophospholipids, are often overlooked as immediate response elements yet they exert fundamental control over cell fate. Inside cells, processing of a subclass of glycerophospholipids, choline-containing membrane phospholipids, by proteins called enzymes generate powerful smaller lipid molecules, the second messengers. Each messenger controls distinct cellular processes yet little is known about the daily changes of these key lipids in the brain. Here, we use the state-of-art technology to profile changes of choline-containing second messengers over a 24 h period in the mouse brain. Our data demonstrate that some specific lipid messengers exhibit a daily rhythm in the brains of mice housed on a 12 h dark-light schedule but, surprisingly, that the global metabolism of the lipid second messengers is not altered. These findings represent the first molecular identification of a specific day-night lipid second messenger rhythm in the mouse brain. These changes of discrete lipid species may lend a potential explanation to the daily fluctuation of cognitive state of Alzheimer's patients.

\*Supported by CIHR MOP-89999 and the CIHR Training Program in Neurodegenerative Lipidomics (TGF-96121) to DF and SALB. HX was supported by the Institute of Aging and CIHR Training Program in Neurodegenerative Lipidomics.

## SESSION 1 CONT

### VALIDATING THE IMPACT OF CREE MEDICINE ON THE GLYCEROPHOSPHOCHOLINE LIPIDOME IN PERIPHERAL DIABETIC NEUROPATHY\*

Camille A. Juzwik<sup>1,2</sup>, Ashleigh C. McLean<sup>1,2</sup>, Carolina Cieniak<sup>1,2</sup>, Fida Ahmed<sup>1,2</sup>, Alexandre P. Blanchard<sup>1,2</sup>, Despina Harbilas<sup>3</sup>, Antoine Brault<sup>3</sup>, Pierre Haddad<sup>3</sup>, Daniel Figeys<sup>2</sup>, Steffany A.L. Bennett<sup>1,2</sup>

<sup>1</sup>Neural Regeneration Laboratory, <sup>2</sup>Ottawa Institute of Systems Biology, Department of Biochemistry, Microbiology, and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, ON K1H 8M5, Canada, <sup>3</sup>Natural Health Products and Metabolic Diseases Laboratory, Department of Pharmacology, Université de Montréal, P.O. Box 6128, Downtown Station, Montreal, Quebec, H3C 3J7, Canada

The Cree Nation of Eeyou Istchee is experiencing a type II diabetes incidence rate 3 to 5 times higher than that of the remaining adult Canadian population. In this study, traditional Cree medicines were studied as preventative diabetic medicines using an unbiased lipidomics approach. Choline-containing lipid second messengers in dorsal root ganglia from mice fed a high fat diet with and without plant extracts identified by Cree elders and healers as potentially anti-diabetic at 250 mg/kg were profiled to determine whether these treatments are capable of restoring glycerophosphocholine metabolism. Glycerophosphocholine second messenger changes may potentially influence the on-set of type II diabetes as they are implicated in diabetic neuropathy, a major diabetic complication. We found that specific lipid species accumulate in peripheral nerves as a result of a high fat diet and that one of the plant extracts tested was able to restore balance of specific metabolites.

\*This project was supported by the (CIHR) Training Program in Neurodegenerative Lipidomics Undergraduate Summer Student Research Award to CAJ and a research travel award to ACM.

### SPATIAL AND TEMPORAL MODELING OF THE ENTRY OF LIPID SECOND MESSENGERS INTO NEURAL STEM CELLS OVER THE COURSE OF SPECIFICATION: STEP ONE\*

Crystal Hanley<sup>2†</sup>, Matthew Cooke<sup>1†</sup>, Nico Valenzuela<sup>2</sup>, Steffany A.L. Bennett<sup>1</sup>, Stephen Fai<sup>2</sup>

<sup>1</sup>Neural Regeneration Laboratory and Ottawa Institute of Systems Biology, Department of Biochemistry, Microbiology and Immunology, University of Ottawa, <sup>2</sup>Carleton Immersive Media Studio, Azrieli School of Architecture and Urbanism, Carleton University

Gap junctions provide a means for small metabolites and signaling molecules to pass between adjacent cells. Connexin proteins, the structural units that make up gap junctions, can also form non-junctional hemichannels open to the extracellular space. Passage of lipid second messengers through these channels appears to play a role in a wide range of neurological events including neurogenesis, gliogenesis, and inflammation. Unfortunately, no pharmacological tools currently exist to test this hypothesis as we lack highly specific modulators of connexin proteins. Here, we sought to visually represent the role these channels play in neural stem cell specification using 3D animation and compositing software. Along with concurrent high throughput targeted screening for small molecule connexin inhibitors, this work serves as a first step towards communicating the capacity of connexin proteins to regulate the intracellular accumulation of lipid second messengers and influence neural cell specification.

\*This project was supported by the Institute of Aging and a Canadian Institutes of Health Research (CIHR) Training Program in Neurodegenerative Lipidomics Undergraduate Summer Student Research Award to CH and MC and a CIHR Training Program in Neurodegenerative Lipidomics Post-Professional Scholarship to NV.

†Authors contributed equally.

### UNDERSTANDING THE ROLE OF ABNORMAL MITOCHONDRIAL LIPID SPECIES IN PARKINSON'S DISEASE\*

M. Emdadul Haque<sup>1</sup>, Maxime Rousseaux<sup>1</sup>, Alexandre P. Blanchard<sup>2</sup>, Andrew Syrett<sup>2</sup>, Hu Zhou<sup>3</sup>, Rudolfo Zunino<sup>4</sup>, Heidi M. McBride<sup>4</sup>, Daniel Figeys<sup>3</sup>, Steffany A.L. Bennett<sup>2</sup>, David S Park<sup>1</sup>

<sup>1</sup>Department of Cellular and Molecular Medicine and <sup>2</sup>Neural Regeneration Laboratory, <sup>3</sup>Ottawa Institute of Systems Biology, Department of Biochemistry, Microbiology, and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, ON, K1H 8M5, Canada, <sup>4</sup>University of Ottawa Heart Institute, Ottawa, Canada.

Parkinson's disease (PD) is a progressive neurodegenerative disorder that is characterized by the progressive loss of a selective population of dopamine neurons in the substantia nigra pars compacta (SNc) which results in physical and cognitive deficits. It has been reported that mitochondria play a critical role in developing PD. Here, we asked whether mitochondrial apoptosis is associated with a shift in the glycerophosphocholine second messenger lipidome at the level of the organelle. To understand the role of abnormal lipid species generation in the damaged mitochondria, we have initiated a study measuring the choline-containing lipid second messengers in isolated healthy or damaged mitochondria following staurosporine treatment. This approach will be extended to determine the role of these mitochondrial lipids in a very specific genetic model of Parkinson's disease.

\*This project was supported by the Insitutte of Aging and Canadian Institutes of Health Research (CIHR) Training Program in Neurodegenerative Lipidomics Postdoctoral Fellowships to MEH and CIHR Training Program in Neurodegenerative Lipidomics Graduate Scholarships to MR and AS.

### MAPPING AND VALIDATING THE LATE-STAGE ALZHEIMER DISEASE GLYCEROPHOSPHOLIPIDOME\*

Leigh Anne Swayne<sup>1,2</sup>, Weimin Hou<sup>2</sup>, Alexandre P. Blanchard<sup>1,2</sup>, Hongbin Xu<sup>1,2</sup>, Daniel Figeys<sup>1,2</sup>, and Steffany A.L. Bennett<sup>1,2</sup>

<sup>1</sup>Neural Regeneration Laboratory and <sup>2</sup>Ottawa Institute of Systems Biology, Department of Biochemistry, Microbiology, and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, ON K1H 8M5, Canada

Emerging evidence from our laboratory and others suggest there are complex lipid changes in the brain in Alzheimer disease (AD). AD involves nerve cell death in the areas of the brain involved in memory. We are particularly interested in the role of a family of lipids called glycerophosphocholines (GPCs), which consist of a three-carbon glycerol backbone, a phosphocholine headgroup and one or two fatty acid chains of variable length and characteristics. Nerve cell membranes are composed of large GPC molecules referred to as 'structural GPCs'. Pathological processes in AD contribute to the breakdown of structural GPCs into smaller GPCs, also known as 'bioactive GPCs' that can trigger changes in nerve cells. The generation of bioactive GPCs with fatty acids of different lengths and characteristics can ultimately result in the preservation or the death of nerve cells. We have profiled these GPC second messengers in the brains of AD post-mortem human tissue, and in disease models to identify the distinct GPCs, at the detailed molecular level, that are altered by AD. We have found changes in specific GPCs that exert either neurotoxic or neuroprotective responses. This work provides new insight into how targeting bioactive lipids could alter progression of AD.

\*This project was supported by the Insitutte of Aging and Canadian Institutes of Health Research (CIHR) Training Program in Neurodegenerative Lipidomics Postdoctoral Fellowships to LAS and HX, and CIHR Training Program in Neurodegenerative Lipidomics Graduate Scholarships to WH and APB.

## SESSION 2 (CONT)

### VISUALIZATION OF LIPID-PROTEIN INTERACTIONS\*

Nicolas E. Valenzuela<sup>3</sup>, Steffany A.L. Bennett<sup>1,2</sup>, Stephen Fai<sup>3</sup>

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A comprehensive understanding of lipid changes, lipid metabolism, and lipid-protein interactions is constrained by the use of 2-dimensional (2D) representations to describe 3-dimensional (3D) microenvironments. Moreover, the legibility of these images is made difficult to those unaccustomed with their particular codes and conventions. Architectural modes of representation coupled with visualization technologies offer a way of decoding this language in such a way as to make lipidomic datasets more accessible and immediately engaging while concomitantly providing a new means of representing concepts and hypotheses relevant to targeted research in Neurodegenerative Lipidomics.

\*This project was supported by a Canadian Institutes of Health Research (CIHR) Training Program in Neurodegenerative Lipidomics Post-Professional Fellowship to NEV.

### PROFILING THE IMPACT OF OPA1 NULL-MUTATION ON THE MITOCHONDRIAL GLYCEROPHOSPHOLIPIDOME USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY ELECTROSPRAY IONIZATION MASS SPECTROMETRY (LC-ESI-MS)\*

Ruthanna Okorosobo<sup>1</sup>, David Patten<sup>1</sup>, Jason MacLaurin<sup>1</sup>, Andrew Syrett<sup>2,3</sup>, Camille A. Juzwik<sup>2,3</sup>, Daniel Figeys<sup>3</sup>, Steffany A.L. Bennett<sup>2,3</sup>, and Ruth S. Slack<sup>1</sup>

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Optic Atrophy-1 or Opa1 is an inner mitochondrial membrane protein critical for maintaining the mitochondrial cristae architecture. Previous studies have shown that in the absence of proper neuronal Opa1 processing, the mitochondrial cristae junction is no longer tightly sealed, thereby allowing the release of cytochrome c into the cytoplasm, where it ultimately leads to apoptosis. Furthermore, Opa1 interacts with various mitochondrial membrane lipids, which has been implicated in regulating the function of Opa1. Hence, using the Opa1 knockout mouse embryonic fibroblast (MEF) as a model for neurodegeneration, we identified specific changes in discrete glycerophosphocholine second messenger species between the mitochondrial lipid pools of Opa1 knockout and wild-type MEFs. Future studies will indicate if these changes support the role of mitochondrial membrane phospholipid metabolism in Opa1 functioning.

\*This project was supported by the Institute of Aging and a Canadian Institutes of Health Research (CIHR) Training Program in Neurodegenerative Lipidomics Undergraduate Summer Student Research Award to RO, CAJ, and a CIHR Training Program in Neurodegenerative Lipidomics Graduate Scholarship to AS.

### IDENTIFICATION AND VALIDATION OF THE CELLULAR TARGETS INVOLVED IN GLYCEROPHOSPHOCHOLINE LIPID SIGNALLING\*

Michael A. Kennedy<sup>1</sup>, Nazir Kabania, Jean-Philippe Lambert<sup>1</sup>, Leigh Anne Swayne<sup>1</sup>, Daniel Figeys<sup>1</sup>, Steffany A. L. Bennett<sup>1</sup>, Jennifer Bryan<sup>2</sup>, Kristin Baetza<sup>1</sup>

<sup>1</sup> Ottawa Institute of Systems Biology, Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa, Ontario K1H 8M5;

<sup>2</sup> Michael Smith Laboratories and Department of Statistics, University of British Columbia, Vancouver, BC, Canada V6T 1Z4

Accelerated cognitive decline in Alzheimer's patients is associated with accumulation of choline-containing lipids. One of these lipids, called C16:0 platelet activating factor (PAF), is present at increased levels in brains of Alzheimer's patients. Prolonged exposure to increased levels of C16:0 PAF ultimately kills neuronal cells therefore it is crucial to identify the mechanism(s) that prevent the toxic effects of this lipid. We have exploited the similarities between man and baker's yeast to identify key genes that are essential to prevent the toxic effects of C16:0 PAF. We found that two genes involved in controlling the activity of a key component of lipid signaling, phospholipase D (PLD), are essential for preventing the toxic effects of C16:0 PAF in yeast. Further, we demonstrate that PLD is also important for preventing the toxic effects of C16:0 PAF in mammalian cells as well. Our study suggests that therapeutic strategies aimed at modifying PLD activity may be helpful in reducing the impairments associated with Alzheimer's Disease which we believe are due to changes in normal lipid metabolism.

\*This project was supported by the Insitutte of Aging and Canadian Institutes of Health Research (CIHR) Training Program in Neurodegenerative Lipidomics Postdoctoral Fellowships to MAK and LAS.

### EVIDENTIAL STATISTICAL ANALYSIS FOR NEUROLIPIDOMICS\*

Corey M. Yanofsky<sup>1</sup>, Steffany A.L. Bennett<sup>1</sup>, Daniel Figeys<sup>1</sup>, and David R. Bickel<sup>1</sup>

<sup>1</sup>Ottawa Institute of Systems Biology, Department of Biochemistry, Microbiology, and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, ON K1H 8M5, Canada

In lipidomics experiments, a large number of hypotheses are under simultaneous investigation, and some hypotheses may appear plausible due to unexplained biological variation. Reliable statistical methods are required to determine which hypotheses are worthy of investigation by more focused validation experiments. In the current research, we applied recently developed statistical methods to lipidomics and proteomics data. We analyzed abundance levels of 46 lipids and 914 proteins in the brains of Alzheimer's disease and control individuals. Lipid abundance levels were available for both temporal cortex and hippocampus, enabling comparisons between brain regions; for proteins, only temporal cortex samples were analyzed. Our statistical analysis used a flexible approach suitable to both kinds of data by treating each of the analyte abundance differences as either strictly zero or drawn from a shared distribution, thereby obviating corrections for multiple comparisons. Statistical evidence was reported on an easily interpreted scale as "decisive," "very strong," "strong," "moderate" or "weak," either in favor of or against the hypothesis of differing lipid abundances.

\*This project was supported by the Canada Foundation for Innovation, the Ministry of Research and Innovation of Ontario, and a Canadian Institutes of Health Research (CIHR) Training Program in Neurodegenerative Lipidomics Postdoctoral Fellowship Award to CMY.

## SESSION 3 (CONT)

### MODELING LIPIDOMIC VISUALIZATION IN REPRESENTATIONAL THEORY

Sarah B. Gelbard<sup>3</sup>, Stephen Fai<sup>3</sup>, Steffany A.L. Bennett<sup>1,2</sup>

<sup>1</sup>Neural Regeneration Laboratory, <sup>2</sup>Ottawa Institute of Systems Biology, Department of Biochemistry, Microbiology, and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, ON K1H 8M5, Canada and <sup>3</sup>Carleton Immersive Media Studio, Azrieli School of Architecture and Urbanism, Carleton University, Ottawa ON K1S 5B6, Canada

The technological advances that allow medical researchers both to enter into a scale beyond the capacity of the eye to observe directly, and to create virtual models and visualizations affect how researchers represent what is observed. The representations have, in turn, affected how they conceive, perceive, and interpret that which is observed. Like any methodology, visualization demands a critical consideration of both the applications and the implications of the various forms, processes and techniques, its communicative function, and limitations. An examination of the conceptual framework for the application of representational theories and techniques from architecture to medical research and the historical precedent for this collaboration provides a greater understanding of how the visualization process and the resulting images may facilitate new conceptual insights. This project involves comparative analysis of two visualization projects developed by researchers in the Neurodegenerative Lipidomics program with historic parallels: (1) digital 3D modeling of a mouse brain and the projective reconstruction technique for modeling wax embryos developed by Wilhelm His in the mid-nineteenth century; and (2) visualization of microenvironments and lipid-protein interactions and the representational implications and effects of Robert Hooke's seventeenth-century *Micrographia*.

\*This project was supported by the CIHR Institute of Aging and a Canadian Institutes of Health Research (CIHR) Training Program in Neurodegenerative Lipidomics

### THE LIPIDOMICS PROFILE OF DEPRESSED CAD PATIENTS TREATED WITH OMEGA-3 FAS\*

Allison B. Gold<sup>1,2</sup>, Nathan Herrman<sup>1,2,3</sup>, Steffany A.L. Bennett<sup>4,5</sup>, Krista L. Lanctot<sup>1,2,3,6,7</sup>

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Depression is a common problem associated with Coronary Artery Disease (CAD). About 47% of patients with CAD suffer from major or minor depression within one year following an Acute Coronary Syndrome (ACS). Amongst patients with CAD, major and minor depression significantly increase the risk of death or heart attack. A large proportion of CAD patients with depression do not respond to current treatments. Non-response to treatment is associated with a higher rate of another ACS. Randomized controlled trials (RCTs) in patients with major depression suggest that omega-3 fatty acids ( $\omega$ -3 FAs) may be effective as an add-on to other treatments and may be helpful for persistent depressive symptoms or treatment-resistant depression. The subgroup of patients most likely to benefit has not been defined. Inadequate intake or modification of essential  $\omega$ -3 FAs may predispose patients to depressive disorders. Patients with CAD have been shown to have decreased plasma concentrations of  $\omega$ -3 FAs, however, a recent RCT of omega-3 fatty acid supplementation in CAD patients with depression was negative. A patient's lipid profile may determine the ability of  $\omega$ -3 FAs to reverse depressive symptoms. Leading-edge lipidomic techniques may define an endophenotype of patients who might respond to  $\omega$ -3 FAs.

\*This project was supported by the Ontario Mental Health Foundation and Canadian Institutes of Health Research (CIHR) Training Program in Neurodegenerative Lipidomics Post-doctoral Supplemental Scholarship to ABG.

Project not presented at Neurolipidomics 2010 and will be presented at an alternate sponsored event TBD.

### **SPECIFIC ALTERATIONS OF GLYCEROPHOSPHOCHOLINE SECOND MESSENGERS IN A TRANSGENIC MODEL OF ALZHEIMER DISEASE**

Alexandre P. Blanchard, Leigh Anne Swayne, Camille A. Juzwik, Daniel Figeys, Steffany A.L. Bennett

Neurodegenerative Lipidomics Training Program, Neural Regeneration Laboratory and Ottawa Institute of Systems Biology, Dept of Biochemistry, Microbiology, and Immunology, University of Ottawa, Ottawa ON, Canada

The deficit in brain communication underlying the dementia experienced by Alzheimer disease (AD) patients is attributed to the pathological hallmarks of the disease: senile plaques and neurofibrillary tangles (NFT). Accumulation of toxic A $\beta$  fragments, the principal constituent of the senile plaques, is understood to be the causative factor of AD onset. However, the severity of AD is highly correlated with the rate of NFT. With the support of the CIHR Training Program in Neurodegenerative Lipidomics, we found using an unbiased lipidomics approach that A $\beta$ 42 causes the aberrant intraneuronal accumulation of specific alkylacylglycerophosphocholine metabolites (O-16:0 PAF and its immediate precursor O-16:0 lyso-PAF) that, in turn, signal the hyperphosphorylation of tau in human neurons (Ryan et al., PNAS, 2009). Using the TgCRND8 mouse model, we are currently mapping the glycerophosphocholine second messenger lipidome to identify temporal changes in lipid metabolism and their mechanistic impact upon AD etiology.

\*This project was supported by the CIHR Institute of Aging and a Canadian Institutes of Health Research (CIHR) Training Program in Neurodegenerative Lipidomics

### **HIGH-FAT DIET AND ALTERED CEREBROVASCULAR FUNCTION IN TGCRND8 MICE.\***

Jenna Boulanger<sup>1</sup>, Vian Peshdary<sup>2,3</sup>, Steffany A.L. Bennett<sup>2,3</sup>, and Claude Messier<sup>1</sup>

<sup>1</sup>Department of Psychology, Faculty of Social Sciences, University of Ottawa, Ottawa, ON, K1N 6N5 and <sup>2</sup>Neural Regeneration Laboratory, <sup>3</sup>Ottawa Institute of Systems Biology, Department of Biochemistry, Microbiology, and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, ON K1H 8M5, Canada

Diet and exercise are well-known modulators of cerebral health and function. While the high lipid content and low nutritional value of the typical Western diet represent important risk factors for the development of cardiovascular disease and Alzheimer's disease (AD), regular exercise reduces the incidence of AD and increases the number and diameter of cerebral blood vessels. It is unclear whether this angiogenic effect of exercise is positive in AD, as angiogenic vessels are not as effective as longstanding vessels. A diet rich in lipids also has an angiogenic effect, as hypoperfusion and inflammation are also known to stimulate the formation of new blood vessels. Since more new blood vessels are found in the brain of AD patients, it is important to understand whether angiogenesis contributes to the pathogenesis of AD or is a reactive compensating process that slows down functional impairment. Preliminary results suggest that a high-fat diet, as well as a high-fat diet combined with access to a running wheel, induce neovascularisation in a transgenic mouse model of AD before these mice start showing behavioural deficits. Thus, angiogenesis, in the early stages of disease progression, may have a protective effect on AD.

\*This project was supported by a Natural Sciences and Engineering Council of Canada (NSERC) Graduate Scholarship and by a Canadian Institutes of Health Research (CIHR) CIHR Training Program in Neurodegenerative Lipidomics Supplementary Graduate Scholarship to JB; NSERC funding to CM; and CIHR funding to SALB.

## POSTER SESSION (CONT.)

### DEFINING THE PLATELET-ACTIVATING FACTOR RECEPTOR INTERACTOME\*

Brett M. Hawley<sup>1,2</sup>, Steffany A.L. Bennett<sup>1,2</sup>, Daniel Figeys<sup>1,2</sup>

<sup>1</sup>Ottawa Institute of Systems Biology, <sup>2</sup>Department of Biochemistry, Microbiology, and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, ON K1H 8M5, Canada

In recent years, platelet-activating factors have been studied extensively with regard to neuronal cell death, and survival, and its capability to exert both pro- and anti-apoptotic effects in the central nervous system. PAF's ability to exert anti-apoptotic effects is only possible in the presence of the PAF receptor (PAFR). Because of this ability we would like to determine the mechanism by which PAFR interactors are able to promote neuronal survival or trigger cell death by the defining the PAFR interactome in different lipid environments. In order to define the interactome, we will be using mass spectrometry to identify these interacting proteins. PAFR is cloned into an expression vector containing the FLAG tag, which is transfected into the human cell line, HEK 293T. Following transfection, proteins are purified using FLAG coupled agarose beads, and then they are digested and then analyzed using the LTQ-MS. We hope to identify possible interactors and determine the pathway or mechanism by which neural cells are able to protect themselves from PAF-caused apoptosis.

\*This project was supported by the Insitutte of Aging and Canadian Institutes of Health Research (CIHR) Training Program in Neurodegenerative Lipidomics Graduate Student Award to BMH.

### LYSO-FORM FRAGMENT IONS FACILITATED THE DETERMINATION OF STEREOSPECIFICITY OF DIACYL GLYCEROPHOSPHOLIPIDS\*

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In this work we reported the development of a novel methodology that uses MS<sup>2</sup> recorded on a hybrid quadrupole time-of-flight mass spectrometer to determine the stereospecificity of diacyl glycerophospholipids based on the lyso-form fragment ions, attributed to the neutral loss of fatty acyl moieties. The fragmentation patterns of a variety of diacyl glycerophospholipid standards were first fully examined over a wide range of collision energy. We observed that lyso-form fragment ions corresponding to the neutral loss of fatty acyl moieties attached to the sn2 position as free fatty acids and as ketenes exhibited consistently higher intensity than their counter part ions due to the neutral loss of fatty acyl moieties attached to the sn1 position. We concluded that an empirical fragmentation rule can be used to precisely determine the stereospecificity of diacyl glycerophospholipids, primarily on the basis of relative abundance of the lyso-form fragment ions. Combining the novel methodology reported in this work with the currently widely practiced mass spectrometric techniques such as multiple precursor ion scans, fatty acyl scans, and multidimensional mass spectrometry-based shotgun lipidomics should enable a reliable and convenient platform for comprehensive glycerophospholipid profiling in the context of neurodegeneration using human samples and murine models of disease.

\*This project was supported by the Insitutte of Aging and Canadian Institutes of Health Research (CIHR) Training Program in Neurodegenerative Lipidomics Graduate Student Award to WH

## POSTER SESSION (CONT.)

### PROTEIN INTERACTIONS WITH OLIGODENDROCYTIC CX32 INFLUENCE PRODUCTION AND MAINTENANCE OF THE MYELIN SHEATH\*

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Gap junction channels are composed of connexin proteins, and are important mediators of intercellular signaling. Recently, their rich protein associations have also implicated gap junctions and the connexin family of proteins in many intracellular signaling processes. The purpose of this work was to identify and verify protein interactions with the oligodendrocytic connexin32 (Cx32) that influence generation of the myelin sheath, notably sphingolipid production, and lipid second messenger passage through Cx32 channels that sustain oligodendrocyte survival. I was awarded travel funding to attend an advanced microscopy course at McGill University (Montreal Light Microscopy Course) that provided expert training on the acquisition of laser scanning confocal microscopy images, and advanced image processing and co-localization techniques. This training was essential to the successful verification of protein interactions with Cx32, and this information will be required to assess the impact of Cx32 on oligodendrocytic cell replacement in disease.

\* This project was supported by a travel grant from the CIHR Training Program in Neurodegenerative Lipidomics to SLF.

### EXAMINING THE ROLE OF CX32 IN THE REGULATION OF MYELIN SPECIFIC LIPID LEVELS FOLLOWING SPINAL CORD INJURY\*

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Neurons in the central nervous system (CNS) depend upon oligodendrocytes to communicate. Their ability to propagate an axon potential over long distances requires that their nerve fibers are properly myelinated. Myelin is the lipid-rich sheath produced by oligodendrocytes in the central nervous system (CNS) and Schwann cells in peripheral nervous system (PNS). Spinal cord injury leads to a permanent loss of motor, sensory and autonomic function. Connexin proteins have been shown to propagate death signals between cells, causing neuronal death and demyelination. Dysregulation of connexins may be leading to the damage of healthy neurons and contributing to oligodendrocyte myelin-lipid loss. We will determine whether restoring normal connexin expression will reduce neurodegeneration and rescue demyelination. Modulating endogenous connexin expression may prove useful in influencing secondary cell injury and survival necessary to restore lipid levels, presenting a novel approach to spinal cord repair.

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## POSTER SESSION (CONT.)

### UNCOVERING THE GLYCEROPHOSPHOLIPIDOME IN AN ANIMAL MODEL OF PARKINSON'S DISEASE\*

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Parkinson's disease (PD), a neurological movement disorder affecting 1-2% of the population over 50 in Canada, is characterized by gait and movement defects. This neurodegenerative disorder is typically characterized by the gradual and constant loss of neurons in the substantia nigra and in the striatum, an area of the brain responsible for reward responses. Little is known about disease etiology and current treatments consist of symptom relief. In order to better understand the metabolic components that contribute to PD, we have taken a lipid-targeted approach. As lipid changes and dynamics have been shown to play an important role in neurodegeneration, we sought here to establish whether turnover of small lipid second messengers is altered in PD. To do this in a relevant context, we performed an unbiased tissue-targeted screen for lipid changes in "parkinsonian" mice. In short, we analyzed lipid profiles in two areas of the brain affected in PD in an animal model of the disease. We are currently further exploring these lipid alterations by understanding what role they play in the cell. By taking this approach, and understanding new lipid species involved in the neurodegenerative process, we seek to identify new targets for therapeutic intervention.

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### BIOACTIVE GLYCEROPHOSPHOLIPIDS AND THEIR ROLE IN MODULATING NEURONAL VULNERABILITY FOLLOWING CEREBRAL ISCHEMIA\*

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Stroke is an acute neurodegenerative condition resulting from an obstruction to cerebral blood flow leading to an immediate phase of necrotic neuronal death and a delayed phase of neuronal apoptosis, or "programmed cell death". The necrotic phase occurs rapidly as cells are deprived of the oxygen and glucose they require for energy production. The mechanisms underlying the apoptotic phase, however, are more controversial. Recently, the advent of specialized lipid analyzing techniques has revealed new, highly specialized physiological roles for lipids and their metabolites. Glycerphosphocholine second messengers, including the Platelet-activating factor (PAF) family of alkylacylglycerophosphocholines have been implicated in the development and progression of neurodegenerative conditions, including Alzheimer's disease, HIV dementia, meningitis and more. Here we identify spatial changes in discrete glycerophosphocholine second messenger species in adult mouse brain following middle cerebral artery occlusion, a reproducible rodent model of stroke. These data will provide insight into the role these potent pro-inflammatory lipid mediators play in the progression of stroke injury, and may represent novel targets for therapeutic intervention.

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