# Visions In Pharmacology

Presents

# This Year's Theme:

# Addiction

Distinguished Visiting Lecturer:

**Dr. Edythe London**

Pharmacology & Toxicology
UNIVERSITY OF TORONTO

Pharmacology & Toxicology
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# Visions in Pharmacology 2018

## Research Symposium & Distinguished Lecture

Presented by:

**Department of Pharmacology and Toxicology**
**Faculty of Medicine**
**University of Toronto**

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# Acknowledgments

**This year's Vision of Pharmacology Research Symposium was organized by the members of the 2017-2018 Pharmacology Graduate Students Association (PGSA):**

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**The VIP organizing committee would like to thank the Department of Pharmacology and Toxicology. We would especially like to thank Dr. Ali Salahpour, Dr. Ruth Ross, Dr. Michelle Arnot, Dr. Peter McPherson, Elaine Jack and Phyllis Thawe for their support in organizing this conference. In addition, we would like to thank Dr. Rachel Tyndale for nominating our distinguished lecturer this year.**

# Program Schedule

**9:00 AM – 9:30 AM**
Registration and Poster Setup at Great Hall

**9:30 AM – 9:35 AM**
Opening Remarks, Dr. Ali Salahpour

**9:40 AM – 10:30 AM**
Poster Session I

**10:30 AM – 11:20 AM**
Poster Session II

**11:25 AM – 1:00 PM**
Student Seminars, Hart House at Great Hall

Ahmed El-Boraie **(11:25 AM - 11:45 PM)**
Martino Gabra **(11:50 PM - 12:10 PM)**
Marie N.S. Gendy **(12:15 PM - 12:35 PM)**
Justin Matheson **(12:40 PM - 1:00 PM)**

**1:00 PM – 2:00 PM**
Lunch at Lower Gallery

**2:00 PM – 3:00 PM**
Distinguished Lecture by Dr. Edythe London at Great Hall
*Stimulant Use and Dopamine Receptor Subtypes: Brain Function and Therapeutic Approaches*

**3:00 PM – 3:30 PM**
Awards Presentation and Closing Remarks
Chairs: Lucia Zhang and Celina Liu

**3:30 PM - 5:00 PM**
Reception

# Distinguished Lecture


**Dr. Edythe London** holds the Thomas P. and Katherine K. Pike Chair of Addiction Studies, and is a Professor in the Departments of Psychiatry and Biobehavioral Sciences, and Molecular and Medical Pharmacology at UCLA.

She is a pioneer in the application of brain imaging to the study of addiction. Her laboratory uses a combination of imaging modalities, including PET, activation and resting-state fMRI and structural MRI, to explore the links between dopaminergic signalling and behavioural functions that can influence the course of addiction and recovery.

Edythe London will be giving a talk about her research on the **Stimulant Use and Dopamine Receptor Subtypes: Brain Function and Therapeutic Approaches.**

# Award Descriptions

## The Malle Jumira-Romet Award

**Dr. Malle Jurima-Romet**
**January 8th 1959 - October 3rd 2014**

The Romet Award was created to honour the memory of Dr. Malle Jurima-Romet, an outstanding Ph.D. graduate of our Department who balanced her scientific training with a passion for music and the arts. Following her Ph.D. training in drug metabolism and pharmacogenetics, Malle enjoyed a successful career in research and executive positions with Health Canada, Phoenix International, MDS Pharma Services, and Celerion. She was also a talented administrator, devoting her time and expertise to the Society of Toxicology of Canada for many years. The value of the award is $2,000 and is meant to offset costs associated with extracurricular pursuits in the arts by a graduate student in our Department. Applicants are considered on the basis of academic excellence and extracurricular pursuits in the arts, described and documented in a short essay.

## The Amar Sen Memorial Award for Excellence in Academic Achievement

**Dr. Amar Kumar Sen**
**March 14, 1927 – March 14, 2004**

Dr. Amar K. Sen, Professor Emeritus of the Department of Pharmacology, University of Toronto, was a tremendously respected scientist, teacher, colleague, mentor and friend in the Department from the 1960s until his retirement in the early 1990s, and he continued to maintain regular contact with us since that time. He will be remembered not only for his seminal scientific contributions which include his important studies of the biochemistry and pharmacology of the Na/K-ATPases, but also for his tireless commitment to the Department's mission over the past 40 years, and for his friendship among his colleagues, students and peers throughout the Department, the University and the scientific community.

The Amar Sen Memorial Award is offered by the Department of Pharmacology to a PhD student who graduated in 2017 in recognition of his/her achievements during their time with the department.

# Award Descriptions

## The Fiona Smillie Memorial Award for Departmental Service

**Fiona Smillie**
**October 2, 1945 - December 19, 2009**
Fiona was a greatly respected and much loved member of the administrative staff of the Faculty of Medicine for the past 30 years, of which the last 8 years were spent as the Business Officer and senior administrator within the Department of Pharmacology and Toxicology. Fiona's kind spirit, enthusiasm and empathy made her a cherished colleague and friend of many within the department. In keeping with Fiona's exemplary dedication and service to the department, the Fiona Smillie Memorial Award for Departmental Service is given to a graduate student who graduated in the 2016-2017 academic year and who has significantly contributed to the department by improving experience of graduate studies and/or advancing the interests of graduate students.

## The Visions in Pharmacology Travel Award

To be awarded to the Graduate Student with the most outstanding poster presentation at the annual VIP Research Day. The funds shall be used by the student to cover travel expenses to present their work at a conference or meeting during the 12-month period following the receipt of the award.

Awards will be given to the best Masters and PhD student Poster and best Oral Presentation.

# Award Descriptions

## Dr. Walter Roschlau Memorial Award in Pharmacology

**Dr. Walter Roschlau**

Dr. Walter H.E. Roschlau was a distinguished medical doctor, researcher and professor, whose work included the development of the first high-efficiency artificial kidney in Canada with Canadian surgeon Dr. Gordon Murray, the development of anti-coagulants at the Connaught Laboratories, and research on blood clotting mechanisms and fibrinolysis, joining the Department of Pharmacology at the University of Toronto in 1966. He played a major role in teaching medical, arts & science, dentistry, pharmacy and graduate students, and was co-editor of the textbook Principles of Medical Pharmacology for the 3rd (1980) to the 6th (1998) editions. Walter was a dedicated and thorough teacher, who set high but fair standards for his students, was always available to help them, and earned the gratitude and esteem of many who interacted with him, even years after their graduation.

The Dr. Walter Roschlau Memorial Award was established to recognize his dedication to teaching by honouring students with the best academic records in the graduating class of the Arts & Science Specialist programs in Pharmacology & Toxicology.

## W. Mac Burnham Award

This award is presented by the Department for Outstanding Achievement in a Pharmacology or Biomedical Toxicology Major Program. This award recognizes the highest academic achievement in our courses by a student graduating from one of our Major programs. This is the inaugural year of the award and highlights the excellent and long-standing nature of Professor W. Mac Burnham's contributions to The Department of Pharmacology and Toxicology's Educational Programs.

## Dezso Kadar Student Achievement Award

This award is presented by the Division of Teaching Laboratories and recognizes the top student within the Undergraduate Laboratory course.

## Evaluation of a Weighted Genetic Risk Score for the Prediction of Biomarkers of CYP2A6 Activity

Ahmed El-Boraie(1), Taraneh Taghavi(1), Meghan J. Chenoweth(1), Koya Fukunaga (2), Taisei Mushiroda (2), Michiaki Kubo (2), Caryn Lerman(3), Nikki Nollen(4), Neal L. Benowitz(5), & Rachel F. Tyndale(1)(6)

(1) Department of Pharmacology & Toxicology, University of Toronto; (2) Center for Integrative Medical Sciences, RIKEN; (3) Department of Psychiatry and Abramson Cancer Center, University of Pennsylvania; (4) Department of Preventive Medicine and Public Health, University of Kansas; (5) Departments of Medicine and Biopharmaceutical Sciences, Division of Clinical Pharmacology and Experimental Therapeutics, Medical Services and Center for Tobacco Control Research and Education, University of California; (6) Addictions Division, Centre for Addiction and Mental Health and Division of Brain and Therapeutics, Department of Psychiatry, University of Toronto

**Background and Aims:** The nicotine metabolite ratio (NMR; 3-hydroxycotinine/cotinine) is an index of CYP2A6 activity; CYP2A6 is responsible for nicotine's metabolic inactivation and variation in the NMR/CYP2A6 is associated with several smoking behaviours. Our aim was to integrate established alleles and novel GWAS signals to create a weighted genetic risk score (wGRS) for the CYP2A6 gene for European-ancestry populations. The wGRS was compared to a previous CYP2A6 gene scoring approach designed for an alternative phenotype (C2/N2; cotinine-d2/cotinine-d2+nicotine-d2).

**Methods:** CYP2A6 genotypes and the NMR were assessed in European-ancestry participants. The wGRS training set included N = 933 smokers recruited to the Pharmacogenetics of Nicotine Addiction and Treatment clinical trial [NCT01314001]. The replication cohort included N = 196 smokers recruited to the Quit 2 Live clinical trial [NCT01836276].

**Findings:** In both the training and replication sets, the wGRS, which included seven CYP2A6 variants, explained 33.5% of the variance in NMR, an improvement to previous CYP2A6 gene scoring approaches. The wGRS demonstrated a significant ability (AUC=0.79) to discriminate between NMR slow and normal metabolizers, and it replicated previous NMR-stratified cessation outcomes showing unique treatment outcomes between metabolizer groups. We also demonstrate that rare CYP2A6 gene variants identified by sequencing may contribute a source to the unexplained variation in the NMR.

## Evaluating MicroRNA Mediated Chemo-resistance in Acute Myeloid Leukemia (AML)

Martino Gabra(1), Leonardo Salmena(1,2)
(1) Department of Pharmacology, U. of T., 1 King's College Cir., Toronto, M5S 1A8; (2) Princess Margaret Cancer Centre, 610 University Ave, Toronto, ON M5G 2M9

AML describes blood malignancies that arise due to genetic alterations in hematopoietic cells. Despite advances in our understanding of AML, long-term survival remains poor and daunorubicin (DNR) & cytarabine (ARA-C) resistance remains to be a major hurdle. microRNAs (miRNA) are small non-coding RNAs with gene regulatory functions implicated in AML and may be therapeutic targets. Despite this, little is known about a role for miRNAs in AML drug resistance. I hypothesize that alterations in miRNA expression can promote drug resistance which will be investigated in the following aims:

1. Screening of Resistant AML cell lines. Using a standardized dosing protocol, I generated DNR or Ara-C resistant progeny of HL60, OCI-AML2, OCI-AML3, U 937 and MV4-11 AML cell lines. I will then use miR-seq to identify miRNA dysregulations in chemoresistance. To date, I have created all resistant cell lines and collected samples to examine miRNA changes.

2. miRNA-CRISPR library screen. I created a CRISPR knockout library that targets 1865 miRNAs and screened for resistance promoting genes by knocking-in the library in treatment naïve cells before dosing as in aim 1. I present data for 3 cell lines that demonstrate that miRNA such as miR-376a and -17, known oncomiRs, are maintained while other miRNA such as mIR-153 and -122 are lost.

3. Validation of miRNAs of resistance. I will validate the findings by knocking-down or overexpressing the miRNA to examine drug resistance effects. Altogether, this project evaluates miRNAs as genes of drug resistance in AML and in turn contributes to miRNA-based therapies.

## Lab-based and Web-based Randomized Clinical Trials testing novel treatments for smoking cessation and alcohol-related sleeping problems

Marie N.S. Gendy(1,5), Patricia Di Ciano(1), William J. Kowalczyk(8), Sean P. Barrett(9), Tony P. George (3,6,7,10), Stephen Heishman(8), Bernard Le Foll(1-7)

(1) Translational Addiction Research Laboratory, Centre for Addiction and Mental Health, Toronto, Ontario, Canada; (2) Alcohol Research and Treatment Clinic, Addiction Medicine Services, Ambulatory Care and Structured Treatments, Centre for Addiction and Mental Health, Toronto, Ontario, Canada; (3) Addictions Division, Campbell Family Mental Health Research Institute, Centre for Addiction and Mental Health, Toronto, Ontario, Canada; (4) Department of Family and Community Medicine, University of Toronto, Toronto, Ontario, Canada; (5) Department of Pharmacology, University of Toronto, Toronto, Ontario, Canada; (6) Department of Psychiatry, Division of Brain and Therapeutics, University of Toronto, Toronto, Ontario, Canada; (7) Institute of Medical Sciences, University of Toronto, Toronto, Ontario, Canada; (8) Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, Baltimore, MD, USA; (9) Department of Psychology & Neuroscience, Dalhousie University, Halifax, Nova Scotia, Canada; (10) Biobehavioural Addictions and Concurrent Disorders Research Laboratory (BACDRL) Centre for Addiction and Mental Health (CAMH), Toronto, Ontario, Canada

In Canada, 15% are Tobacco smokers and the available treatment success is limited. Also, substance use disorder is found in 21 % of Canadians and alcohol comes 1st substance to be used. Over 70 % of alcohol use disorder patients have alcohol-related sleeping problems and the available treatment have major health risks. Therefore, randomized clinical trials testing novel treatments are needed.

**RCT 1**: Testing the PPAR hypothesis of tobacco use disorder in humans: A randomized trial of the impact of gemfibrozil in smokers.

Previous pre-clinical studies demonstrated a promising role of alpha-type peroxisome proliferator-activated receptors (PPARα) agonists in decreasing nicotine self-administration and nicotine-seeking behavior in animals. Our goal was to investigate the potential of gemfibrozil, a PPARα agonist, on reducing tobacco smoking in humans.

**Methods**: This was a double-blind, placebo-controlled, cross-over study evaluating the effects of gemfibrozil (1200 mg/day) on smoking in 27 treatment-seeking smokers. The study had two 2-week phases separated by a washout period of at least 1 week. In each phase and after 1 week on medication, participants underwent a lab session where cue reactivity and forced choice paradigms were conducted. Physiological responses and self-reported craving were monitored during the presentation of smoking and neutral cues. In addition, two types of cigarettes were used in the forced choice paradigms: the Nicotinized cigarettes (Nic) and the Denicotinized cigarettes (Denic). The number of quit days was calculated during the two quit attempts weeks (one while taking gemfibrozil and one while taking placebo) of the study.

**Results**: There were no significant differences between gemfibrozil and placebo in the percentage of choice of Nic cigarettes, the cue-reactivity (both physiological and subjective measures), or in the number of days of abstinence over placebo.

**Conclusions**: Although preclinical studies with PPAR α agonists showed promising results regarding smoking cessation, this preliminary study did not demonstrate positive effect of gemfibrozil on tobacco use and cessation indices.

## Comparing the Acute Cognitive effects of Cannabis and Alcohol: An Exploratory Analysis

Matheson J (1,2), Mann RE (1,2), Le Foll B (1,2), Wickens C (1,2), & Brands B (1,2).
(1) University of Toronto, Department of Pharmacology and Toxicology (2) Centre for Addiction and Mental Health

**Aim**: To examine differences in the acute effects of cannabis and alcohol on memory, attention, and psychomotor function.

**Methods**: Data came from two placebo-controlled, double-blind, randomized, parallel-design clinical trials assessing the effects of smoked cannabis (Study 1; 12.5% THC) or alcohol (Study 2; target 0.08% breath alcohol content) on simulated driving and cognition. Participants (19-25 years) used cannabis 1-4 days/week (62 active, 31 placebo) or experienced a binge drinking episode in the past 6 months (20 active, 10 placebo). A battery of four tasks (verbal memory, sustained attention, processing speed, dexterity) was administered before and 60 minutes after drug exposure. Difference scores were calculated for each outcome, and differences within each study (active vs. placebo) were analyzed using independent samples t-tests, followed by nested one-way ANOVA to compare alcohol and cannabis effects.

**Results**: Cannabis (vs. placebo) significantly impaired verbal memory alone, whereas alcohol (vs. placebo) impaired performance in all four tasks. A priori contrasts indicated that alcohol had a stronger effect than cannabis on aspects of verbal memory ($p<0.001$), sustained attention ($p=0.026$), processing speed ($p=0.033$), and dexterity ($p=0.032$).

**Conclusion**: Cannabis seemed to have a specific effect on verbal memory, while alcohol had a larger and more general effect across the four domains assessed.

## Behavioural Pharmacology

### Sex Differences in Brain Oxycodone Metabolism and Analgesia

Nicole Arguelles (1,2), Fariba Baghai Wadji (1,2) , Maria Novalen (2), Sharon Miksys (1,2), Rachel F Tyndale (1,2,3)

(1) Campbell Family Mental Health Research Institute, CAMH; (2) Departments of Pharmacology & Toxicology, University of Toronto; (3) Department of Psychiatry, University of Toronto

**Background**: Sex differences are recognized in opioid analgesia, abuse, and dependence in animals and humans. The underlying mechanism is unclear but may involve differences in pharmacokinetics and/or pharmacodynamics. Numerous opioids are substrates of CYP2D, and variation in this enzyme's activity alters opioid response. We previously showed that inhibiting brain CYP2D, leaving hepatic CYP2D unchanged, in male rats increased oxycodone analgesia and oxycodone brain levels, measured by in vivo microdialysis, but not plasma levels; together, this suggests a role for brain CYP2D in oxycodone-induced analgesia. Our pilot data indicate that female rats have lower ex vivo brain CYP2D activity than males, suggesting a possible role in sex differences in opioid response. We hypothesize that females will have higher brain oxycodone levels, and thus, will experience greater analgesia in the dose-response compared to their male-counterpart.

**Methods**: Male and female rats, at different estrous cycle stages, were administered varying amounts of oxycodone orally by gavage. Oxycodone-induced analgesia was measured using tail-flick latency. Brain drug levels were measured over time through in vivo brain microdialysis. Data were analyzed by one-way or two-way ANOVA, with post hoc analysis for multiple comparisons.

**Results**: Female rats experienced greater peak analgesia and overall analgesia (AUC(0-120)), compared to male rats at 7.5, 10, and 12.5 mg/kg oral oxycodone. Consistent with this, female rats had higher brain oxycodone levels than male rats. These differences were greatest in females in diestrus phase, both in oxycodone-induced analgesia and brain oxycodone levels. For example, at 10 mg/kg oxycodone, females in diestrus phase had a 2.0-times higher peak and 2.9-times higher AUC(0-120) analgesia relative to males, while females in estrus phase had a 1.2-times higher peak and 1.5-times higher AUC(0-120) analgesia relative to males. These were also observed at the higher and lower dose of oxycodone. We are currently characterizing the relative contribution of CYP2D to these observed sex differences.

**Conclusion**: These data, consistent with our pilot ex vivo data, suggest that females may have lower brain CYP2D activity which may vary with estrous cycle. Thus, females appear to metabolize less oxycodone in brain resulting in higher brain oxycodone levels and greater analgesia than males, which is even more pronounced during diestrus.

### Stress-induced Astroglial Vulnerability Correlates with Specific Synaptic Dysfunctions and Depressive-like Phenotypes.

Dipashree Chatterjee (1,2), Thomas Prevot (1,2), Patricia Gali (2), Etienne Sibille (1,2), and Mounira Banasr (1,2)
(1) University of Toronto; (2) Centre for Addiction & Mental Health

Cellular deficits revolving around pathological tripartite synapses in the prefrontal cortex (PFC) have been implicated in major depressive disorder (MDD). Indeed, GABAergic, glutamatergic, and astroglial dysfunctions have consistently been observed in MDD brains and rodent models of stress. However, these changes are usually studied independently and often at endpoints. Here, we aim to characterize the kinetics of cell-specific changes in PFC and correlate them with depressive behaviours, using mice subjected to chronic restraint stress (CRS) 1h, twice a day, for 1-5 weeks. CRS effects on anxiety (response to a 1h spotlight challenge), anhedonia (sucrose consumption reduction), and PFC GABAergic, astroglial, and synaptic alterations (western blot analysis) were measured at multiple time points. We found that anxiety behaviour emerged immediately, and anhedonia after week 3 of CRS. Reduced GABAergic (week 1) and astrocytic (week 3) marker expression paralleled this time course, but synaptic changes were only present after week 4. Correlation analysis showed GABAergic changes linked with anxiety, synaptic changes with anhedonia, whereas astrocytic changes correlated with both anhedonia and anxiety deficits. Altogether, our results suggest that chronic stress induces time- and cell-specific alterations that govern the emergence of specific phenotypes. This work highlights a potential role of astrocyte dysfunction as a turning-point in stress response associated with MDD.

### Sex-specific Effects of Social Isolation on Dorsal Raphe Serotonin Neurons and Behaviour

Katheron Intson(1), David K. Oliver(2), Sanghavy Sivakumaran(2), Saige K. Power(2), Derya Sargin(5,6), Amy J. Ramsey(1,2), Evelyn K. Lambe(2,3,4)
1. Department of Pharmacology & Toxicology, University of Toronto; 2. Department of Physiology, University of Toronto; 3. Department of Psychiatry, University of Toronto; 4. Department of Obstetrics & Gynaecology, University of Toronto;5. Department of Physiology & Pharmacology, University of Calgary; 6. Hotchkiss Brain Institute, University of Calgary

Early social isolation is a well-validated stressor linked to the induction of depression and anxiety in adulthood. In biomedical research, isolation following weaning in animals has persisted as an environmental model of those mood disorders. Previously, we characterized the behavioural profile of male mice socially isolated at weaning, and reported electrophysiological findings that their raphe neuron excitability is decreased (Sargin et al., Elife, 2016). Here, we investigated whether these findings were also true in female mice. In male socially-isolated mice, we found behavioural changes on tests relevant to a depressive-like state. By contrast, we observed either no change or opposite change in the female groups. We also found that dorsal raphe serotonin neurons in the socially-isolated females are more excitable than those in group-housed females, contrasting with our previous findings in male animals. Of note, dorsal raphe neurons from group-housed female mice had an increased propensity to enter depolarization block upon stronger stimulation. Ongoing experiments are

examining a number of cell and circuit factors that may contribute to these observations. Based on our previous findings, we are particularly interested in the functional role of SK2 and SK3 channels by sex in the different housing conditions. These results highlight likely sex-specificity in the effects of chronic isolation on behaviour, and within the serotonergic system.

### Brain cytochrome P450 2D6 (CYP2D6) is Neuroprotective in a Harmine Model of Neurotoxicity.

Marlaina R. Stocco(1)(2), Fariba Baghai-Wadji(1)(2), Sharon Miksys(1)(2), Edgor C. Tolledo(1)(2), Bin Zhao(1)(2), Rachel F. Tyndale(1)(2), Frank J. Gonzalez(3)

(1) Pharmacology and Toxicology Department, University of Toronto, Ontario; (2) Campbell Family Mental Health Research Institute, CAMH, Toronto, Ontario; (3) National Cancer Institute, NCI, Bethesda, Maryland

**Rationale**: Cytochrome P450 2D enzymes (CYP2D) metabolically inactivate neurotoxins, including harmine. Variation in brain CYP2D metabolism affects CNS drug levels, which may alter susceptibility to neurotoxicity caused by drugs (e.g. haloperidol) and toxins (e.g. pesticides). In vivo, propranolol (PRL) is a mechanism-based inhibitor of the human CYP2D6 enzyme, but not of mouse CYP2D isoforms. A 24 hr intracerebroventricular (ICV) pretreatment with PRL inhibits local brain (but not liver) human CYP2D6 expressed in humanized transgenic mice (Tg-2D6), without inhibiting the mouse CYP2Ds. 24 hr ICV PRL pretreatment has no effect on brain or liver mouse CYP2D activity in C57BL/6 wild type mice (WT). We hypothesized that inhibiting human CYP2D6 in the brain of Tg-2D6 in vivo with ICV PRL pretreatment would exacerbate measures of harmine neurotoxicity: hypothermia and tremor. We expect no effect of ICV PRL pretreatment in WT, used here as a control comparison group.
**Methods**: Tg-2D6 and WT mice were pretreated with 80 µg ICV PRL (or cyclodextrin vehicle) 24 hrs before ex vivo CYP2D activity was assessed to confirm that ICV PRL inhibited brain, but not liver, human CYP2D6 in Tg-2D6, and had no effect in WT. ICV pretreatment was administered 24 hrs before a 7.5 mg/kg IP injection of harmine; order was randomized and conditions were crossed over following a 7 day washout period (n=25 Tg-2D6; n=24 WT). Core body temperature and tremor were assessed for 90 mins.
**Results**: ICV PRL reduced ex vivo CYP2D velocity in Tg-2D6 brain (unpaired t-test; $p=0.057$), but not in liver; there was no change in ex vivo CYP2D velocity in brains or livers of WT. ICV PRL exacerbated hypothermia in Tg-2D6, but not in WT (Repeated Measures ANOVA; $p<0.0001$). There was no effect of ICV PRL on tremor in Tg-2D6 or WT. Tg-2D6 display less severe hypothermia and tremor than WT (RM ANOVAs; $p<0.01$), as expected due to their faster CYP2D metabolism.
**Conclusion**: The severity of harmine-induced hypothermia, but not tremor, was exacerbated by inhibiting human CYP2D6 metabolism of harmine in the Tg-2D6 brain, suggesting a significant role of brain CYP2D6 metabolism in neurotoxicity. Overall, these findings implicate brain CYP2D6 as a protective factor in neurotoxic responses.

### A Mouse Model for Investigating Human Brain CYP2D6

Edgor C Tolledo [1], Marlaina Stocco [1], Sharon Miksys [1], Frank Gonzalez [2], Fariba B Wadji [1], Bin Zhao [1], Rachel F Tyndale [1]

[1] Department of Pharmacology and Toxicology, Department of Psychiatry, University of Toronto & Campbell Family Mental Health Research Institute, CAMH, ON, Canada; [2] Laboratory of Metabolism, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA

**Background**: Genetically polymorphic human CYP2D6 metabolizes drugs, neurotransmitters, and neurotoxins. In addition to genetic variation, brain CYP2D is inducible, and brain CYP2D metabolism impacts response to centrally acting substrates. Injecting propranolol, a rat and human CYP2D mechanism-based inhibitor, directly into rat cerebral ventricles (icv), selectively inhibited brain, but not hepatic, CYP2D. Brain, but not plasma, drug levels were altered and behavioural-drug responses were changed. We have established a paradigm to investigate the role of human brain CYP2D6 using transgenic-CYP2D6 mice (Tg), which express both the mouse CYP2D and human CYP2D6 genes.

**Methods**: (1) Propranolol inhibition of in vitro CYP2D-mediated O-demethylation of dextromethorphan, a CYP2D-selective probe drug, to dextrorphan was investigated using C57BL/6 wild-type (WT) and Tg liver microsomes. (2) Propranolol inhibition of in vivo brain dextromethorphan O-demethylation was investigated by giving WT and Tg mice propranolol icv pre-treatment (80 µg) 24-hours prior to intraperitoneal injection of dextromethorphan (30 mg/kg). Dextromethorphan and dextrorphan levels were assessed ex vivo in brain tissue and plasma, and dextromethorphan O-demethylation was assessed in vitro in brain membranes and liver microsomes.

**Results**: (1) In vitro, propranolol inhibited dextromethorphan O-demethylation in WT and Tg ($IC50_{,WT}$=0.081 µM, $IC50_{,Tg}$=0.393 µM) liver microsomes, and inhibition was further increased by pre-incubation with propranolol and NADPH ($IC50_{,WT}$=0.007 µM and $IC50_{,Tg}$=0.059 µM), suggesting mechanism-based inhibition in vitro. This propranolol inhibition was dose- and time-dependent for WT ($K_{I,WT}$ = 6.1 nM and $k_{inactivation,WT}$ = 0.20 min-1) and for Tg ($K_{I,Tg}$ = 3.9 nM and $k_{inactivation,Tg}$ = 0.10 min-1). (2) In vivo in Tg given propranolol icv 24-hr pre-treatment, compared to Tg given vehicle pre-treatment, the brain dextrorphan/dextromethorphan ratio was decreased by 27% ($p=0.053$) and in vitro brain dextromethorphan O-demethylation was decreased by 33% ($p<0.05$); neither the brain dextrorphan/dextromethorphan ratio nor in vitro dextromethorphan O-demethylation were altered in WT (unpaired two-tailed t-test, $p>0.1$). In addition, giving propranolol icv 24-hr pre-treatment to either WT or Tg altered neither plasma dextrorphan/dextromethorphan levels nor in vitro hepatic dextromethorphan O-demethylation.

**Conclusion**: In vitro, propranolol is a mechanism-based inhibitor of both WT and Tg liver microsomes, but in vivo, icv propranolol is a mechanism-based inhibitor of only Tg brain CYP2D, and not WT brain CYP2D; this suggests that in vivo, propranolol inhibits human brain CYP2D6 but not mouse brain CYP2Ds. This provides a novel approach to study the potential role of human brain CYP2D6 in the metabolism of centrally-acting substrates and behavioural outcomes in vivo.

### Measuring Changes in Resting-state Functional Connectivity before and after Smoking Cessation Treatment

Paul Wannas, BSc*, Nancy Lobaugh, PhD, Peter Selby, MBBS, Laurie Zawertailo, PhD.
University of Toronto, Centre for Addiction and Mental Health

**Background**: Resting state functional connectivity (rsFC) has been shown to correlate with duration of smoking history and underlies persistent smoking in dependent individuals. Smokers have altered rsFC within the salience (SN), default mode (DMN), and executive control networks (ECN); increased SN-DMN network strength has been associated with smoking abstinence and may be reversed in satiety. Although the strength of these functional circuits at baseline is associated with quit outcomes during smoking cessation attempts, the influence of smoking cessation treatment and long-term abstinence on these circuits remains unclear. We hypothesise that smoking cessation treatment will result in reduced SN-DMN connectivity and increased SN-ECN connectivity.
**Methods**: Smokers enrolled in a 12-week NRT treatment study underwent 3 fMRI scans following overnight abstinence: at baseline, end of treatment, and 6-month follow-up. Participants completed an rsFC paradigm during which they were instructed to focus their gaze on a rest cross while allowing their mind to wander freely.
**Results**: 23 baseline, 15 end-of-treatment, and 14 follow-up scans were completed. We will present preliminary results of rsFC analyses exploring the influence of smoking cessation treatment with NRT and quit status on functional network strength at end of treatment.
**Conclusions**: Changes in functional connectivity associated with long-term abstinence will help to elucidate the neural mechanisms of addiction recovery.

## Clinical Pharmacology

### Investigating Whether A Dysfunctional Glutathione Antioxidant System is Associated with Cognitive Impairment in Coronary Artery Disease Patients

Mehnaz Ahmed, HBSc (1,2); Dr. Krista Lanctot, PhD (1, 2); Dr. Nathan Herrmann, MD (1, 2)
1: University of Toronto; 2: Sunnybrook Research Institute

Oxidative stress products accumulate from prodromal to more severe dementia states, representing a common pathological characteristic of neurodegenerative diseases, and contribute to symptomatic declines in cognitive performance. The glutathione antioxidant system counteracts the formation of oxidative stress products in the body. In this secondary analysis of the Ceramides and CAROTID studies, we are investigating whether the glutathione antioxidant system may be impaired in patients with a prodromal condition for vascular dementia, known as Vascular cognitive impairment-no dementia (VCIND). Coronary artery disease (CAD) patients (age = 62.0 ± 7.1, 80% male, MMSE = 28.76 ± 1.2) with probable VCIND (n = 50, z-scores < -1 on assessments of executive function and/or verbal memory) were matched to CAD patients who did not meet the criteria for VCIND (n=50) on the basis of age, gender, BMI, and smoking history. Plasma samples were collected at baseline. Executive function was assessed using the Trails B, Animal naming and FAS verbal fluency tests at

baseline. Verbal memory was assessed using the California Verbal Learning Test-II at baseline. Markers of the antioxidant system: glutathione peroxidase, glutathione reductase, and glutathione S-transferase will be measured using Western Blot analysis. It is hypothesized that baseline levels of these markers will be lower in patients with probable VCIND, compared to control patients with coronary artery disease who did not meet the criteria for VCIND. Moreover, lower baseline antioxidant marker levels are expected to associate with lower executive function and verbal memory scores. Results consistent with this hypothesis would suggest that a dysfunctional glutathione antioxidant system is a potential etiological factor for the decline in cognitive performance observed in these patients.

### The Role of the Dopamine D3 Receptor in Alcohol Use Disorder

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**Background**: While animal models have implicated the dopamine D3 receptor (D3R) in alcohol use, human research provides limited understanding on the topic. This project aims to expand on previous research by exploring D3R in alcohol use disorder (AUD) subjects.
**Objectives**: (1) to examine D3R levels in AUD subjects compared to controls; and (2) to explore how craving and alcohol consumption relate to D3R levels. We hypothesize an upregulation of D3R in AUD subjects, and a positive association between D3R levels and our behavioral measures.
**Methods**: D3R levels in AUD subjects (n=10) (and healthy controls, n=18) were estimated by Positron Emission Tomography (PET) using the D3R preferring radiotracer, [11C]-(+)-PHNO. AUD subject's D3R levels were then correlated with measures of craving (assessed by a cue-exposure paradigm) and alcohol consumption (assessed by computer-assisted intravenous alcohol self-administration under a progressive ratio schedule).
**Results**: Preliminary data show no differences in [11C]-(+)-PHNO binding between groups. While analysis in the AUD group revealed no relationship between self-administration peak blood alcohol concentration and [11C]-(+)-PHNO binding, there was a positive association between craving score increases and binding in several brain regions (e.g., globus pallidus, $r=0.74$, $p<0.05$; dorsal striatum, $r=0.89$, $p<0.001$).
**Conclusions**: While early data show no differences in D3R binding between groups, they do suggest a role of D2/3R in alcohol craving.

### Circulating Osteocalcin and Verbal Memory Performance in People with Type 2 Diabetes Mellitus

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**Background**: Osteocalcin circulates in carboxylated (cOCN) and undercarboxylated (ucOCN) forms, and ucOCN is understood to have widespread neuroendocrine effects. In type 2 diabetes mellitus (T2DM), circulating ucOCN concentrations are lower, and cognitive decline is accelerated. We aim to determine whether ucOCN is associated with verbal memory performance in people with T2DM.
**Methods**: Fasting serum concentrations of cOC and ucOCN were assayed using isoform specific ELISAs. Verbal memory performance was assessed using the California Verbal Learning Test, 2nd Ed (CVLT-II), from which a composite Z-score was calculated from verbal learning, short-delayed free recall and long-delayed free recall.
**Results**: In 30 people with T2DM (age 63.3±8.9, 60% women, HbA1c 7.64±0.01%, duration of diabetes 8.2±8.6 years), ucOCN (β=0.423, p=0.019), but not cOCN (β=0.052, p=0.789) or total osteocalcin (β=0.193, p=0.319), was associated with memory performance, in models controlling for age and gender. ucOCN was associated with fasting insulin concentrations (β=0.454, p=0.021); however, fasting insulin was not significantly associated with verbal memory performance (β=0.278, p=0.181).
**Conclusions**: The results suggest the possible relevance of a bone-derived neuroendocrine mediator to memory performance in people with T2DM.

### Respiratory Illness (RI) And Respiratory Syncytial Virus (RSV)-related Hospitalization (RSVH) in Infants with Congenital Diaphragmatic Hernia (CDH) in the CARESS Registry (2005-2017)

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Infants with congenital diaphragmatic hernia (CDH) may experience an increased risk of severe respiratory infection (RI) and respiratory syncytial virus (RSV) infection because of co-existing anomalies impacting pulmonary function. This study compared the hazards for RI hospitalization (RIH) and RSVH in CDH infants versus: 1) non-risk infants (NR; those received RSV prophylaxis as part of multiple births) and 2) infants prophylaxed for standard indications (SI; prematurity, chronic lung disease or significant congenital heart disease) in the Canadian RSV Evaluation Study of Palivizumab (CARESS), a registry of infants who received of palivizumab. Cox proportional hazards analyses compared RIH and RSVH risks across groups, adjusted for potential confounders. 21,053 of 25,003 infants in the CARESS were included (163 CDH, 339 NR, and 20,551 SI). Crude RIH rates were 8.6%, 2.4%, 6.2% for CDH, NR, and SI, respectively. CDH infants had a significantly increased RIH risk versus NR (HR=2.7, p=0.04) but not SI (HR= 1.0, p=0.95) infants. Crude RSVH rates were: 0.6% (CDH), 0.3% (NR), and 1.3% (SI), with no significant difference in RSVH risk between CDH infants and the other two groups (NR: p=0.94, SI: p=0.93). Similar hazards for RSVH between CDH, NR, and SI groups suggests that CDH infants may benefit from palivizumab in reducing RSVH during the RSV season.

Clinical Trial Registration: ClinicalTrials.gov NCT00420966

### Sex Differences in the Effects of Cannabis on Simulated Driving Performance

A Fares, RE Mann, B Le Foll, C Wickens, B Brands

**Aim**: To assess sex differences in driving simulator performance under the influence of cannabis.
**Methods**: Young cannabis smoking (1-4 days/week) adults (19-25 years old) were randomized to smoke an active or placebo cannabis cigarette. Blood samples, to measure THC levels, and driving occurred at baseline and after cannabis smoking. Main outcome variables included mean speed and standard deviation of lateral position for the overall scenario and on a straightaway. One of two driving assessments was conducted under conditions of divided attention. Difference scores and two-way ANOVAs were conducted to evaluate if cannabis led to sex differences in driving behavior.
**Results**: At the time of driving the males in the active group had a mean whole-blood THC concentration twice as high as females (10.6 vs. 4.8 ng/mL; $t(59)=2.4$, $p=0.018$). For mean speed on a straightaway under divided-attention, there was a significant main effect of condition [$F(1,87)=7.385$, $p=0.008$] and of sex [$F(1,87)=7.289$, $p=0.008$]. There was also a main effect of condition for overall mean speed under divided-attention [$F(1,87)=10.013$, $p=0.002$]. There were no significant sex by drug condition interactions ($p>0.1$).
**Conclusion**: No sex differences were apparent in simulated driving under the influence of cannabis, despite males achieving a significantly higher level blood THC concentration, suggesting that impairment may emerge in female drivers at lower blood concentrations of THC.

### The Incidence and Risk Factors for Cardiovascular Disease in Kidney Transplant Recipients with Low Bone Mineral Density

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The association between bone mineral density (BMD) and cardiovascular disease (CVD) in kidney transplant recipients (KTR) is unclear. This study investigated if low BMD was associated with CVD, and elucidate risk factors. In a retrospective cohort study 888 first-time KTRs from January 1, 2000 – December 31, 2012 and had a BMD test one-year post-transplant were followed until December 31, 2015. T-scores, Major Adverse Cardiac Events (MACEs), total graft failure (TGF), death-censored graft failure (DCGF) and death with graft function (DWGF) was obtained from medical records. Cox models, Kaplan-Meier curves and restricted cubic splines were used to analyze data trends. BMD was not associated with MACE, but history of diabetes or MACE before one-year post-transplant increased risk of MACE after adjustments. Higher risk of TGF was associated with lower eGFR ($p<0.001$), lumbar spine t-scores ($p=0.03$), Tacrolimus prescription at discharge ($p=0.01$), older age, and history of MACE ($p=0.04$). Also, higher risk of DCGF was associated with lower eGFR ($p<0.001$), non-white race ($p\leq0.001$), no induction ($p=0.05$), Tacrolimus prescription at discharge ($p=0.01$), younger age ($p=0.01$), and

history of MACE (p=0.01-0.04). Lastly, higher risk of DWGF was associated with lower lumbar spine t-scores (p=0.05), and older age (p<0.001). BMD was not associated with CVD however, lumbar spine t-scores was significantly associated with TGF and DWGF. Therefore, BMD may be prognostic of poor graft outcomes.

### The Effect of Baseline Depressive Symptoms on Smoking Cessation Outcomes in Daily Smokers Treated with Bupropion or Varenicline: A Retrospective Analysis of MATCH data

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**Background**: Results from the Medication Aids for Tobacco Cessation and Health (MATCH) study suggest that Varenicline is a more effective smoking cessation aid than Bupropion at end of treatment (12 weeks) and 6 months, however, it is unclear whether these medications are as effective for individuals experiencing depressive symptoms (DS) at the start of their quit attempt. Bupropion, which is also used to treat depression, may be equally as effective as Varenicline in this sub-population. We hypothesize that MATCH study participants with baseline (BL) DS will have significantly lower quit rates compared to MATCH study participants with no BL DS and that these outcomes will not significantly differ between medication groups.
**METHODS**: MATCH study participants that received medication were grouped by total PHQ-2 score at BL (No DS at BL: PHQ-2 = 0; DS at BL: PHQ-2 >0). Associations between cessation outcomes, PHQ-2 group and medication assignment were analyzed using chi-square tests.
**RESULTS**: Individuals with DS at BL (n=684) were found to have significantly lower quit rates compared to individuals with no DS at BL (n=279) at 12 weeks (p>0.05). In addition, quit rates significantly differed between assigned medication groups.
**CONCLUSIONS**: Smokers with DS at the start of a quit attempt may be less likely to succeed than smokers with no DS at the start of a quit attempt. Further research is needed to determine the most effective smoking cessation treatments for this sub-population.

### Establishment of The Canada Mitochondria Network (mitoNET) to Transform Health through Understanding the Mitochondria

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Mitochondria are organelles responsible for energy production. Bioenergetic homeostasis is pivotal for cellular health and the disruption of this system via mitochondrial dysfunction has primarily been associated with mitochondrial disease. In addition, mitochondria may play a role in numerous complex human diseases such as neuropsychiatric disorder, cardiovascular disease, metabolic disorder, cancer, and aging. Despite their importance, the role and extent to which the mitochondria contribute in each illness is poorly understood. Better understanding of the mitochondria could transform the ability to prevent, detect, diagnose,

and treat common diseases.

The Canada Mitochondrial Network (mitoNET) was recently established to unite patients, researchers, and clinicians of different fields to advance comprehensive and interdisciplinary research. Collaboration is prioritized to address the complex nature of the mitochondria through multiple perspectives and lead in breakthroughs by understanding mitochondrial genetics and the development of a large-scale informatics platform (known as mitoCODE); advancing technologies such as patient-derived screening methods; and developing novel therapeutics. The national research coalition is rooted to promote research in mitochondrial biology; revolutionize diagnosis, prevention, and treatment of all diseases; partner with patient advocates to develop policy and public awareness; and prepare the leaders of tomorrow.

### The Evaluation of practice and Risks from Administration of Pharmacotherapies into Intravascular devicES (THERAPIES)

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**Introduction**: Vascular access devices (VAD) enable monitoring and provision of therapy to critically ill children. Experience suggests that multiple infusions are administered through a limited number of available lumens. VAD complications and lumen dysfunction may complicate the effective provision of therapy.
**Objective**: Describe the frequency, nature and factors associated with VAD complication and lumen dysfunction.
Methods: Prospective observational study of patients in two pediatric intensive care units. Data from patient VADs, lumens and lines were abstracted twice daily. Proper documentation was determined, and data collection reliability was assessed.
**Results**: 47 patients, observed for a mean of 4.5 days, had 122 devices (56 central venous (CVL), 19 arterial (ART), 47 peripheral venous (PIV)) with 175 unique lumens during 563 lumen-days through which 1051 continuous and 258 intermittent infusions were administered via 1454 lines. 14(11%) devices had VAD complications: 1 infection, 5 device mal-placements, 1 occlusion and 7 insertion-site complications (irritation 4, bleeding 1, leaking 1, impaired local circulation 1). Complications, in unique lumens, included 48(27%) with patency intervention (28 CVL, 7 ART, 13 PIV) and 27(19%) unable to aspirate. 14(8%) partial and 1(0.6%) complete occlusion occurred.
**Conclusion**: Complete occlusion was uncommon, however, patency interventions occurred in 27% of lumens. Requirement of patency intervention for proper function could be due to various risk factors; evaluated in future work.

### Complement Proteins in Alzheimer's disease; a Meta-Analysis

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**Purpose**: Genome-wide association studies implicate complement pathway activity as a cause of Alzheimer's disease (AD); however reports of complement protein levels in peripheral blood and cerebrospinal fluid (CSF) have conflicted. This meta-analysis seeks to quantitatively summarize blood and CSF complement data.
**Methods**: Literature was searched via Medline, PubMed, Embase, PsycInfo, Cochrane Controlled Trials Register, and Cochrane Database of Systematic Reviews. Original peer-reviewed studies measuring complement and complement regulator protein concentrations in AD and healthy elderly control (NC) subjects were included. Standardized mean differences were calculated with random effects models. Results: 73 studies have been included thus far. Preliminary results show increased CSF concentrations of complement component 1q (C1q; 151/117, Z=2.46, p=0.01; I^2=54%), increased clusterin concentrations in CSF (371/437, Z=4.18, p<0.0001; I^2=31%) and blood (1269/1651, Z=2.06, p=0.04; I^2=97%), and increased CSF amyloid P (AP) concentrations (283/109, Z=2.94, p=0.003; I^2=0%) in AD compared to NC.
**Conclusions/Implications:** CSF results support involvement of complement in AD; elevated C1q and AP levels may promote activity early in the complement cascade, while elevated clusterin could inhibit membrane attack complex (MAC) formation in the cascade's final stages. Increased blood clusterin concentrations in AD may imply brain-periphery coordination of immunoregulatory activities.

### Evaluating the Effects of Single-Session transcranial Direct Current Stimulation electrode (tDCS) Placement on Cognition in Mild Cognitive Impairment (MCI) and mild Alzheimer's disease (AD): A Pilot Study

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**Objectives**: While single-session transcranial direct current stimulation (tDCS) is an emerging tool to predict response to therapeutic neurostimulation, little is known about optimal electrode placement for those with early cognitive impairment. The primary objective of this study is to evaluate the cognitive effects following a single session of bifrontal, bitemporal, and sham tDCS in Mild Cognitive Impairment (MCI) and mild Alzheimer's disease (AD).
**Materials and Methods:** MCI and mild AD patients (N=13) were enrolled in a randomized, cross-over trial comparing single sessions of bifrontal, bitemporal, and sham tDCS (20 minutes, 2mA) with a 1-week washout period between stimulations. Outcomes included the Montreal Cognitive Assessment (MoCA), the Word Recall and Word Recognition subscales of

the Alzheimer's disease Assessment Scale-Cognitive, and the n-back. Repeated measures analyses of variance were used to analyse differences in cognitive change scores between stimulation groups.
**Results**: There was a statistically significant difference in 2-back accuracy between stimulation types ($F(2,24)=4.75$, $p=0.02$). Post-hoc analysis using Least Significant Difference correction revealed a statistically greater improvement in 2-back accuracy following bitemporal stimulation compared to sham ($p=0.03$) and bifrontal stimulation ($p=0.03$). There were no differences in 2-back accuracy between bifrontal and sham ($p=0.91$). No differences in MoCA, recall, or recognition were found between groups.
**Conclusions**: In our preliminary results, working memory improved following a single session of bitemporal tDCS compared to sham. Recruitment is ongoing (target sample; N=20). Single-session tDCS may be able to help predict optimal electrode placement and create a profile of responders to guide future personalized treatments with tDCS.

### The Gut Microbiome In Schizophrenia and Antipsychotic-Induced Metabolic Side Effects: A Pilot Study

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**Background**: Emerging evidence has suggested an important role of the human gut microbiome in psychiatry and neurodevelopmental disorders. An increasing body of literature comprised of animal studies have reported the gut microbiome to influence brain development and behavior by interacting with the gut brain axis. Furthermore, as the gut microbiome has an important role in metabolism and is known to interact with pharmaceuticals, recent evidence suggests a role for the microbiome in antipsychotic induced metabolic side effects in animals and humans.
**Purpose**: Here we present a protocol for a two-phase study investigating the gut microbiome in healthy controls and patients with schizophrenia treated with antipsychotics.
**Methods**: Phase I of our study involves exclusively humans. We are recruiting 25 patients who are chronically treated with clozapine compared with 25 healthy controls matched for age, sex, BMI and smoking status. A second cohort will consist of 25 patients newly starting on clozapine, all of whom will be prospectively assessed for up to 6 weeks. Phase II of this study incorporates a germ free mouse model to examine the influence of human fecal transplant on metabolic parameters and the gut brain axis.
**Progress and future directions:** We are underway with the first participants enrolled in all phase I treatment cohorts. This study will contribute to elucidate the role of the gut microbiome in schizophrenia and metabolic side effects. In addition, this might help to explore potential therapeutic targets for AP induced metabolic side effects.

### Clusterin Concentration in Alzheimer's Disease in Cerebrospinal Fluid and Plasma: A Meta-Analysis

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**Introduction**: Genetic evidence implicates the complement system in Alzheimer's disease (AD). Clusterin, as a regulator of complement system, prevents the formation of the membrane attack complex (MAC), which may affect amyloid clearance and neuroinflammation. The present study is intended to compare clusterin concentrations between AD patients and elderly controls.
**Methods**: Eligible literature was identified from MEDLINE (Ovid), PubMed, Embase, Cochrane Database of Systematic Reviews, Cochrane CENTRAL and PsychINFO (Ovid) databases. Abstracts were screened and the mean and SD clusterin concentrations, sample sizes and other study details were extracted from full text articles. Meta-analysis was conducted in Revman using random effects models of standardized mean differences (SMD).
**Results**: Eleven studies were included for meta-analysis, of which 3 measured clusterin level only in cerebrospinal fluid (CSF), 7 measured only plasma level, and 1 measured both CSF and plasma level. Significantly higher concentrations of clusterin were found in CSF ($Z = 4.19$, $p< 0.00001$, $n = 416$ AD vs. $n = 354$ controls) and plasma ($Z = 2.06$, $p< 0.05$, $n = 1651$ AD vs. $n = 1269$ controls) compartments. Substantial heterogeneity was detected between plasma ($Q=223.44$, $I2 =97\%$, $p<0.00001$), but not CSF ($Q=4.37$, $I2 =31\%$, $p>0.05$) studies.
**Conclusion**: Higher concentrations of clusterin were found in the plasma and CSF of AD patients compared to controls, with highly consistent results in CSF, supporting the involvement of the complement system in AD. Further studies to evaluate the role of clusterin in AD pathogenesis and its utility as a diagnostic or prognostic biomarker are indicated.

### Randomized trial of Empagliflozin on Cardiac Structure, Function and Biomarkers in Patients with Type 2 Diabetes and Cardiovascular disease

Tamique Mason, Subodh Verma, Andrew Yan, Kim Connelly, David Mazer

Cardiovascular disease is a leading cause of death in patients with Type 2 diabetes. Empagliflozin, an inhibitor of glucose re-absorption in the kidney, has been found to improve cardiovascular outcomes and reduce mortality. The mechanism by which this is accomplished remains unclear, but in preliminary studies, empagliflozin treatment was associated with a reduction in left ventricular mass. It is hypothesized that its effect to lower mortality may be due to a reduction in heart failure and that via a diuretic and natriuretic effect, empagliflozin directly affects cardiac structure, geometry and function. This research employs a double blinded randomized clinical trial design, cardiac magnetic resonance imaging, and bio-marker assessments, to rigorously study any changes in cardiac structure and geometry in patients with diabetes and cardiovascular disease being treated for 6 months with empagliflozin versus placebo. To date, we have recruited, randomized, and completed baseline cardiac magnetic resonance scans for 90 patients and have stored their baseline serum samples at -80 C for bio-marker analysis. For study completion, we need to conduct 6 month post

randomization cardiac scans and bio-marker analyses for all patients. Our study is of profound significance in providing critical and definitive insight into the mechanistic basis of empagliflozin's cardio-protective effect and may open doors to harnessing its potential in the care of patients with heart failure.

### Associations Between Electronic Cigarette (e-cigarette) Use and Smoking Cessation: Effects of use Patterns in Treatment-Seeking Smokers

Ng G, Liu M, Mobin A, Ng G, Baliunas D, Selby P, Zawertailo L

**Objective**: To assess the association of e-cigarette use and smoking cessation in smokers enrolled in the STOP program, a cessation program involving free counseling and NRT implemented across Ontario.
**Methods**: Data was collected from smokers enrolled in the STOP program between April 2016 and January 2017. Participants were classified as: 1) non-users (no e-cigarette use); 2) non-daily users (used e-cigarettes up to 3 times/week; or 3) daily users (used e-cigarettes daily). The primary outcome was self-reported quit status (7-day PPA) at 6-months and multivariable logistic regression was used to assess the association between e-cigarette use and quit status.
**Results**: At the 6-month follow-up, 77.3% (n=4717) of participants were non-users and 22.7% (n=1382) reported e-cigarette use in the past 6-months, of which 46.8% (n=647) were daily users and 53.2% (n=735) were non-daily users. Quit rates were significantly different between groups: 31.2% of non-users, 25.5% of daily users, and 15.4% of non-daily users quit. While the odds of daily users quitting smoking were not significantly different compared to non-users, OR 1.15 (95%CI, 0.81-1.61, p=0.44), the odds of non-daily users quitting smoking was 0.50 (95%CI, 0.33-0.73, p<0.001) times that of non-users.
**Conclusion**: Non-daily e-cigarette use appears to be detrimental to quit attempts while daily e-cigarette use is neither detrimental nor beneficial. Further research on the mediating effects of e-cigarette use on NRT use is needed.

### Different Measures of Varenicline Adherence, Nicotine Metabolite Ratio and Smoking Abstinence

Annie R. Peng, B.Sc (1), Robert Schnoll, Ph.D (2), Caryn Lerman, Ph.D (3), Rachel F. Tyndale, Ph.D (1), on behalf of the PGRN-PNAT Research Group

**Background**: In smoking cessation trials, pill counts have traditionally been used as an indirect measure of adherence; very few studies have examined the use of a biological measure. We hypothesize: (1) salivary varenicline levels will not correlate with self-reported pill count, (2) adherence based on varenicline levels, but not self-reported pill count, will be predictive of smoking abstinence, and (3) among those treated with varenicline, the association between nicotine metabolite ratio (NMR), a genetically informed biomarker of nicotine clearance, and smoking abstinence will be stronger in adherent individuals as defined by salivary varenicline levels.
**Methods**: Baseline blood NMR, Week 1 salivary varenicline levels, 3-, 7- and 14-day self-report pill counts, and multiple measures of biochemically-verified abstinence (exhaled carbon

monoxide CO, salivary cotinine COT) were assessed (N=376) (NCT01314001). Receiver operating characteristic curve and logistic regression analyses were conducted.
**Results**: 3-, 7-, and 14-day pill counts were highly correlated with each other, but very weakly correlated with salivary varenicline levels (Spearman's rhos ranging from .10 to .15, p's≤.05). Salivary varenicline levels were significant predictors of Week 1 abstinence (ORs 2.75 to 3.16, p's<.001). In contrast, when using a traditional cut-point of 80% of pills taken in the preceding 3, 7, or 14 days (as determined by pill count) to classify adherence, there were no statistical differences in Week 1 abstinence rate between adherent and non-adherent individuals (ORs 1.40 to 2.96, p's≥.05). Normal metabolizers of nicotine (NMR≥0.31) were significantly more likely to be abstinent at end-of-treatment in adherent individuals determined by varenicline levels (OR 2.00, 95%CI 1.23-3.24, p=0.005), but not by self-reported pill counts (ORs 1.27 to 1.36, p≥.18).
**Conclusions**: The concordance between pill counts and salivary varenicline levels is poor; pill counts did not predict abstinence while varenicline levels did. Amongst adherent individuals by drug levels, normal metabolizers of nicotine have higher abstinence rates than slow metabolizers. Proper assessment of medication adherence may enhance our ability to identify individuals who may experience difficulties quitting, and improve pharmacogenomics associations with outcomes.

### Association between Inflammatory Markers and Neurocognitive Flexibility among Adolescents with and without Bipolar Disorder

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**Introduction**: Peripheral inflammatory markers are elevated among adolescents and adults with bipolar disorder (BD), particularly during symptomatic episodes. In adults with BD, inflammatory markers are negatively associated with neurocognitive functioning. This relationship has not been investigated in BD adolescents.
**Methods**: Participants were 13-20 years old, 63 with BD (31 symptomatic hypomania and/or depression, 32 euthymic) and 60 HC. Diagnoses were confirmed using the K-SADS semi-structured interview. Serum levels of three pro-inflammatory markers (interleukin (IL)-1, IL-6, and tumor necrosis factor) and an anti-inflammatory marker (IL-10) were measured using commercial ELISA kits. Neurocognitive flexibility was assessed via the CANTAB intra/ extradimensional shift (IED) task. Multivariate linear regression controlled for IQ and lifetime ADHD.
**Results**: IL-1, IL-6, TNF and IL-10 protein concentration levels did not differ by diagnosis. Significant interactions were observed: within symptomatic BD adolescents, but not asymptomatic BD or HC adolescents, lower IL-1/IL-10 ratio was significantly associated with more errors prior to the extra-dimensional shift (p=0.023). Similarly, among symptomatic BD

adolescents, but not asymptomatic BD or HC adolescents, lower IL6/IL10 ratio was associated with significantly more trials to complete the IED task (p=0.012). The models accounted for 13.8% and 13.5% of variance in neurocognitive flexibility, respectively.
**Conclusion**: Anti-inflammatory predominance was unexpectedly associated with better neurocognitive flexibility among symptomatic BD adolescents, but not among euthymic adolescents or HCs. Prospective, repeated measure studies are warranted to verify the direction of these findings.

### Cerebrocholesterol, a Marker of Agitation Severity and Treatment Response to Nabilone in Patients with Moderate-to-Severe Alzheimer's Disease

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**Background**: Alzheimer's disease (AD) progression is associated with reduced brain cholesterol metabolism, which increases free cholesterol and reduces membrane fluidity. This may attenuate endocannabinoid system signalling, which has been implicated in agitation, and may also interfere with receptor binding of cannabinoid pharmacotherapies for agitation, such as nabilone. We assessed whether cerebrocholesterol (Cchol), the only brain cholesterol metabolite able to cross the blood brain barrier, was associated with agitation and treatment response to nabilone.
**Methods**: Serum Cchol was collected from moderate-to-severe AD patients participating in a 14-week, double-blind cross-over trial comparing 6 weeks of nabilone to placebo with a 1-week washout between phases. Samples were collected at the start and end of each treatment phase. We assessed (1) the relationship between Cchol and Neuropsychiatric Inventory-Agitation/Aggression subscore (NPI-A/A), at baseline (BL); (2) the relationship between BL levels of Cchol and change in NPI-A/A, following nabilone treatment; and (3) the longitudinal relationship between changes in Cchol levels and changes in NPI-A/A, following nabilone treatment, in all patients, and in patients who demonstrated improvement on the Clinicians' Global Impression of Change (CGIC).
**Results**: Thirty-nine patients (mean±SD age=86.7±10, 77% male, NPI-A/A=7.1±3.3, Mini-Mental Status Exam=6.3±6.3, Cchol=14.3±5.2 ng/mL) were randomized. There was no significant association between Cchol and NPI-A/A at BL (OR=0.098, 95% CI 0.002 to 5.423, p=0.257). However, in those with severe agitation/aggression (NPI-A/A≥6) (N=27), lower Cchol levels were significantly associated with greater NPI-A/A at BL (OR=0.005, 95% CI 0.00003 to 0.63, p=0.03). Lower BL Cchol levels had an associative trend with improvements in NPI-A/A with nabilone compared to placebo (b=-5.3, 95% -11.02 to 0.35, p=0.065). Increases in Cchol levels from BL had an associative trend with a reduction in NPI-A/A with nabilone compared to placebo (b=-4.0, 95% -8.24 to 0.27, p=0.067). In those with minimal to marked improvement on the CGIC during nabilone (N=17), increases in Cchol levels from BL were significantly associated with a reduction in NPI-A/A in nabilone compared to placebo (b=-8.1, 95% CI -12.7 to -3.5, p=0.001).

**Conclusions**: These findings suggest that serum Cchol may be a marker of agitation severity in AD, and a marker of treatment response to nabilone.

### Reduced Endocannabinoid Metabolism in Schizophrenia: in vivo Imaging of Fatty Acid Amide Hydrolase with C-11 CURB

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**Purpose**: The endocannabinoid (eCB) system modulates brain responses to factors related to psychosis risk and relapse including cannabis and stress. In vivo imaging of the eCB system in psychosis has been limited to cannabinoid CB1 receptors. We investigated the eCB system in antipsychotic-naïve first episode psychosis (FEP) patients while controlling for FAAH genetic variation and cannabis use, using PET imaging with C-11 CURB, a ligand for fatty acid amide hydrolase (FAAH), the enzyme that metabolizes the endocannabinoid anandamide.
**Methods**: In this pilot study, we recruited antipsychotic-naïve FEP and age-matched healthy volunteers (HV) participants. Diagnosis of FEP and active symptoms were confirmed by a by clinical interview. HV had no current or past DSM IV axis I diagnosis and no family history of psychotic disorders. The FAAH genetic polymorphism rs324420 affects FAAH protein levels, thus rs324420 genotype was obtained for each subject.
**Results**: FEP had lower C-11 CURB binding than HV in the amygdala and striatum, with trends toward lower binding other regions including prefrontal cortex. FAAH genotype influenced C-11 CURB binding in FEP and HV, with lower binding observed in A-allele carriers.
**Conclusions**: Data from this pilot sample provide the first in vivo evidence that FAAH is altered in psychosis, and this change is present near the time of disease onset. Overall, these data provide preliminary support for a link between psychosis and the eCB system, which regulates brain responses to stress and cannabis, two factors implicated in psychosis risk and relapse.

### Swelling-Induced Chloride Current in Glioblastoma Proliferation, Migration and Invasion

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**Background**: Glioblastoma (GBM) remains the most common and aggressive malignant brain tumor originating in the central nervous system. Diagnosis is lethal with a median survival of <15 months. Aberrant swelling-induced chloride channel ICl,swell expression has been linked to GBM cellular functions (i.e. proliferation, migration and invasion).
**Objectives**: We hypothesize that inhibition of the swelling-induced chloride channel ICl,swell suppresses GBM cellular functions. The purpose is to establish ICl,swell as a potential drug target by evaluating DCPIB, a specific antagonist for the swelling-induced chloride channel ICl,swell, on GBM cellular functions.

**Methods**: We used the human GBM cell lines U251 and U87. First, with the whole-cell patch-clamp technique to measure activity of ICl,swell. GBM proliferation and viability were assessed with MTT and colony formation assays. Moreover, GBM migration and invasion were assessed with scratch wound and Matrigel invasion assays, respectively. With Western immunoblots, we also assessed in GBM the protein levels of p-Akt/t-Akt, p-JAK2/t-JAK2, and p-STAT3/t-STAT3 in order to examine the underlying mechanism.
**Results**: We demonstrated that DCPIB enhanced the endogenous swelling-induced chloride channel ICl,swell. GBM proliferation and viability were reduced with DCPIB treatment. DCPIB also suppressed GBM migration and invasion. We found that DCPIB inhibited the JAK/STAT as well as the PI3k/Akt signaling pathways, which could potentially be the underlying swelling-induced chloride channel ICl,swell-dependent mechanism.
**Conclusions**: Because potentiated swelling-induced chloride channel ICl,swell activity contributes to the devastating proliferative, migratory and invasive characteristics of GBM, our study establishes the involvement of ICl,swell in GBM cellular functions.

### Lipoxygenase Derived Metabolites Are Differentially Associated with Alzheimer's Disease and Subcortical Ischemic Vascular Disease; Preliminary Evidence from a Multimodal Stratified Cohort Study

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**Background**: Neuroinflammation plays an essential role in the advancement of both Alzheimer's disease (AD) and subcortical ischemic vascular disease (SIVD). Polyunsaturated fatty acids and their oxidized metabolites, in particular the bioactive oxylipins, are multifunctional molecules involved in the regulation and resolution of inflammation. The relationships between oxylipins, AD and SIVD remain to be clarified.
**Methods**: The present study applied a targeted lipidomics platform to quantitatively probe for 72 oxylipins in AD (n=30) and non-AD (n=54) participants, including strata of extensive (n=43, SIVD) or minimal (n=41, Non-SIVD) white matter hyperintensities (WMHs). WMHs were identified through multimodal MRI, and quantified using a personalized semi-automatic processing pipeline (Lesion Explorer). The oxylipins were extracted from serum using solid phase extraction, and quantified with UPLC-MS/MS. Executive function was assessed using the Stroop and Trail-Making B (TMT-B) tests.
**Results**: In a multivariate analysis of covariance model controlling for age and sex, the lipoxygenase (LOX)-derived dihomo-gamma-linoleic acid metabolite, 15(s)-hydroxyeicosatrienoic acid (15(s)-HETrE; detectable in n=81) was lower in SIVD ($F_{1,80}$ =11.51,

p=0.001) but not AD (F1,80 =2.74, p=0.102). The LOX derived arachidonic acid metabolite, 15-hydroxyeicosatetraenoic acid (15-HETE; detectable in n=65), was lower in AD (F1,64=4.98, p=0.032) but not SIVD (F1,64=0.05, p=0.821). In participants with extensive SIVD but no AD (n=28), two LOX derived linoleic acid metabolites, 9-hydroxyoctadecadienoic acid (9-HODE) and 13-HODE, were found to be positively associated with Z-scores on the Stroop (ρ=0.523, p=0.004 and ρ=0.444, p=0.018, respectively) and TMT-B (ρ=0.595, p=0.001 and ρ=0.557, p=0.003, respectively), and negatively associated with periventricular WMH volume (ρ=-0.386, p=0.042; ρ=-0.393, p=0.039, respectively).
**Conclusions**: The generation of LOX metabolites may be compromised differently in AD and SIVD. These metabolites might be protective against white matter injury and cognitive decline caused by subcortical ischemic vascular disease.

### Circulating Osteoprotegerin and Breast Cancer Risk in BRCA1 and BRCA2 Mutation Carriers

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**Background**: Emerging evidence suggests aberrant signaling in the receptor activator of nuclear factor kappa B (RANK)/RANK ligand (RANKL) pathway in the pathogenesis of BRCA-associated breast cancer. Osteoprotegerin (OPG) is the endogenous decoy receptor for RANKL which antagonizes RANK signaling. Lower circulating OPG levels among BRCA mutation carriers compared to non-carriers has been reported. Furthermore, we observed an inverse relationship between circulating OPG and breast cancer risk. We sought to validate our findings in a larger group of BRCA mutation carriers with longer follow-up period.
**Methods**: This prospective study measured serum OPG (pg/ml) in 606 BRCA mutation carriers through enzyme-linked immunosorbent assay. Kaplan-Meier survival analysis was used to estimate the cumulative incidence of breast cancer by median OPG levels.
**Results**: Over a mean follow-up of 2.7 years (range 0.003-7.5), 20 incident breast cancer cases were diagnosed. The mean serum OPG level was 124.4 pg/ml (range 32.1-368.6). We stratified women by median OPG levels (<116.7; n = 303 vs. ≥116.7 pg/ml; n = 303). There were 14 incident cases among women with OPG levels below the median and 6 incident cases among women with OPG levels above or equal to the median. The cumulative incidence of breast cancer for women with OPG levels <116.71 pg/ml was 31% compared to 3% for women with OPG levels ≥116.71 pg/ml (P-log rank = 0.11).
**Conclusions**: These findings support the role of aberrant RANK-signaling in BRCA-breast cancer development and suggest that RANKL-blockade is a likely candidate for non-surgical prevention. Consequently, circulating OPG levels may identify women who are ideal candidates for RANKL-chemoprevention.

### Medication Compliance in Smoking Cessation: Self-Report vs Biochemical Confirmation as a Predictor of Quit

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**Background and Objectives:** Adherence to medication has been shown to predict treatment outcomes for smoking cessation. The purpose of this analysis was to compare the reliability of self-reported compliance to biochemically confirmed medication compliance.
**Methods**: Participants were recruited and enrolled via the internet. Eligible participants were randomized 1:1 to receive a 12 week supply of either bupropion (150 mg b.i.b.) or varenicline (1mg b.i.d.). Follow-up surveys were conducted at weeks 4, 8 and 12 to assess self-reported medication compliance. Participants also submitted a saliva sample after 4 weeks of medication, which was analyzed by GC/MS for varenicline or bupropion and its three metabolites (BUP-OH, E-BUP and Tert-BUP).
**Results**: A total of 964 participants received medication (VAR=499 and BUP= 465) with 383 submitting a saliva sample at 4 weeks. At end of treatment (EOT), quit rates were 30% and 20% for VAR and BUP respectively. Self-reported compliance at 4 weeks was a significant predictor of quit at EOT for both BUP and VAR ($p<0.001$). Biochemical VAR compliance was a significant predictor of quit outcomes (OR=5.85; 95%CI: 3.2-10.7, $p<0.001$), but BUP compliance was not (OR=0.3; 95%CI: 0.1-1.0, $p=0.055$).
**Conclusions**: Medication compliance significantly predicts treatment outcomes. Self-reported medication compliance is widely used in clinical trials; however, biochemical confirmation of varenicline compliance should be implemented to increase the reliability of compliance measures.

### Development of a Novel Screening Method in White Blood Cells for the Nod-like Receptor 3 Protein (NLRP3) Inflammasome: a Biological Target for Psychiatric and Metabolic Diseases

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Several psychiatric and metabolic illnesses exhibit both mitochondrial dysfunction and elevated inflammation; yet treating each complication separately has not been clinically effective. The NLRP3 inflammasome is a node of intracellular stress pathways and a druggable target which integrates mitochondrial stress and inflammatory cascades. This study explored mitochondrial dysfunction as a biological source of NLRP3 activation in peripheral blood mononuclear cells (PBMC) from patients with bipolar disorder (BD), major depressive disorder (MDD), and type 2 diabetes mellitus (T2DM).

Immunocytochemistry has shown aggregation of ASC, the NLRP3 adaptor protein, into dense specks. Following brief culture of PBMCs, baseline levels of intracellular ASC specks were quantified in each disease group and compared to stimulation with lipopolysaccharide and nigericin, an NLRP3 agonist. For all disorders, results show an increase in baseline proportion of cells expressing ASC specks compared to controls. A progressive increase in stimulated cells was also observed, ranging from lowest in BD, followed by MDD, and highest in T2DM.

These findings present a spectrum of inflammation across BD, MDD, and T2DM. Intracellular ASC specks may serve as a biomarker for diagnosing and monitoring inflammatory diseases. Future experiments will evaluate the levels of mitochondrial complexes and its relevance to NLRP3, a promising and novel target for rational drug development in inflammatory diseases.

## Molecular and Biochemical Pharmacology

### Investigating Novel Mechanisms of Inositol Polyphosphate-4-phosphatase Type II (INPP4B)-mediated Phosphoinositide Signalling Leading to The Activation of Autophagy

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Autophagy, a pro-survival process that recycles damaged organelles and non-essential cellular components to provide energy upon stress, is frequently upregulated in chemoresistant cancers. Phosphatidylinositol 3-monophosphate (PI(3)P) is essential for autophagy since it recruits and binds critical FYVE-domain containing proteins required for autophagosome formation. INPP4B is a phosphoinositide 4-phosphatase that generates PI(3)P by hydrolyzing PI(3,4)P2. High levels of INPP4B in some cancers, is associated with poor patient survival and chemoresistance. Thus, we hypothesized that by increasing cellular PI(3)P levels, INPP4B promotes autophagy and thereby confers chemoresistance in cancer. To test this hypothesis, we have developed cell models to study INPP4B-mediated phosphoinositide signaling and associated autophagy. By confocal microscopy in cells stably expressing GFP-FYVE fusion proteins, we have observed that siRNA-mediated INPP4B knockdown dramatically reduces fluorescent puncta identifying a specific role for INPP4B in PI(3)P homeostasis. Conversely, INPP4B overexpression promoted autophagy levels under various conditions, as observed by LC3II by Western blots and CytoID flow cytometry. Our future experiments are designed to confirm the role of INPP4B in both PI(3)P signaling and autophagy activation in novel cell systems. Overall, we aim to shed light on cancer-promoting mechanisms associated INPP4B overexpression as putative targets for cancer therapies.

### Using C. elegans to Screen for Drugs that Cure the Childhood Liver Disease, PFIC3

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The hepatocytes secrete bile acids, which strip phosphatidylcholine (PC) away from the outer membrane of hepatocytes to form micelles. The hepatocytes rely on the ABC transporter called ABCB4 for a steady supply of PC. Mutations in ABCB4 result in progressive familial intrahepatic cholestasis 3(PFIC3), which leads to bile duct obstruction and cirrhosis. Ursodeoxycholic acid (UDCA) is the only drug available but its use is limited to mild cases. In most cases, patients require a liver transplant to survive. C. elegans has a homolog of ABCB4 called PGP-14. Mutations in PGP-14 allow the worms to resist the lethal effect of a molecule called wact-190. This molecule forms crystals in worm anterior pharynx. However, both crystals and lethality are not observed in the pgp-14 mutants. My objective is to find compounds that can circumvent the loss of PGP-14 and possibly ABCB4 activity. I have screened 2560 molecules and identified 7 hits that suppress wact-190 resistance. These hits also induced crystal formation in the anterior pharynx of pgp-14 when co-incubated wth wact-190, suggesting that wact-190 is the main mediator of lethality. I am currently performing forward genetic screens to find the potential target of my hits. Characterization of the molecules and the suppressor genes will provide valuable insights into membrane biology. Successful hits will be tested in a mouse ABCB4 deficiency model to investigate their potential as a novel PFIC3 management option.

### Investigating Structure-Activity Relationships (SAR) of Pharmacological Chaperones of the Dopamine Transporter

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The dopamine transporter (DAT) is an SLC6, Na+/Cl- dependent transporter that is the main mediator of extracellular dopamine uptake. Loss-of-function mutations of DAT cause an autosomal recessive condition known as dopamine transporter deficiency syndrome (DTDS). DTDS is characterized by parkinsonism-dystonia along with elevated dopamine metabolites. In addition, when expressed in-vitro, DTDS mutations of DAT display decreased dopamine uptake along with ER retention. A series of experiments by Beerepoot et al. 2016, have shown that ibogaine, a natural psychoactive compound, and bupropion, a smoking cessation drug, possess pharmacological chaperone activity of DTDS DAT mutants. Pharmacological chaperones or pharmacochaperones are drugs that selectively act to stabilize a protein during folding, ultimately enhancing its maturation and surface expression.
We therefore examine the structure-activity relationships that facilitate chaperoning of DAT in order to discover more efficacious and potent compounds. An SAR-based screen of bupropion and ibogaine analogs demonstrated necessity for a secondary amine and single halogen substitution on bupropion analogs along with a flexible heterocyclic ring, chair-like substituent and hydroxyl substitution on ibogaine analogs. Lead candidates noribogaine and PAL594 were able to rescue mature DAT. We also report that there is no link between DAT inhibition and chaperoning, suggesting that chaperoning may be mediated allosterically.

### Schizophrenia Related Protein Fxr1 Controls Homeostatic tuning of Neuronal Activity

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**Background:** Genetic variants of the fragile X mental retardation syndrome-related protein 1 (FXR1) are associated with mood regulation, schizophrenia, and bipolar disorders. Nonetheless, the genetic association does not indicate a functional link to neuronal activity and plasticity. Mental illnesses are believed to be associated to a miss-regulation of excitation/inhibition balance and disruption in homeostatic plasticity could be one of the causes.
**Methods:** We used CRISPR/Cas9 mediated knockout and overexpression to investigate the impact of Fxr1 and its negative regulator Gsk3β on homeostatic plasticity in vitro and in vivo.
**Results:** We have discovered that synaptic upscaling accompanied with an increase of synaptic GluA1 and a decrease in expression of Fxr1. Augmentation of Fxr1 expression and CRISPR/ Cas9 mediated knockout of Gsk3β, the negative regulator of Fxr1, was sufficient to completely abolish upscaling.
We have identified that sleep deprivation induced increase in synaptic strength is also accompanied with an increase of synaptic GluA1 and a decrease in expression of Fxr1 and can be blocked by augmentation of expression of Fxr1 and reduction of its negative regulator Gsk3β.
**Conclusions:** These results underscore a regulatory role of Fxr1 across different types of homeostatic regulation of neuronal activity in vitro and in vivo. This also suggests how Fxr1 can contribute to neuronal plasticity in response to environmental conditions, such as sleep deprivation, and in illnesses like mood disorders and schizophrenia.

### Lactate in Bipolar Disorder: A Systematic review and Meta-Analysis

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Bipolar disorder (BD) is a debilitating mood disorder with no specific biological marker and no novel treatment. Although BD pathophysiology remains unclear, there is strong evidence in the literature supporting the role of mitochondrial dysfunction in BD. In this systematic review, we identified and investigated twelve studies that measure lactate (a direct marker for mitochondrial dysfunction) in BD patients and healthy controls. Of the six studies that measured brain lactate levels, five reported significantly elevated lactate levels in BD patients. Two studies reporting cerebrospinal fluid lactate levels also found significantly elevated lactate in BD. Two other studies that reported peripheral lactate levels did not demonstrate significant findings. The meta-analysis, using standardized means and random-effect model for five studies that measured brain lactate levels, corroborated the findings of the systematic review. Although the meta-analysis had a nearly significant overall effect ($Z = 1.97$, $p = 0.05$), high statistical heterogeneity ($I2 = 86\%$) and possible publication bias suggest that the results should be interpreted with caution. Further studies with larger sample size, more inclusive

patient populations, and standardized methodologies are required to validate lactate abnormalities in BD. Peripheral lactate and other bioenergetic markers should be thoroughly studied to better understand mitochondrial dysfunction in BD and help develop objective diagnostic tools.

### Osteoblast-specific Overexpression of Gαs or Gα11 leads to Differential Fracture Healing Responses

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Despite the ability of bone to self-repair, the normal process of fracture healing is sometimes compromised resulting in non-union that requires additional interventions that are costly and lead to loss of productivity. G protein-coupled receptor signaling in osteoblasts plays a regulatory role in skeletal development. Here, we show the effects of Gαs and Gα11 overexpression in osteoblasts on bone healing from stabilized transverse osteotomy of tibiae using wild-type and two transgenic mouse models developed in our lab, Gs-Tg that has increased bone and G11-Tg that has osteopenia. Our Micro-CT analysis revealed that increased Gαs signaling leads to rapid fracture healing with enhanced callus mineralization and new woven bone formation, while increased Gα11 signaling leads to delayed fracture healing with reduced bone formation but early transition into the remodeling phase. Histologically, Gs-Tg mice showed increased formation of cartilage and immature woven bone in the reparative phase, but persistence of the irregular morphology of woven bone in the remodeling phase indicating defective bone remodeling. Increased formation of cartilage, which was rapidly resorbed in the remodeling phase, was also seen in G11-Tg mice, but no obvious differences in the remodeling of woven bone into mature lamellar bone were seen compared with WT mice. Altogether, our results indicate that osteoblast-specific overexpression of Gαs and Gα11 leads to different fracture healing responses.

### Maternal Separation Stress Leads to Depressive and Anhedonia-Like State With Dysregulation of Dopamine Receptor Heteromeric Complex

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Postpartum depression is a subset of depression that is commonly overlooked though it affects as many as 15% of new mothers. Studies have shown that activation of the dopamine D1-D2 receptor heteromeric complex triggers depression-like and anxiety-like effects in rats and disruption of this heteromer leads to antidepressant and anxiolytic outcomes. Female rats express more heteromer in nucleus accumbens than male. The present study investigated the role of the D1-D2 heteromer in a rodent model of postpartum depression. After delivery, Sprague-Dawley dams were assigned to animal-facility rearing (AFR) conditions or a daily 180-minute separation (MS180) from their pups from postnatal day (PND) 2-15. Maternal behavior

was tested on PND 3, 9 and 15. After weaning, dams were subjected to the sucrose preference test, elevated plus maze (EPM) and forced swim test (FST). MS180 dams displayed higher levels of depression- and anhedonia-like behaviour with decreased sucrose preference and increased immobility on the FST. However, MS180 dams showed less anxiety on the EPM and increased overall maternal care immediately after separation. Proximity ligation assay revealed lower levels of D1-D2 heteromer in the nucleus accumbens of MS180 dams compared to AFR dams, in fact, AFR dams had higher levels than virgin rats. These findings show that maternal separation stress lowered the level of heteromer that would naturally be occurring postpartum.

### Effects of Pamidronate Treatment on Cortical and Trabecular Bone in HOM-Gs Mice

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**Objective:** Mice overexpressing Gas (HOM-Gs) in osteoblasts have high bone mass but the cortical bone is weakened by a high level of porosity. The objective of this study is to determine whether pamidronate administration during early development in HOM-Gs mice will prevent the formation of cortical pores.
**Methods:** WT FVB mice and HOM-Gs mice were given saline or pamidronate a dose of 3mg/kg s.c from 6 to 12 weeks of age. Calcein green was injected 9 and 2 days before sacrifice for measure of bone formation rates. Bone marrow stromal cells were extracted from femurs, to assess osteoclasts in vitro. Osteoclasts were stained with tartrate-resistant acid phosphatase and cell numbers quantified. Micro-computed tomography (μCT) of femurs was used to assess bone morphometry. Humeri were taken for RNA analysis of a panel of genes by RT-PCR. Decalcified tibiae were embedded in wax for bone histological analysis. Undecalcified tibiae were embedded in a plastic for dynamic histomorphometry. Biomechanical testing will be performed on femurs and vertibrea following μCT analysis.
**Results & Conclusions:** Pending.

### Investigating BRCA1 and PTEN Tumour Suppressor Protein Regulation to Identify Novel Avenues for Cancer Prevention and Treatment

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The Breast cancer susceptibility gene 1 (BRCA1) and the Phosphatase and tensin homolog gene (PTEN) are tumour suppressor genes (TSG) that suppress the formation of numerous cancers. BRCA1 or PTEN heterozygosity increases the risk of developing or progressing to more aggressive forms of cancer. Emerging studies suggest that the associated risk can be modified in these individuals by physiological and cellular factors which may alter gene expression from the remaining wild-type TSG allele. Thus, we hypothesize that TSG up-regulation can prevent or decrease cancer risk by promoting the molecular mechanisms of

onco-suppression. The overarching goal of this project is to identify mechanisms of regulation and novel agents with the capacity to modify TSG expression. Towards this goal our first objective is to generate reporter cell lines where TSGs are endogenously tagged with turbo-GFP. The second objective will use these reporter cell lines in genome-wide CRISPR-KO screens to characterize the "regulome" for PTEN and BRCA1 expression. The third objective entails the use of our reporter cell lines in high content screens to identify agents (drugs, small molecules, nutritional compounds) that can modify TSG expression. Overall, our project aims to characterize the mechanisms regulating expression of BRCA1 and PTEN and discover novel agents that can modify gene expression. Together, our project will shed light on novel avenues for preventative or treatment therapies in cancer.

### Mitochondrial Function in Individuals at Clinical High Risk for Psychosis

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Alterations in mitochondrial function have been implicated in the etiology of schizophrenia. Most studies have investigated alterations in mitochondrial function in patients in which the disorder is already established; however, whether mitochondrial dysfunction predates the onset of psychosis remains unknown. We measured peripheral mitochondrial complex I–V function and lactate/pyruvate levels in 27 antipsychotic-naïve individuals at clinical high risk (CHR) for psychosis and 16 healthy controls. We also explored the association between mitochondrial function and brain microglial activation and glutathione levels using a translocator protein 18kDa [18F]FEPPA PET scan and 1H-MRS scan, respectively. There were no significant differences in mitochondrial complex function and lactate/pyruvate levels between CHR and healthy controls. In the CHR group, mitochondrial complex III function ($r=-0.51$, $p=0.008$) and lactate levels ($r=0.61$, $p=0.004$) were associated with prodromal negative symptoms. As previously reported, there were no significant differences in microglial activation and glutathione levels between groups, however, mitochondrial complex IV function was inversely related to microglial activation in the hippocampus in CHR ($r=-0.42$, $p=0.04$), but not in healthy controls. In conclusion, alterations in mitochondrial function are not yet evident in clinical high risk, but may relate to the severity of prodromal symptoms, particularly negative symptoms.

### Investigating the Role of Inositol Polyphosphate-4-Phosphatase Type II (INPP4B) in Pancreatic Cancer

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Pancreatic tumours are one of the deadliest forms of cancer with 5-year survival rates of less than 5% worldwide. Survival analyses in pancreatic cancer patient databases revealed that high expression of Inositol Polyphosphate- 4-Phosphatase Type II (INPP4B) was significantly associated with poor survival suggesting that INPP4B promotes pancreatic cancer aggressiveness. Since a causal role for INPP4B overexpression in pancreatic cancer has not been explored, we have established several models of INPP4B-knockdown and overexpression in pancreatic cancer cell lines. Using a battery of cellular experiments to evaluate hallmarks of cancer, including assays to measure proliferation rates, anchorage-independent colony formation, wound healing and migration, we have observed that INPP4B overexpression promoted each of these features, which is consistent with the highly metastatic nature of aggressive pancreatic cancer. In contrast, INPP4B knockdown reduced cellular proliferation, migration, and anchorage-independent colony formation. In order to elucidate a direct role for INPP4B in pancreatic cancer, future studies will couple these molecular signaling studies with in vivo studies on the effects of INPP4B in pancreatic tumour models. Finally, we will explore the roles of INPP4B in pancreatic cancer therapy, both as a modulator of sensitivity to chemotherapy and as a putative novel target.

### Effects of Osteoblast-Specific Gαs Over-Expression on Skeletal Responses to Anabolic and Catabolic Stimuli using a Transgenic Mouse Model

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**Objective:** To examine the effects of increased osteoblastic G(alpha)s expression on responses to anabolic bone therapies and osteocatabolic conditions that mimic human pathologies.
**Methods:** We treated wild type (WT) mice and mice that have osteoblast-specific G(alpha)s over-expression (HOM-Gs mice) with osteoanabolic stimuli (i.e. exercise and intermittent parathyroid hormone) and osteocatabolic stimuli (i.e. low calcium diet (LCD) and continuous parathyroid hormone (cPTH)).
**Results:** In response to osteoanabolic stimuli, HOM-Gs mice displayed enhanced increases in trabecular bone mass compared to WT mice. In response to osteocatabolic stimuli, HOM-Gs mice displayed similar responses to LCD as WT mice (e.g. loss of bone mass), but were resistant to the catabolic effects of cPTH (e.g. increase rather than decrease in trabecular bone mass).
**Conclusions:** Our findings suggest that stimulation of G(alpha)s signalling in the presence of additional G(alpha)s protein drives bone anabolism when sufficient calcium levels are present. Despite the increases in bone mass, no biomechanical improvements were observed indicating that bone formation was favoured at the cost of bone quality.

## Neuropharmacology

### Mitochondrial genetics and protein function in post mortem brains of Bipolar Disorder patients

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Bipolar Disorder (BD) is a severe disorder characterized by alternating episodes of mania and depression, which significantly impact the quality and lifestyle of the patient. Previously in our laboratory, we have found that BD patients compared to non-psychiatric controls have significantly decreased level and activity of the mitochondrial complex I in postmortem brains, specifically in the prefrontal cortex (PFC). This study aims to measure mitochondrial electron transport chain complex activity, mitochondrial DNA (mtDNA) copy number variation and deletion, and lipid oxidation in postmortem brains of BD and schizophrenia (SCZ) patients compared to non-psychiatric controls. Broadmann area 24 (BA24), cerebellum (CE), hippocampus (HYP), and PFC were used to measure mitochondrial complex activity, and mtDNA copy number variation and deletion. Lipid peroxidation was only measured in BA24, CE, and HYP. Results will be controlled for patient age, sex, body mass index, brain pH, and post-mortem interval.

### Characterization of Mitochondrial Function in Cerebral Organoids Derived from Human Pluripotent Stem Cells

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Mitochondrial dysfunction is a key player in a variety of human disorders including primary mitochondrial diseases, psychiatric disorders, and neurodegenerative diseases. One of the major problems in understanding mitochondrial pathology across several brain disorders is that we cannot directly access the human brain. Here, we aim to develop an in vitro model of the developing brain such as three-dimensional (3D) brain organoids to characterize mitochondrial function and neurotransmission. Following directed differentiation of human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) to brain organoids, we were able to simultaneously track mitochondrial morphology using MitoTracker Red CMXRos and perform whole cell patch clamp recordings in a live 6-month old hESC-derived brain organoid. We were also able to successfully characterize mitochondrial morphology in brain organoids by immunohistochemical fluorescent detection of TOMM-20, a marker of the outer mitochondrial membrane and COX-IV, a marker of the inner mitochondrial membrane. While further investigation of mitochondrial function should also include a combination of other assays such as oxygen consumption, ATP synthesis, assessment of mitochondrial membrane potential and respiratory complexes I – V activity, our preliminary work provides a

3D model for characterizing the mitochondria, which will enhance our understanding of the role of mitochondrial dysfunctions across a variety of diseases.

### Characterizing Novel Inhibitors of Neuromuscular Function that May Arrest Worm Infections

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Approximately 20% of the global population is currently infected with a parasitic nematode (Pulan et al., 2014). Aside from being important therapeutics for humans, drugs that clear parasitic nematode infections (anthelmintics) are of agricultural interest for maintaining healthy populations of farmed animals. Our group has previously demonstrated that the non-parasitic nematode Caenorhabditis elegans is a useful model for anthelmintic discovery (Burns et al., 2015). A common effective anthelmintic strategy is to target the nervous system and muscles of parasitic worms; doing so not only provides an effective means of killing these parasites, but also for inhibiting their feeding, fecundity and attachment to tissues of the host animal. Here, we aim to use the egg-laying system of C. elegans to identify small-molecule modulators of neuromuscular function. We have screened through 4644 molecules and revealed 82 molecules modulate C. elegans egg-laying. I am currently focusing on the characterization of 2 molecules that we hypothesize agonize two-pore domain potassium channels and another molecule that we hypothesize agonizes ionotropic acetylcholine receptors. All three of these molecules lack activity in vertebrate systems and are active against two agriculturally relevant parasitic nematodes Cooperia oncophora and Haemunchus contortus suggesting that these molecules have potential as anthelmintic leads.

### Imaging Alterations in Endocannabinoid Metabolism in Clinical High Risk for Psychosis: A Pilot PET study using [11C]CURB for Fatty Acid Amide Hydrolase (FAAH)

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**Purpose**: The endocannabinoid system (eCBS) is involved in brain responses to stress and cannabis use, two risk factors for psychosis. In vivo imaging studies show an alteration of the eCBS in psychosis, but no studies in the clinical high risk (CHR) for psychosis state are available. We investigated the eCBS in CHR with positron emission tomography (PET) imaging using [11C]CURB, a ligand for fatty acid amide hydrolase (FAAH), the catabolic enzyme of the endocannabinoid, anandamide.
**Methods**: So far, we recruited 17 healthy volunteers (HV) and 23 CHR individuals. Regional [11C]CURB binding (lambda k3) was calculated using an irreversible 2-tissue compartment

model and plasma input function. Participants tested negative for drugs of abuse at baseline. Analysis was controlled for the participants' FAAH C385A variant.
**Results**: [11C]CURB binding was reduced in a priori regions dorsolateral prefrontal cortex (DLPFC; p=0.006) and striatum (p=0.016), and exploratory regions medial prefrontal cortex (mPFC; p=.057), anterior cingulate cortex (p=0.011) and hippocampus (p=0.004), in CHR versus HV. Analysis revealed a main effect of past cannabis exposure on [11C]CURB binding in the DLPFC, striatum, amygdala and hippocampus, and a positive correlation between striatal [11C]CURB binding and depression scores in CHR.
**Conclusions**: This is the first exploration of FAAH in CHR. This study suggests the eCBS might be alerted in CHR, and could be related to past cannabis use and depression.

### Exploiting Locomotor Phenotypes for the Characterization of Novel Neuroactive Compounds in C. elegans

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We are working towards the identification of novel neuromodulators that can be exploited as anthelmintic leads. Towards this end, we have developed a pipeline to identify neuroactive compounds using C. elegans. A collection of 4644 molecules that showed bioactivity in C. elegans, yeast, and/or fish were screened for their ability to modulate the egg-laying rate of worms, which is regulated by conserved neural signaling pathways. The hits from these screens were then re-screened for their ability to perturb locomotion. We found a structural family of molecules, called the wact-45 family, that induces three locomotor phenotypes upon acute exposure; jerky locomotion, coiling and paralysis. Several lines of evidence suggest that the wact-45 family inhibits the vesicular acetylcholine transporter (VAChT), which is encoded by unc-17 in C. elegans. First, wact-45 supresses the acute paralysis induced by the acetylcholine esterase inhibitor aldicarb, suggesting antagonism of cholinergic signaling. Second, hypomorphic unc-17 mutants phenocopy the effects of wact-45. Third, wact-45 is structurally similar to vesamicol, a known inhibitor of vertebrate VAChT. Lastly, vesamicol-resistant unc-17 mutants also resist the effects of wact-45. Unlike vesamicol, preliminary analyses indicate wact-45 is not toxic to vertebrate models. We are excited to continue developing wact-45 as a candidate anthelmintic. Further work will be done to investigate the wact-45 interaction with VAChT biochemically

### Functional Mapping of Cortical Dopamine D2 Receptor Expressing Neurons

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The dopamine D2 receptor (Drd2) is a G-protein coupled receptor (GPCR), a direct target of antipsychotics and may contribute indirectly to the action of mood stabilizers. DRD2 gene variants have been identified as risk factors for schizophrenia. A majority of studies focused on prefrontal cortex pyramidal neurons and interneurons, however expression of Drd2 in other cortical areas has also been reported, albeit with severe technical limitations due in part to the relatively low expression of Drd2 in most cortical neurons. To overcome this limitation, we used mice expressing a Cre activated RiboTag, specifically in Drd2 positive (Drd2+) cells. RiboTag mice carry a ribosomal protein Rpl22 allele with a floxed wild-type C-terminal exon followed by an identical C-terminal exon that has three HA epitopes inserted before the stop codon. Thus, in contrast to analog systems in which reporter protein expression is proportional to Drd2 promoter activity, these mice allow for a highly sensitive digital (all or none) detection of Drd2+ neurons throughout the brain. Furthermore, the possibility to perform cell specific translational profiling provides a direct quantification of endogenous Drd2 mRNA translation only in neurons expressing the RiboTag reporter. We used these mice to establish a multimodal map of Drd2+ neurons, establish their complete translational profile and evaluate the impact of chronic antipsychotic treatment on these profiles in the medial prefrontal cortex (mPFC).

### Impact of a GWAS SNP in MIR137 on Neurodevelopment in a Novel Transgenic Mouse Model for Schizophrenia

MengYi Xu, Albert HC Wong

A recent large genome-wide association study identified miR137 as the leading candidate gene for schizophrenia (SCZ). MiR137 directly participates in the regulation of numerous well-characterized genes related to SCZ. MiR-137 is also highly enriched in the brain, particularly in dendritic spines and synapses. Moreover, miR137 regulates signaling pathways crucial for neuronal migration, myelination and GABAergic neurotransmission. These roles suggest the potential of miR137 as a therapeutic target for schizophrenia and other psychiatric disorders, yet little is known regarding the function of miR137 in the etiology of schizophrenia. To address this, we generated a transgenic mouse carrying a miR137 promoter SNP strongly linked to the SCZ-associated miR137 marker identified in the GWAS above. This GWAS single nucleotide polymorphism (SNP) for SCZ has previously been found to be linked with the downregulation of the mature miR137 levels in vitro. Immunohistochemistry will be used to quantify total neuronal and GABA interneuron neuron numbers and location, as well as to quantify astrocytes and white matter integrity. Golgi-cox staining will be use to analyze dendritic architecture and spine morphology/density. Our preliminary results indicate that this miR137 SNP can affect cortical pyramidal neuron dendrite morphology and dendritic spine formation. We hypothesize that the point mutation of miR137 could affect dendritic outgrowth and spine formation and maturation, thereby contributing to the pathoetiology of SCZ.

## Pharmacogenetics

### The Influence of the Genetically Variable alpha4 Nicotinic Receptor on Smoking Cessation Outcomes

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In tobacco users, nicotine exerts its reinforcing effects via brain nicotinic acetylcholine receptors (nAChRs), specifically α4β2 nAChRs, which are also targets of the smoking cessation treatments nicotine replacement therapy (NRT) and varenicline. We examined the effect of the genetic variant rs1044396 (C>T) in the α4 subunit gene (CHRNA4) on smoking cessation using data from a placebo-controlled, NRT patch versus varenicline clinical trial in Caucasian smokers (n= 653). Biochemically-verified abstinence (exhaled CO < 8 ppm) was assessed at 4 time points: week 1, end-of-treatment (EOT), 6-, and 12-month follow-up. As expected, varenicline had the highest efficacy then patch then placebo. There was no overall effect of genotype (CC vs. CT vs. TT) on quit success at any time point (X2-test p=.13-.66), or within the placebo (X2-test p=.69-.97) or NRT (X2-test p=.78-.97) arms. At EOT, among CC individuals, NRT and varenicline were equally efficacious (X2-test p=.90), while varenicline produced higher quit rates vs. NRT for T-allele individuals (CT: OR 2.15, 95% CI 1.17-3.94, p=.01; TT: OR 2.12, 95% CI 1.04-4.34, p=.04). These data suggest that smokers with the CC genotype should get patch (equally efficacious/cheaper/safer), while T allele will benefit more from varenicline. Following replication, this CHRNA4 variation may be useful for optimizing smoking cessation treatments based on genotype, ultimately improving cessation rates and reducing tobacco-associated harm.

### Effects of CYP1A2 and Cholinergic Receptor Muscarinic 1 (CHRM1) Gene Variants on Plasma Ratio of Clozapine to N-desmethylclozapine and Cognitive Performance in Schizophrenia

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**Introduction**: Cognitive deficits are a core feature in individuals with schizophrenia (SCZ) that is associated with severe consequences on their quality of life and recovery process. However, antipsychotic medications show minimal effects on cognitive symptoms of SCZ, including clozapine (CLZ), which is generally recognized to be the most effective antipsychotic. One explanation for the mixed effects of CLZ on cognition has been proposed to involve (1) the conversion of CLZ to a metabolite (NDMC) by a metabolizing enzyme (CYP1A2) and (2) their opposing effects on the muscarinic 1 receptor (CHRM1) that supports cognitive processes. Higher ratios of CLZ/NDMC have been associated with poorer working memory performance,

indicating CLZ/NDMC ratio as a strong predictor of cognitive effects.
Objectives: The present study will investigate the genetic variants of the CYP1A2 and CHRM1 genes contribution to clozapine/NDMC ratio and improvements in cognitive performance, respectively.
**Methods**: Individuals with SCZ on CLZ monotherapy (n=75) will be assessed for performance on seven cognitive domains (MATRICS Consensus Cognitive Battery), CLZ/NDMC ratio, symptom scores (PANSS), and education level. Genetic analyses will include genome-wide coverage of genetic markers to allow for extensive coverage of the genetic variation in the two target genes.
**Progress**: Preliminary analysis on our first cohort (n=30) revealed, that after adjusting for age, education and CLZ/NDMC ratio, rs2075748 of the CHRM1 gene was significantly associated with working memory.
**Implications**: This study has major clinical relevance, as the optimal CLZ/NDMC could be achieved through dietary or pharmacological interventions for the improvement of cognitive symptoms while on CLZ.

## Toxicology

### Characterising the Immune Response to Carbamazepine: Understanding the Development of Idiosyncratic Drug Reactions

Alison Jee (Dept of Pharmacology), Jack Uetrecht (Faculty of Pharmacy, Department of Pharmacology)

Idiosyncratic drug reactions (IDRs) can be serious, life-threatening reactions. While most IDRs appear to be immune-mediated, the exact mechanisms remain unclear. It has long been observed that the most severe forms of IDRs (e.g. toxic epidermal necrolysis or liver failure) occur with a very low incidence, while a similar but milder form of the IDR occurs in response to the same drug with a greater incidence (e.g. maculopapular skin rash, mild liver injury). Medications that cause IDRs may elicit an even milder, subclinical immune response that spontaneously resolves with an even greater incidence. The focus of this study is carbamazepine, which causes a range of serious IDRs involving the skin, liver, and blood. Our hypothesis is that drugs that elicit IDRs cause a subclinical immune response in most patients that resolves through immune tolerance. We have developed a dosing protocol that produces a therapeutic serum level in mice and causes a delayed increase in alanine aminotransferase in PD-1 knockout mice, a model utilising immune checkpoint inhibition to induce idiosyncratic drug-induced liver injury. This is consistent with the hypothesis that carbamazepine-induced liver injury is immune-mediated. Further studies will be performed to characterise the cell populations involved in this injury by histology and qPCR. Ultimately, we aim to find a biomarker to predict whether a drug will cause IDRs, which would save patients' lives and reduce risk during drug development.