**9th Annual Child Health and Human Development Research Day**

**Full Abstract Book**

**Murielle Akpa**

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**May 10, 2024**

**Cruess Amphitheatre and McConnell Atrium**

**Word of Welcome from the Child Health and Human Development Program**

Dear colleagues and friends of the Child Health and Human Development (CHHD) Program at the Research Institute of the McGill University Health Centre,

It is a pleasure and honor to welcome all participants and attendees to the 9th Edition of the **CHHD Research Day**. This significant event brings together the CHHD community to celebrate and highlight the outstanding research conducted by CHHD trainees and research staff. Events like this are crucial inn fostering collaboration, sharing knowledge, and promoting advancements in research. It is a pleasure to witness the commitment of the CHHD community to pushing the boundaries of knowledge. As always, the diversity and quality of abstract received this year are a testament to the excellence within the CHHD community.

This year, a special welcome to Dr. Beatrice Latal whose expertise and contributions to the Child Development Center of the university Children’s Hospital Zurich have undoubtedly enriched the field of child health development. We are fortunate to have her as our keynote speaker and are eager to learn from her insights into the developmental outcomes of newborns and children at risk for neurodevelopmental disorders.

We would like to express our gratitude to all participants, judges, volunteers, and sponsors whose support and dedication have been instrumental in making this event a success. Your contributions are invaluable and underscore the collaborative spirit that defines the CHHD community.

Sincerely,

The CHHD Program

**The CHHD Team / L’Equipe du SEDH**

Dr. Kolja Eppert, Program Leader / Leader du Programme

Dr. Isabelle Gagnon, Program Co-leader / Co-leader du Programme

Dr. Murielle M. Akpa, Interim Program Manager / Gestionnaire de Programme par Intérim

Angela Roussos, Program Assistant / Assistante de Programme

Rosanna Camarda, Program Assistant / Assistante de Programme

**Mot de bienvenue du Programme de Santé de l’Enfant et Développement Humain**

Chers collègues et amis du Programme de Santé de l’Enfant et du Développement Humain (SEDH) à l’Institut de Recherche du Centre Universitaire de Santé McGill,

C’est un plaisir et un honneur d’accueillir tous les participants à la 9e édition de la Journée de Recherche du SEDH. Cet événement rassemble notre communauté pour célébrer et mettre en valeur l’exceptionnel travail mené par les étudiants et le personnel de recherche. Des événements comme celui-ci sont d’excellents catalyseurs de collaboration, de partage des connaissances et de promotion du progrès en recherche. C’est un plaisir de constater l’engagement du SEDH pour la connaissance. La qualité et la diversité des résumés reçus cette année témoigne de l’excellence au sein de notre communauté.

Cette année, nous avons la chance d’accueillir Dre Beatrice Latal, dont l’expertise et les contributions au Centre de Développement de l’Enfant de l’Hôpital pour Enfant de l’Université de Zurich ont sans aucun doute enrichi le domaine. Nous avons la chance de l’avoir comme conférencière et sommes impatients d’en apprendre davantage sur ses connaissances des résultats développementaux des nouveau-nés et des enfants à risque de troubles neurodéveloppementaux.

Nous tenons à exprimer notre gratitude à tous les participants, juges, bénévoles et sponsors dont le soutien et le dévouement ont contribué au succès de cet événement. Vos contributions sont inestimables et soulignent l’esprit de collaboration qui définit la communauté du SEDH.

Sincèrement,

Le Programme de SEDH

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Rosanna Camarda, Program Assistant / Assistante de Programme

**Abstract Review Committee / Comité d’évaluation des Résumé**

Dr. Kolja Eppert

Dr. Isabelle Gagnon

Dr. Murielle M. Akpa

**Research Day Committee / Comité de la Jounée de Recherche**

Dr. Murielle Akpa: CHHD Interim Program Manager / Gestionnaire de Programme par Intérim

Atafeh Masoumipour: CHHD Technical Coordinator / Coordinatrice Technique du Programme

Angela Roussos: CHHD Program Assistant / Assistante de Programme

Rosanna Camarda: CHHD Program Assistant / Assistante de Programme

Yanchen Dong: CHHD Trainee Committee – President / Comité Etudiant du SEDH - Présidente

Diego Loggia: CHHD Trainee Committee – VP Finance / Comité Étudiant du SEDH – VP Finance

Neha Kamath: CHHD Trainee Committee – VP Administration / Comité Étudiant du SEDH – VP Administration

**Program / Programme**

Friday May 10, 2024 / Vendredi 10 mai 2024

8:00 – 15:00

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| --- | --- |
| 8:00 - 9:00 | Registration and Poster set up |
| **Welcome and Opening Remarks** | |
| 9:00 - 9:10 | **Dr. Kolja Eppert** CHHD Program Leader |
| **Keynote Speaker** | |
| 09:10 - 10:10 | **Dr. Beatrice Latal**  **Co-Director, Child Development Center**  University Children’s Hospital Zurich |
| Title | Neurodevelopmental outcome and imaging correlates in children with congenital heart disease |
| 10:10 -10:30 | **Coffee Break - Atrium** |
| 10:30 - 11:15 | **Senior Oral Presentations (8min talk/2min Q&A)** |
| 10:30 - 10:40 | Alice Le Moël (PhD Candidate - Lumedlab) |
| 10:41 - 10:51 | Wajih Jawhar (PhD Candidate – Drs. Nada Jabado and Livia Garza Lab) |
| 10:52 - 11:02 | Nadia Deville-Stoetzel (Post-Doctoral Fellow – Dr. Deborah Da Costa Lab) |
| 11:03 - 11:13 | Mahsa Jalali (Post-Doctoral Fellow – Dr. Janusz Rak Lab) |
| 11:15 - 12:15 | **Poster Presentation Session and Lunch Break** |
| 12:00 – 13:00 |  |
| **CHHD Principal Investigator Presentation** | |
| 13:00 - 13:40 | **Dr. Janusz Rak**  Senior Scientist, CHHD |
| Title | Cancer ‘nanosphere’ – How cancer genes shape the vascular tumour microenvironment? |
| 13:45 - 14:30 | **Junior Oral Presentations (8min talk/2min Q&A)** |
| 13:45 – 13:55 | Zeynep Yalcin (MSc Candidate – Dr. Rima Slim Lab) |
| 13:56 – 14:06 | Jennelle Smith (MSc Candidate – Loydie Jerome-Majewski) |
| 14:07 – 14:17 | Zainab Ahmed (Medical Student – Drs. Patricia Li and Evelyne Constantin Lab) |
| 14:18 – 14:28 | Iris Liu (MSc Candidate – Dr. Indra Gupta) |
| 14:30 – 14:45 | **Coffee Break - Atrium** |
| 14:45 - 15:00 | **Dr. Isabelle Gagnon**: Closing Remarks and Award Presentation |
| 15:00 - 16:30 | **Closed CHHD PI Session** |

**Awards**

**Oral Presentations**

Senior Oral Presentation: $250

Junior Oral Presentation: $250

**Junior Poster Presentations**

First Place: $250

Second Place: $175

Third Place: $100

**Junior Poster Presentations**

First Place: $250

Second Place: $175

Third Place: $100

Employee

**1 - Stress and Quality of Life of Parents of Children with POLR3-related Leukodystrophy**

Laura Lentini1, Geneviève Bernard1, Simon Fournier1, Helia Toutounchi1, Xiaoru Chen1

1Division of Medical Genetics, McGill University Health Centre; Departments of Neurology and Neurosurgery, Pediatrics and Human Genetics; Child Health and Human Development Program, Research Institute of the McGill University Health Centre, Montreal, Quebec, Canada

*POLR3*-related leukodystrophy (or 4H LD) is a rare, genetic, hypomyelinating disorder caused by biallelic pathogenic variants in genes that encode for RNA Polymerase III subunits: *POLR3A, POLR3B, POLR1C,* and *POLR3K*. Clinically, it is characterized by hypomyelination, hypodontia and hypogonadotropic hypogonadism. Disease-onset often occurs in childhood, leading to progressive disability and premature death.

Despite its debilitating nature not only for patients but their caregivers, studies investigating parental stress and quality of life (QoL) of parents of patients with *POLR3*-related LD are especially lacking compared to other diseases. Therefore, the aim of this project was to study the stress and QoL of mothers and fathers of patients with *POLR3*-related LD. We hypothesized that parental stress would be higher and QoL would be lower relative to parents of healthy children, and certain clinical features would influence these scores. Thus, questionnaires and clinical assessments were collected cross-sectionally. Questionnaires assessed parents' well-being, stress-impacting factors, perceptions of injustice, and coping mechanisms. Stress and QoL scores were compared to healthy populations using one-sample t-tests. Perceived injustice scores were compiled into percentile ranges. Correlational and chi-square analyses determined relationships between modifiable factors and parents’ scores.

Parents of children with *POLR3*-related LD had lower QoL compared to normative samples, yet 80% of parents’ stress scores fell within normal ranges. Mothers’ and fathers’ perceived injustice scores were high. Correlations were found between and within mothers’ and fathers’ scores. Helpful coping mechanisms were those where parents were directly involved in their child’s care. Relationships were found between mothers’ stress scores, years since disease onset and certain life circumstances.

While stress scores remained normal, QoL scores for parents with children with *POLR3*-related LD was shown to be lower than parents of healthy children. These results show the importance of implementing services and social support to improve the QoL of these parents.

MSc Candidate

**2 - A Report of Two Homozygous TERB1 Protein-Truncating Variants In Two Unrelated Women With Primary Infertility**

Eric Bareke1, Amira Nabil2, Seang-Lin Tan3, 4, Ibrahim M. Abdelrazek2, Corinna Friedrich5, Manqi Liang1, Zeynep Yalcin1, Ebtesam Abdalla2, Frank Tuttelmann5, Rima Slim2, 4, 6, Jacek Majewski1

1Department of Human Genetics, McGill University Health Centre, Montreal, QC, Canada., 2Department of Human Genetics, Medical Research Institute, Alexandria University, Alexandria, Egypt., 3OriginElle Fertility Clinic, Montreal, QC, Canada., 4Department of Obstetrics and Gynecology, McGill University, Montreal, QC, Canada., 5Institute of Reproductive Genetics, University of Münster, 48149, Münster, Germany., 6Research Institute of the McGill University Health Centre, 1001 Décarie Blvd, Montréal, Québec, H4A 3J1, Canada.

Purpose: To investigate the genetic etiology of patients with female infertility.

Methods: Whole exome sequencing was performed on genomic DNA extracted from the patient's blood. Exome data were filtered for damaging rare biallelic variants in genes with possible roles in reproduction. Sanger sequencing was used to validate the selected variants and segregate them in family members.

Results: A novel homozygous likely pathogenic variant, c.626G>A, p.Trp209\*, was identified in the *TERB1* gene of the patient. Additionally, we report a second homozygous pathogenic *TERB1* variant, c.1703C>G, p.Ser568\*, in an infertile woman whose azoospermic brother was previously described to be homozygous for her variant.

Conclusions: Here, we report for the first time two homozygous likely pathogenic and pathogenic TERB1 variants, c.626G>A, p.Trp209\* and c.1703C>G, p.Ser568\*, respectively, in two unrelated women with primary infertility. TERB1 is known to play an essential role in homologous chromosome movement, synapsis, and recombination during the meiotic prophase I and has an established role in male infertility in humans. Our data add TERB1 to the shortlist of Meiosis I genes associated with human infertility in both sexes.

PhD Candidate

**3 - Endometriosis, Anxiety, and Atherosclerosis: A study of eight million young hospitalized women in United States**

Hormoz Nassiri Kigloo1, Eva Suarthana1, Togas Tulandi1, Tina Montreuil2

1Department of Obstetrics and Gynecology, McGill University, 2Department of Psychiatry McGill University, Montréal, Québec, Canada

**Background:** In recent years, several studies have proposed an association between endometriosis and various cardiovascular diseases.

**Objective:** Our study evaluated the association between endometriosis, anxiety, and atherosclerosis using a large population database.

**Design:** This was a retrospective population-based study.

**Setting**: We used the data of more than eight million hospitalized women from the Healthcare Cost and Utilization Project (HCUP) databases between 2007 and 2014, in the United States. The prevalence of endometriosis, atherosclerosis, and related conditions, such as psychiatric conditions, were estimated, and logistic regression model was used to examine the association.

**Participants**: Individuals under 35 years of age who were registered in one of the hospitals participating in the HCUP during the study period of 2007 to 2014. Patients with cancer were excluded from the study.

**Interventions**: We compared the prevalence of endometriosis and anxiety in patients with atherosclerosis.

**Results:** In the period of study, we noted an upward pattern for the prevalence of atherosclerosis and a downward trend for endometriosis. Adjusting the analysis for sociodemographic characteristics and comorbidities, the probability of being diagnosed with atherosclerosis was 43% higher in patients with endometriosis (OR= 1.430; 95%CI 1.065-1.920); 35% higher in patients with anxiety (OR= 1.349; 95%CI 1.246-1.461); and 3 times higher in women with both endometriosis and anxiety (OR= 3.076; 95%CI 1.971-4.806) compared to women without those conditions.

**Conclusion:** The strong association between endometriosis and atherosclerosis suggests that they may share a similar mechanism possibly endothelial dysfunction related to chronic inflammation. Further studies on the potential role of psychiatric conditions, such as anxiety, on systemic inflammatory diseases are also deemed timely and important.

PhD Candidate

**4 - Usability of the Patient-Generated Index (PGI): Insights from Children, Young People, and Clinicians in the Context of the Long-term Follow-up of Esophageal Atresia**

Dan Poenaru1, 2, Zanib Nafees1, 2, Julia Ferreira1, Nancy Mayo3, Elena Guadagno1, Nikki Ow4

1Harvey E. Beardmore Division of Pediatric Surgery, The Montreal Children’s Hospital, McGill University Health Centre, Montreal, Quebec, Canada, 2Department of Surgical and Interventional Sciences, McGill University Faculty of Medicine and Health Sciences, Montreal, Quebec, Canada, 3School of Physical & Occupational Therapy, James McGill Professor, McGill University Health Centre, 4Occupational Science and Occupational Therapy, Faculty of Medicine, The University of British Columbia

**Background:**

This study investigates the quality of life (QoL) of pediatric patients following neonatal repair for Esophageal Atresia (EA) using the Patient-Generated Index (PGI) alongside standard measures. The PGI assesses individualized perceptions of QoL, while the standard measures assess health-related QoL.

**Study design:**

The study used 3 Patient-Reported Outcome Measures (PROMs): PGI, EuroQol-5D (Youth) (EQ-5D-Y), and the pediatric Patient-Reported Outcome Measurement Information System (PROMIS). These measures were answered by children aged 3-17 years who underwent EA repair at the Montreal Children's Hospital. Cognitive interviews with children and parents assessed PGI completion experience, while interviews with clinicians determined the optimal measure for EA outcomes.

**Results:**

The PGI identified 58 text threads from 15 interviews, covering 14 impairments, 15 activity limitations, and 3 environmental factors. Seven out of the 58 text threads were “looking after one's health,” highlighting a strong focus on health management. QoL scores from the PGI differed from standardized measures, with weak correlations observed between the PGI and both EQ-5D-Y (*r* = 0.20) and PROMIS (*r* = 0.11). The PGI demonstrated construct validity, aligning with relevant disease-specific QoL measures. Patient feedback on the PGI was generally positive, with 8 finding it straightforward, while 2 encountering difficulties with the coin allocation system. Clinicians also provided perspectives on the usability of the measures.

**Conclusion:**

The study highlights the PGI's value in capturing the QoL of children and young people following EA repair. The PGI holds promise for improving communication between healthcare providers and pediatric patients, and guiding personalized treatment decisions in this unique population.

PhD Candidate

**5 - Novel Raman spectroscopy fiber optics system enables real-time intraoperative focal cortical dysplasia detection**

Trang Tran1, Roy Dudley2, Jason Karamchandani2, Alice Le Moël1, 3, Alexander Weil4, Frederic Leblond1, 3

1University of Montréal Hospital Research Center, Montréal, QC, Canada, 2Montréal Children's Hospital, Montréal, QC, Canada, 3Polytechnique Montréal, Montréal, QC, Canada, 4University Hospital Complex Sainte-Justine, Montréal, QC, Canada

**Purpose.** Focal cortical dysplasia (FCD), a malformation of cortical development, is the most common cause for medically refractory focal epilepsy in children. Medications to control seizures exist, however, the failure rate is approximately 30%. Surgery to resect the epileptogenic zone (EZ; the region of the brain producing the seizures) is the most effective treatment for refractory epilepsy, if this region of the brain can be found and removed safely (i.e., without causing harm). Yet, sufficiently detecting the EZ in the case of FCD remains extremely challenging, even with the most advanced neuroimaging and electrophysiological techniques, because in many cases FCD cannot be seen on MRI, and if it is seen, its borders remain poorly defined. Complete resection of the EZ is necessary to allow for seizure freedom, but this occurs in only 40-60% of cases; sometimes three or more follow-up surgeries are required. Thus, new techniques for detecting FCD and its borders are needed. Raman spectroscopy (RS) is a non-destructive, label-free optical method that allows for the molecular characterization of tissues. In a previous ex vivo study, we demonstrated the ability of RS to distinguish between FCD and normal tissue with a sensitivity, specificity, and accuracy of 100%, 95%, and 96%, respectively. The goal of this study is to investigate, in vivo (i.e., during epilepsy surgeries), the ability of RS to distinguish FCD from normal brain tissue to develop a machine learning algorithm which could be used as a real-time, intraoperative FCD detection probe. We aim to assess 2500 specimens from 250 patients over the next 5 years.

**Methods.** Based on each patient’s presurgical evaluation (i.e., multiple neuroimaging and electrophysiology tests), a preoperative surgical plan is made to resect the hypothesized EZ (hEZ). Within this hEZ, the surgeon selects areas of interest (e.g., from the epicenter, borders, and at different depths) and takes an RS measurement with a single point probe before biopsying these individual tissue regions and sending for histopathological analysis. Individual Raman spectra are paired with their corresponding histopathologic labels, RS bands showing differences between FCD and normal are selected, and a support vector machine (SVM) learning model is trained based on these peaks. Sensitivity, specificity, and area under the curve (AUC) are calculated.

**Results.** 145 raman spectra and corresponding surgical specimens were acquired from 19 pediatric patients with intractable focal epilepsy. The results acquired thus far show similarities with our previous ex vivo study. We are able to distinguish tyrosine, phenylaniline, amide and lipides bands associated with FCD and normal brain. An SVM learning model trained with four of these features (1119 cm-1 (lipids), 1171 cm-1 (tyrosine), 1211 cm-1 (C-H methine deformation) and 1251 cm-1 (amide III)) achieved a sensitivity of 74% and a specificity of 78% for classifying FCD from normal brain, with an AUC of 0.81.

**Conclusion.** These preliminary findings suggest that the use of single-point Raman spectroscopy probe can aid delineating FCD borders, which may allow for more complete and safe surgical resections, thus improving surgery outcome for children with focal epilepsy.

MSc Candidate

**6 - POLR3-related leukodystrophy: A Qualitative Study on Parents’ Experiences with the Healthcare System**

Adam Le1, 2, Kelly-Ann Thibault1, 2, Maxime Morsa3, Geneviève Bernard1, 2, 4, 5, 6

1Child Health and Human Development Program, Research Institute of the McGill University Health Centre, Montreal, Canada, 2Department of Neurology and Neurosurgery, McGill University, Montreal, Canada, 3Adaptation, Resilience, and Change Research Unit, Université de Liège, Belgium, 4Department of Pediatrics, McGill University, Montreal, Canada, 5Department of Human Genetics, McGill University, Montreal, Canada, 6Division of Medical Genetics, Department of Specialized Medicine, McGill University Health Centre, Montreal, Canada

RNA polymerase III-related hypomyelinating leukodystrophy (POLR3-HLD) is a rare, genetically-determined, neurodegenerative disorder affecting the white matter of the central nervous system. It is caused by biallelic pathogenic variants in genes encoding subunits of RNA polymerase III (Pol III), namely *POLR3A*, *POLR3B*, *POLR1C*, *POLR3K*, and *POLR3D*. POLR3-HLD is known colloquially as 4H leukodystrophy due to its hallmark clinical features of hypomyelination, hypodontia, and hypogonadotropic hypogonadism. Disease onset typically occurs in childhood and runs a progressive, debilitating, and often fatal course. Patients with this disorder require complex and specialized care, however, due to its rarity and limited widespread awareness, parents are often required to assume additional roles as experts and advocates for their child(ren). Here, we aimed to understand the impact of POLR3-HLD, illustrate parents’ experience navigating the healthcare landscape, and identify potential targets for improvement. 19 semi-structured interviews were conducted with an international cohort of 24 parents to obtain broad perspectives, and this data was analyzed using reflexive thematic analysis to identify patterns of themes that address the research question. Four themes were identified: existing barriers in accessing care, limited knowledge in diagnosis and care, parents as experts and advocates of their child(ren)’s care, and perceived superior care by leukodystrophy specialists. Many parents expressed feeling alone and uncertain with little guidance provided to them. They also identified perceived gaps in their care and challenges they faced which they attributed to the perceived lack of understanding among physicians and healthcare providers regarding POLR3-HLD. They however acknowledged the superior care received at LCEs and found comfort when being treated by leukodystrophy experts in specialty clinics. This study will help better inform healthcare providers, administrators, and policymakers to expand and improve access to quality care for POLR3-HLD patients and their families.

PhD Candidate

**7 - EZHIP; A Novel Oncogene in Osteosarcoma**

Dorothée Dal Soglio1, Nada Jabado2, 3, 4, Claudia L. Kleinman3, 5, Andrea Bajic2, 3, Robert E. Turcotte6, Takeaki Ishii7, Sungmi Jung8, Geoffroy Danieau6, 7, Wajih Jawhar2, 7, 9, Alva Annett3, 5, Ahmed Aoude6, Livia Garzia3, 6, 7

1Department of Pathology, Centre Hospitalier Universitaire Sainte-Justine, Université de Montréal, Montréal, Québec, H3T 1C5, Canada, 2Child Health and Human Development Program, The Research Institute of the McGill University Health Center. Montreal, Quebec, H4A 3J1, Canada, 3Department of Human Genetics, McGill University, Montreal, Quebec, H3A 0C7, Canada, 4Department of Pediatrics, McGill University and the Research Institute of the McGill University Heath Centre, Montreal, Quebec, H4A 3J1, Canada, 5Lady Davis Research Institute, Jewish General Hospital, Montreal, Quebec, H3T 1E2, Canada, 6Department of Surgery, Division of Orthopedic Surgery, McGill University Health Centre, Montreal, Quebec, H3G 1A4, Canada, 7Cancer Research Program, The Research Institute of the McGill University Health Center, Montreal, Quebec, H4A 3J1, Canada, 8Department of Pathology, McGill University Health Centre, Montreal, Quebec., 9Division of Experimental Medicine, Department of Medicine, McGill University, Montreal, Quebec, H4A 3J1, Canada

Osteosarcoma (OS) is an aggressive mesenchymal cancer annually effecting 3-4.5/million individuals worldwide. Incidence rates peak during the pubertal growth spurt as mesenchymal progenitors expand and differentiate to form new bone, suggesting that OS is rooted in altered development. Despite comprehensive characterization of the OS somatic genome, improvements in clinical care have been stagnant and survival rates have not drastically improved in the past 30 years. Conversely, the OS epigenome remains largely uncharted. Given the role of epigenetics in orchestrating development, we hypothesize that epigenomic perturbations play a crucial role in driving OS. Upon evaluating the expression of oncohistones and the oncohistone-mimic EZHIP in 2 independent OS cohorts, we discovered that EZHIP is ectopically expressed in 22% of high-grade tumors. This correlated with the loss of the repressive histone mark H3K27me3. Surprisingly, global loss of H3K27me3 significantly correlated with poor response to neoadjuvant chemotherapy. CRISPR/Cas9 knockout and lentiviral-based overexpression demonstrated that EZHIP is a prominent oncogene in OS xenograft models. Transcriptomic analyses revealed that EZHIP activates oncogenic pathways and maintain stemness programs that permit the expansion of progenitor cells. Testing the differentiation capacity of EZHIP-expressing human mesenchymal stem cells, the putative cell of origin of OS, revealed that EZHIP blocks differentiation into specialized cells such as mature osteoblasts. Instead, transcriptome analysis suggests an expansion of professional cytokine secreting pre-osteoblastic cells that are thought to first expand during puberty in the bone marrow. Finally, although EZHIP promotes an aggressive phenotype, consequential loss of H3K27me3 resulted in enhanced sensitivity to EZH2 inhibitors and degraders including the FDA-approved drug Tazemetostat. Our study reveals that EZHIP and H3K27me3 loss may constitute a novel mechanism of OS pathogenesis by locking mesenchymal progenitors in a cycling state permissive of oncogenic transformation. Further research is warranted to explore targeting EZHIP and H3K27me3 loss for future treatment options.

Research/Medical Fellow

**8 - Cerebral Saturation and Fractional Tissue Oxygen Extraction Associated with Anterior Cerebral Artery Doppler Parameters in Neonates with Congenital Heart Defects**

Gabriel Altit1, Pasinee Kanaprach2

1Neonatal Staff, 2Neonatal Hemodynamic Clinical Research Fellow

**Objectives**: The resistive index (RI) and pulsatility index (PI) of the anterior cerebral artery (ACA), which were assessed via Doppler ultrasound, are blood flow indicators. Cerebral saturation (CSat), measured using near-infrared spectroscopy (NIRS), reflects venous-weighted oxygen saturation. Cerebral fractional tissue oxygen extraction (FTOE) assesses oxygen consumption based on pre-ductal oxygen saturation (SpO2) at CSat measurement. Our objective was to explore the instantaneous relationship between CSat/FTOE and ACA-RI/PI.

**Methods**: This is a prospective study of newborns ≥35 weeks with congenital heart disease (CHD) requiring post-natal intensive care. Daily ACA-Doppler from day 1 to 7 was performed. Continuous CSat/SpO2 monitoring enabled concurrent value retrieval. Doppler data extraction was blinded to NIRS values and participant status.

**Results**: Data from 137 observations on CSat, RI, PI, and cerebral FTOE during the first week of life were collected from 34 patients out of 135 screened. Males constituted 62% of the cohort, with a mean birthweight of 3.2 ±0.6 kg and a gestational age of 38.5 ±1.4 weeks. The mixed effect model showed a significant association between CSat/FTOE and the time-corresponding RI of ACA (p <0.035 and 0.01, respectively). A 2.4% CSat decrease corresponded to a 0.1-point RI-ACA increase (-4.7; -0.2%) and a 3.1-point cerebral FTOE rise (0.7; 5.5). Graphic trends of RI/PI inversely mirrored those of CSat/FTOE.

**Conclusion**: In neonates with CHD within the first week, a significant association exists between RI/PI-ACA, instantaneous CSat and cerebral FTOE. Lower cerebral NIRS and higher cerebral FTOE coincided with elevated RI/PI values of ACA. Future studies should assess whether addressing low CSat or high cerebral FTOE may decrease brain injury burden in this population.

MSc Candidate

**9 - Role of Citrate in the Production of Nitric Oxide During Human Sperm Capacitation**

Diego Loggia1, 2, 3, Cristian O'Flaherty1, 2, 3, 4, 5

1Department of Pharmacology & Therapeutics, 2McGill University, 3Research Institute-MUHC, 4Department of Surgery, 5Department of Anatomy & Cell Biology

Sperm capacitation involves a series of biochemical and morphological changes which are necessary for the spermatozoon to recognize and fertilize the oocyte. The process of sperm capacitation requires the production of low levels of nitric oxide (NO∙), an increase in tyrosine phosphorylation, and sufficient levels of energy metabolites such as citrate. Citrate is abundant in the seminal plasma of fertile men, with low levels being reported in some cases of male infertility. However, the role of citrate in sperm capacitation is largely unknown. Mitochondrial citrate can be exported to the cytosol via the mitochondrial citrate transport protein (CIC). This cytosolic citrate is used as a substrate by ATP-citrate lyase (ACLY) to produce acetyl-CoA and oxaloacetate. Oxaloacetate can then be converted by malate dehydrogenase into malate, which is subsequently converted by the malic enzyme (ME) to yield pyruvate and NADPH. This resulting NADPH produced from citrate metabolism may be used to produce NO∙ via nitric oxide synthase (NOS). We hypothesize that cytosolic citrate supports human sperm capacitation through NO∙production.

The supplementation of spermatozoa with citrate supported sperm capacitation, yet excessive citrate decreased sperm capacitation and impaired sperm motility but not viability. Citrate levels in capacitated spermatozoa were decreased after the 3.5-hour capacitation process, but less citrate was consumed upon incubation with the CIC inhibitor. CIC, ACLY, and ME inhibition prevented FCSu-induced capacitation without affecting total sperm motility or viability. Capacitation levels decreased by adding the NOS inhibitor L-NAME to the capacitation media, both in the presence and absence of citrate. Citrate supported NO∙production in capacitating spermatozoa, but NO∙levels were decreased following ACLY inhibition.

This research has helped to understand citrate metabolism in sperm capacitation and provided the basis to design novel treatment strategies for male infertility.

PhD Candidate

**10 - Lipid Rafts are Signalling Platforms for Intracellular NO• Production During Human Sperm Capacitation**

Cristian O'Flaherty1, 2, 3, 4, Steven Serafini1, 2, 3

1Department of Medicine (Experimental Medicine Division), 2Department of Surgical and Interventional Sciences (Experimental Surgery), 3Research Institute of McGill University Health Center (RI-MUHC), 4Department of Anatomy and Cell Biology McGill University

Worldwide, 17% of couples struggle to conceive due to infertility. Males account for over 50% of these cases, of which 34% are idiopathic. Fertility clinics do not evaluate essential sperm functions like sperm capacitation. The spermatozoon must undergo sperm capacitation to become fertile. The role of lipid signaling in sperm capacitation is largely unknown. Lipid rafts are highly ordered sphingolipid and sterol-rich platforms facilitating protein-protein and protein-lipid interactions. These rafts harbor the components involved in signaling transduction cascades (e.g., G-protein receptors) and membrane trafficking. Sphingomyelin (SM), and Ceramide (Cer) are the two most abundant sphingolipids in lipid rafts. Sphingosine (Sph), Cer, and their phosphorylated forms S1P and C1P are major bioactive signaling lipids implicated in cell viability, germ cell maintenance, reactive oxygen species (ROS) production, and acrosome reaction. We hypothesized that lipid rafts serve as platforms for the organization and regulation of proteins and enzymes that regulate tyrosine (P-Tyr), PI3K (P-PI3K) phosphorylations, and NO● production needed for sperm capacitation. Our objectives were: 1) To determine whether alterations of lipid rafts promote sperm capacitation and its associated phosphorylations and NO● production; 2) To assess the assembly of sphingolipid signaling complexes with lipid rafts and their role in regulating NO● production during human sperm capacitation.

Highly motile human spermatozoa were incubated in BWW medium for 4h at 37°C, with or without our Fetal Cord Serum ultrafiltrate (FCSu, capacitation inducer), Sph or Cer, and pharmacological inhibitors of SphK1/2, CERK, S1PR1/3, PKR, PI3K, AKT, and L-NAME (nitric oxide synthase inhibitor). P-Tyr and P-PI3K levels were determined by immunoblotting. Protein localization was performed using immunocytochemistry. Lipid rafts were modulated using methyl-β-cyclodextrin (MβCD) and analyzed by immunoblotting. NO● production (DAF2-DA probe) was assessed by flow cytometry.

Sph, Cer, and S1P increased P-Tyr, P-PI3K levels. P-SphK1, P-PKR, co-localized with a lipid-raft marker CD59 in the post-acrosomal region in capacitated spermatozoa. Inhibition of PKR decreased P-SphK1 levels. Inhibition of S1PR1, localized in the post-acrosomal region in capacitated spermatozoa, decreased P-Tyr and P-PI3K. NO● is produced downstream of sphingolipid and MβCD signalling.

In conclusion,male infertility may be caused by dysfunction of sphingolipid signalling during capacitation. These studies will allow the development of novel diagnostic tools and treatments for male infertility.

Research/Medical Fellow

**11 - Risks of Malignancy in Patients with Endometrial Polyp: A Systematic Review and Meta-Analysis.**

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Although the majority of endometrial polyps are benign, there exists a subset that carries malignant potential. Therefore, this systematic review and meta-analysis aimed to evaluate the prevalence of endometrial premalignancy/malignancy and factors associated with endometrial malignancy in women with endometrial polyps.

We systematically conducted electronic database research on PubMed, MEDLINE, EMBASE, COCHRANE, and Google Scholar from inception until August 2022 (PROSPERO registration CRD42022361378). Studies of peri and postmenopausal women over 45 years with endometrial polyp were included. We performed a meta-analysis to combine effect estimates as odds ratios (OR) with their 95% confidence intervals (CI). Quality of the studies were evaluated.

Of 1753 identified articles, 20 were included in the meta- analysis. Of 11204 patients with endometrial polyp, we found 287 malignant polyps (2.75%), 182 (1.8%) hyperplasia with atypia, and 520 (5.2%) hyperplasia without atypia within the polyp. Menopausal women had a higher likelihood of pre-malignancy/malignancy than non-menopausal women (OR 5.63 (95CI 3.87, 8.20, I2=0%, P<0.001). Sensitivity analysis by excluding the largest study yielded similar results: (OR 5.76 (95CI 3.65, 9.08, I2=0%, P<0.001). Endometrium in pre-malignancy/malignancy cases was significantly thicker than in the benign polyp (mean difference 4.21 mm, 95% CI 0.77 to 7.64 mm, I2=18%, P=0.02). Women who used tamoxifen or hormone replacement therapy (HRT) had a lower likelihood of endometrial pre-malignancy/malignancy, while women with abnormal uterine bleeding (AUB) had a higher probability of pre-malignancy/malignancy, but the sensitivity analyses suggested their statistical significance were driven by the largest studies.

To conclude**,** menopausal age and thickened endometrium were significantly associated with malignancy changes within endometrial polyps. Our analysis also suggested a reduced likelihood of endometrial malignancy by the use of tamoxifen or HRT, and increased likelihood among those with AUB. Therefore, it is imperative to be vigilant in the management of endometrial polyps, particularly in women exhibiting these characteristics.

PhD Candidate

**12 - Aperiodic Epileptogenic Dynamics in Pediatric Drug-Resistant Epilepsy**

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**Rationale:** Despite the availability of anti-seizure medications, ~1/3 of epilepsy patients continue to suffer from seizures, and surgery remains the only potential curative treatment option. Such drug-resistant cases can extend our understanding of seizure generation and thus improve our identification and delineation of focal seizure onset zones (SOZ). The aperiodic components of neurophysiological activity are indicators of excitation/inhibition balance in brain circuits, which is disrupted during a seizure. We hypothesize that focal perturbations in aperiodic activity occur as a patient approaches a seizure and thus can act as a marker of the SOZ.

**Method:** For 14 (6 male, 4-16 years) pediatric drug-resistant epilepsy patients who underwent presurgical evaluation, we applied the Spectral Parameterization Resolved in Time (SPRiNT) algorithm to extract dynamic aperiodic activity (offset and exponent) of seizures captured non-invasively with magnetoencephalography and intracranial electroencephalography (iEEG). SPRiNT was applied to each of the iEEG contacts and cortical MEG sources.

**Results:** Preliminary results from iEEG recordings show that seizure onsets are preceded by a decrease of the exponent in regions involved in early ictal manifestations. These observations suggest a possible increase in excitatory activity immediately before seizure onset.

**Discussion:** Dynamics in aperiodic activity have the potential to lead toward a new generation of temporal and spatial markers of seizure origination. Such markers would not require the labor-intensive review of recordings by clinical experts. Our approach may also provide a deeper mechanistic insight into the mesoscopic neurophysiological mechanisms of epilepsy and inform more specific and automatic markers of seizure generation.

PhD Candidate

**13 - The Impact of Gender on Pediatric Surgical Care in Africa**

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**Introduction**

Girls, whose care is often affected by barriers steeped in gender inequity, may be at higher risk of poor surgical outcomes. This study explored the impact of gender on pediatric surgical care in Africa.

**Methods**

Differences in access to care and clinical outcomes for boys and girls were examined for pediatric surgical conditions that do not differ by physiological sex. A systematic review of African pediatric surgical studies ensued, followed by a random effects meta-analysis, and risk of bias assessment.

**Results**

Of the 12281 records retrieved, 54 were selected for review. Most studies were retrospective (57.4%), single-site (94.4%), from Egypt, Nigeria, Ghana, or Ethiopia (55.6%), focussed on gastrointestinal conditions (63.0%), published in 2010 or sooner (85.1%), had study durations of 5 years or less (68.5%), and cohorts of less than 200 children (57.4%). Sixty percent reported the outcome of mortality. Meta-analysis odds ratios revealed surgery was performed 3.6 times more often on boys (95% CI: 2.6, 4.9); and mortality was 1.6 times greater for girls (95% CI: 1.3, 2.0).

**Conclusion**

African girls appear to face gender inequities in pediatric surgical care. Findings will be further explored in a mixed-methods study.

MSc Candidate

**14 - Protein CoAlation: A Novel Posttranslational Modification Involved in Human Sperm Capacitation**

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1Department of Pharmacology & Therapeutics, 2Surgery Department (Urology Division), McGill University., 3The Research Institute, McGill University Health Centre.

**INTRODUCTION**

Infertility is rising worldwide, affecting 17% of couples, with half due to male factors. Capacitation, the process through which spermatozoa gain fertilizing function in the female reproductive tract, relies on precise redox signaling, and any dysregulation results in infertility. We have discovered that protein CoAlation, a novel protein modification mediated by Coenzyme A, is involved in the sperm’s antioxidant defense, but its role in redox signaling in capacitation is unknown. This study aims to determine the localization of protein CoAlation and the impact of altered CoASH synthesis on sperm capacitation.

**METHODS**

Highly motile spermatozoa from healthy volunteers were used. Localization of PANK2 and CoASH synthase (COASY) was assessed by subcellular sperm fractionations and immunocytochemistry using spermatozoa incubated with or without oxidative stress induced by 0.5 mM H2O2. To study the effect of altered CoASH synthesis on capacitation, spermatozoa were capacitated with 10% fetal cord serum ultrafiltrate (FCSu) in the presence or absence of different concentrations of pantothenic acid or PANK2 inhibitor for 4h at 37°C. Capacitation was determined by assessing protein tyrosine phosphorylation (P-Tyr) using immunoblotting.

**RESULTS**

CoAlated proteins are primarily present in the treated Triton-soluble and -insoluble fractions. Interestingly, a 45kDa band of CoAlated proteins was also observed in non-treated spermatozoa. Protein CoAlation was observed in the post-acrosomal region and the flagellum of spermatozoa under oxidative stress. PANK2 inhibition increased, and pantothenic acid supplementation decreased P-Tyr levels in capacitated spermatozoa compared to controls only capacitated with FCSu.

**CONCLUSIONS**

CoASH levels modulate sperm capacitation. CoAlated proteins localized in the sperm head and flagella may also be involved in regulating its genetic material and motility, respectively. Ongoing proteomic analysis aims to identify capacitation-related proteins prone to coAlation and to characterize the entire sperm CoAlome during oxidative stress. These findings will aid in developing novel diagnostic tools for male infertility.

PhD Candidate

**15 - ANDROGEN MODULATION OF NEUROBEHAVIORAL OUTCOMES IN GBS-INDUCED CHORIOAMNIONITIS**

Marie-Julie Allard1, Guillaume Sébire1, Bernard Robaire1, Mathilde Chevin1, Seline Vancolen1

1McGill

Group B *Streptococcus* (GBS) infection in the placenta, known as chorioamnionitis, is linked to elevated risks of neurobehavioral deficits in offspring, including autism spectrum disorders, which are more prominent males than females. In our preclinical chorioamnionitis model, males exhibited heightened placental inflammation compared to females, correlating with more severe subsequent neurobehavioral impairments. We hypothesize that androgens upregulate the placental immune response in male fetuses, potentially contributing to GBS-induced autistic traits in male offspring. Our previous findings demonstrated reduced pro-inflammatory cytokines and polymorphonuclear cell infiltration in flutamide (androgen receptor antagonist)-infected compared to vehicle-infected placenta. Expanding on the impact of androgens in our model, we investigated brain injury patterns and neurobehavioral outcomes. Lewis dams received daily injections of corn oil (vehicle) or flutamide from gestational day (G) 18 to 21, followed by saline (non-infected) or GBS (infected) injection on G 19. Behavioral assessments carried out on offspring from postnatal day (P) 9 to 40 revealed impaired communication and reduced social interaction length in infected rats compared to non-infected. Interestingly, flutamide-infected rats exhibited increased social interaction duration compared to vehicle-infected counterparts. Histological analysis of forebrains at P50 indicated trends toward lateral ventricle enlargement and reduced white matter thickness, namely the corpus callosum and external capsule in GBS-infected groups as well as a partial effect with flutamide treatment in infected groups. These findings suggest a potential role for androgens in the skewed sex ratio observed in developmental impairments resulting from perinatal infection/inflammation.

Post-doctoral Fellow

**16 - Murine-derived 3D endometrial organoid model to investigate the role of Nodal signaling in uterine receptivity**

Daniel Dufort1, 2, 3, Laurie Pinel1, 2, 3

1Department of Obstetrics and Gynecology, McGill University, 2Child Health and Human Development Program, 3Research Institute of the McGill University Health Centre

Implantation is a crucial step for the establishment of pregnancy and is dependent on a precise synchronization between the blastocyst and uterus. This is regulated by cellular communication and several signaling pathways. Nodal, a member of the TGF-β family is expressed throughout the glandular epithelium of the endometrium during the peri-implantation period. We showed that uterine inhibition of Nodal leads to implantation failure in 50% of knockout females (NodalKO). Since the uterine epithelium needs to switch from a non-receptive to a receptive state during this period to allow the implantation of the blastocyst, we hypothesized that Nodal plays a role in the regulation of uterine receptivity. **Objective**. The aim is to study the role of Nodal in uterine receptivity. **Methods**. Uterine epithelial cells were isolated from control and NodalKO on day 4.5 of pregnancy (time of implantation) to generate 3D organoid cultures. The number and size of organoids were assessed, and immunofluorescence staining were performed to characterize the organoids. Qualitative-PCR was used to assess the expression of genes implicated in the receptivity of the uterine epithelium. **Results**. The number of organoids was significantly higher in cells isolated from NodalKO uteri compared to the cell culture generated from control mouse uteri, while the sizes were not different. Organoids from control and NodalKO were positively stained for an epithelial cell marker (Ck7), a glandular cell marker (Foxa2) and for the estrogen receptor α. Qualitative-PCR after 7 days of culture indicated a deregulation of several genes (*IHH, ITGAV, ITGB3, MSX1, LTF, ESR1, PGR*) with important functions in establishing the receptive state of the uterus in organoids from NodalKO. **Conclusion**. Our data indicate that Nodal plays an important role into uterine receptivity in the mouse and our next step will be to evaluate its role in the ability of the uterus to support blastocyst adhesion.

PhD Candidate

**17 - Immunotolerance at the maternal-fetal interface during placental development is regulated by uterine Nodal expression**

Daniel Dufort1, 2, Sarah Yull1, 2

1McGill University, 2Research Institute of the McGill University Health Centre

The maintenance of an immunotolerant state at the maternal-fetal interface is necessary to promote placental and fetal development and is highly regulated by leukocytes within the decidua. The dysregulation of immune populations and their secreted factors has been implicated in many pathological pregnancy complications. Recently, our lab has identified a role for the secreted morphogen Nodal during pregnancy as its conditional uterine deletion in mice resulted in significant subfertility. This was shown as severe implantation failure, fetal loss, preeclampsia-like phenotypes, fetal growth restriction and a greater susceptibility for LPS-induced preterm labour. It was hypothesized that these reproductive complications were immune mediated, and that uterine Nodal expression was necessary for maintaining immunotolerance. **Objective**: Characterize the immune landscape of Nodal-deficient females on d10.5 of pregnancy, which coincides with the time of placentation and fetal loss/growth restriction phenotypes. **Methods:** The level of expression of 84 inflammatory genes in Nodal knockout mice will be assessed by RT-qPCR, and flow cytometry will quantify the abundance of decidual leukocytes. Single-cell RNA sequencing of CD45+ cells will provide extensive data on leukocyte expression profiles. **Results:** On d10.5, Nodal knockout females had increased expression of 25 cytokines, TLR4 pathway components and macrophage-associated factors. Further characterization of leukocyte populations revealed a shift towards the pro-inflammatory “M1” macrophage state in Nodal-deficient mice. Current work is now focused on proposing a mechanism of immunomodulation by Nodal signaling. **Conclusion:** The balance between macrophage polarization is critical for the success of artery remodelling and trophoblast invasion, and a dysregulated response has been associated with several pathological complications of the placenta. The sustained, pro-inflammatory state during the expected time of anti-inflammatory tolerance in the Nodal-deficient model demonstrates that Nodal is an important immunomodulator throughout pregnancy. Here, we provide new insights into the mechanisms of placentation, with the overall goal of improving pregnancy outcomes.

MSc Candidate

**18 - Characterization of the role of Cripto in trophoblast cells during placental development**

Daniel Dufort1, 2, Neha Kamath1, 3

1Child Health and Human Development Program, Research Institute of the McGill University Health Centre, 2Department of Obstetrics and Gynecology, McGill University, 3Division of Experimental Medicine, McGill University

Cripto-1, a member of the Epidermal Growth Factor-CFC (*EGF-CFC1*) protein family, plays a crucial role in embryonic development. In addition to embryonic expression, Cripto is expressed in extraembryonic tissues including various trophoblast cell subtypes, including spongiotrophoblast and labyrinth sinusoidal giant cells. In human studies, dysregulation of Cripto expression adversely affects placental development and function, contributing to conditions like placenta accreta and placenta previa. Genetic studies link the EGF-CFC1 gene family to recurrent pregnancy loss. During mouse embryonic development, the homozygous deletion of Cripto in the inner cell mass is embryonic lethal between days 7.5 and 10.5; however, the deletion of Cripto in the extraembryonic trophectoderm, necessary in the placental establishment, is yet to be studied. We generated a Cripto knock-out exclusively in mouse TE cells using Tat-cre. Results mating Cripto floxed females with mTmG males showed high efficiency of Cripto deletion exclusively in TE cells. Homozygous Cripto floxed Tat-Cre-treated embryos were then transferred into pseudopregnant mice for continued development. Dissections of the knockout implantation sites at day 10.5 exhibited fewer implantation sites and significantly decreased implantation site size and placental width compared to the control. Immunohistochemistry staining against Cripto confirmed Cripto deletion in our knockout sites. Hematoxylin and eosin staining revealed substantial differences between the control and the knockout, with the knockouts displaying smaller and positionally tilted placentas. Immunofluorescence staining with TE specific markers, placental lactogen and trophoblast specific protein alpha, displayed aberrant cellular organization and differentiation, with increased trophoblast giant cell expansion and diminished spongiotrophoblast layers. In-situ hybridization analysis of the labyrinth syncytiotrophoblasts using *GCM-1* showed decreased expression in the knockouts compared to the controls. Preliminary results looking at day 8.5 knockout sites show significantly decreased implantation site size and a trending decrease in the viable implantation sites compared to the controls.

Employee

**19 - Effectively leveraging proteomics and molecular analysis in your research: we’re here to help!**

Amy Wong1, Anne-Laure Larroque1, Jenn Nedow1, Jenna Cleyle1, Lorne Taylor1, Ari Gritsas1

1Proteomics and molecular analysis platform

Over the past 8 years, the Proteomics and Molecular Analysis platform at the RI-MUHC has served over 80 labs in various fields including cancer biology, parasitology, and mental health research. We specialize in in identifying and quantifying proteins using advanced mass spectrometry techniques to detect changes in health and disease for biomedical and clinical applications, including biomarker discovery. The molecular analysis branch of our platform analyzes and quantifies peptides, steroids, neurotransmitters, amino acids, prostaglandins and many other “small” molecules. We also perform pharmacokinetic studies, which determine how a drug or compound interacts with the body. Platform staff can also help you design new assays to measure almost any biomolecule, often in a multiplexed assay to help keep costs down. We have extensive experience in study design and execution, quantitative proteomics, data analysis, mapping proteomics data onto biological pathways, and protein functional interaction analysis.

As a platform, our goal is to effectively serve our clients. We customize experimental design to meet the needs of your research question and budget, and meet with our clients before, during, and after their sample analysis. Let us help you make the most of the power of proteomics and molecular analysis to further your research potential!

MSc Candidate

**20 - Unraveling Sex-Specific Epigenetic Regulation: Effects of Sex-chromosome Linked Genes Kdm5c and Kmd5d on DNA Methylation in Mouse Liver**

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The distinct gene dosages on the X and Y chromosomes have profound implications for differences in disease susceptibility and progression between males and females. Sexual dimorphism in phenotypes stems from differences in gene regulation. DNA methylation represents one of the layers of gene regulation. This study aims to elucidate the epigenetic mechanisms that drive sexual dimorphism in mammalian gene expression, particularly highlighting the role of the sex chromosomes. X-linked gene *Kdm5c* and and Y-linked *Kdm5d* encode H3K4 histone demethylases that are crucial for epigenetic regulation of gene expression. Notably, *Kmd5c* escapes X-inactivation in females, leading to a double dose of its RNA in females compared to males.

We hypothesize that KDM5C and KDM5D play significant roles in shaping DNA methylation patterns in somatic cells, leading to sex-specific differences. To test this hypothesis, we performed whole-genome bisulfite sequencing (WGBS) on 28 liver samples from mice that carry mutations in *Kdm5c* or *Kdm5d*: e.g. heterozygous *Kdm5c* mutant females ( X5c+X5c-, n=5), hemizygous *Kdm5c* mutant males (X5c-Y5d+, n=5), *Kdm5d* mutant males (X5c+Y5d-, , n=4), and wildtype controls (X5c+X5c+ , n=5, and X5c+Y5d+, n=9). By comparing methylation levels across these groups, we will identify differentially methylated regions (DMRs) sensitive to the dosage of *Kdm5c* or absence of functional *Kdm5d*. We will also establish the relationship between these DMRs and the sex bias in DNA methylation across the genome. We anticipate that mutant mice lacking functional *Kdm5c* or *Kmd5d* exhibit decreased methylation levels in gene promoter regions that are enriched in H3K4me3 and H3K4me2 (targets of KDM5C and KDM5D action) compared to the wild-type controls. Our data will determine the role of KDM5C or KDM5D in sex-biased DNA methylation in mouse liver, thereby advancing our understanding of the implications of sex chromosome-encoded epigenetic modifiers in male-female disease differences.

MSc Candidate

**21 - A novel Pex16 deficient mouse model for studying and treating Peroxisome Biogenesis Disorders**

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**Introduction:** Peroxisome Biogenesis Disorders (PBD) are inherited autosomal recessive disorders occurring in 1 in 50 000 individuals. PBD is caused by pathogenic variants in one of 13 *PEX* genes, which encode PEX proteins required for peroxisome assembly and function. Peroxisomes are organelles required for multiple vital metabolic processes, such as lipid and reactive oxygen species metabolism. PEX16 is an integral peroxisomal membrane protein required for *de novo* peroxisome formation. While patients with PBD usually exhibit a range of symptoms involving multiple organ systems, mutations in *PEX16* lead to a unique phenotype limited exclusively to the Central Nervous System (CNS), including gait and motor abnormalities. To better understand disease pathophysiology and develop treatments for this form of PBD, we generated a novel *Pex16* deficient mouse model.

**Methods and results:** We generated a postnatal conditional full-body *Pex16-/-* mouse using the tamoxifen-inducible cre-mediated recombination system at 4 weeks of age. 3 weeks post tamoxifen administration, we detected abnormal peroxisome metabolites levels in blood, including elevated very long chain fatty acids and reduced plasmalogen lipids. A subset of mice developed corneal inflammation after 2.5 months. Based on the other postnatal inducible peroxisome disease model (*Pex5*), we expect a functional phenotype to appear after 5 months post gene inactivation. At 6 months post *Pex16* inactivation, there is no obvious motor anomaly. We are currently characterizing brain morphology using histology, and will assess CNS function using Rotarod, CatWalk, and Y-maze tests.

**Conclusion:** We created a *Pex16-/-* mouse model, validated by severely abnormal peroxisome biomarkers. Once functional outcome measures are established, we will treat *Pex16-/-* mice with a CNS-directed *PEX16* gene therapy, and assess the effects.

PhD Candidate

**22 - Exploring the role of Claudin-3 in chick neural tube mechanics**

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The neural tube, the embryonic precursor to the brain and spinal cord, begins as a flat sheet of epithelial cells whose edges, the neural folds, elevate upwards and fuse along the dorsal midline of the embryo. Failure to fuse the neural tube causes neural tube defects, such as anencephaly or spina bifida. We found that depletion of a tight junction protein, Claudin-3 (Cldn3) in chicken embryos, causes spinal neural tube defects due to failure in neural fold fusion. The apical cell surface morphology of Cldn3-depleted cells is altered; there is increased membrane blebbing at cell-cell contacts and smaller apical surfaces. F-actin is reduced at apical bicellular junctions. Our live imaging analyses identified differences in how the neural folds of chick embryos come together in the spinal vs. cranial region. We observed that the neural folds in the future spinal region of chick embryos make contact at distinct points and then the regions between these contact points fuse. This is distinct from the progressive anterior to posterior zippering from the first point of fusion that is observed in the cranial region. Given that depletion of Cldn3 causes spinal neural tube defects, and the loss of F-actin at the apical-lateral cell membrane, we hypothesize that Cldn3 plays a role regulating cell movement and shape changes required for the biomechanical process of spinal neural fold fusion. To test this hypothesis, we are using live imaging to characterize spinal neural fold fusion in control and Cldn3-depleted embryos to quantify apical cell shapes, movements, and junctional rearrangements. Future work will test mechanical properties of cells during spinal neural fold fusion in control and Cldn3-depleted embryos. This research is working towards a better understanding of the morphogenetic events of neural fold fusion and the specific mechanisms controlled by Cldn3.

PhD Candidate

**23 - Modeling Cerebro-Costo-Mandibular Syndrome (CCMS) with Tamoxifen-Induced Snrpb Deletion**

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Heterozygous mutations in *SNRPB*, an essential core component of the five small ribonucleoprotein particles of the spliceosome, are responsible for Cerebro-costo-mandibular Syndrome (CCMS). CCMS is a rare congenital disorder commonly characterized by microcephaly, micrognathia, cleft palate, and varying degrees of rib abnormalities. Previously, we found that *Snrpb* heterozygous mouse embryos arrest shortly after implantation. Although mutants from our previous model, with heterozygous deletion of *Snrpb* specifically in the developing brain and neural crest cells, exhibited craniofacial malformations resembling those observed in CCMS patients, a model that can capture the full spectrum of CCMS symptoms remained elusive.

To address this gap, we employed the tamoxifen (TAM) inducible Cre recombinase system to induce ubiquitous heterozygous deletion of *Snrpb* via tamoxifen injection during mid-gestation and subsequently characterized the phenotypes of mutant embryos across various developmental stages. Notably, skeletal preparation of E17.5 mutant embryos revealed characteristic hallmarks of CCMS, including posterior rib gaps, a bell-shaped thorax, and scoliosis, in addition to the previously described craniofacial phenotypes. These rib and vertebral defects have not been previously modeled, highlighting the novelty and clinical relevance of this CCMS model.

With this model, we aim to investigate the molecular mechanisms underlying these CCMS-like abnormalities. Starting first with axial skeletal anomalies, we conducted bulk RNA sequencing on somite tissues isolated from early-stage (E9.5) mutants. Our preliminary analysis revealed altered splicing of epigenetic modifiers in mutant somites. We hypothesize that this splicing alteration in somites could disrupt the expression of downstream target genes essential for axial skeletal development. To further validate this hypothesis, we intend to conduct ATAC-Seq to assess changes in chromatin accessibility.

PhD Candidate

**24 - A qualitative study exploring the experiences of social stress during the transition to parenthood among Canadian-born and immigrant parents in Quebec, Canada**

Phyllis Zelkowitz1, Sophie Meunier2, Monica Vaillancourt1, 3, Christine Gervais4, Tamarha Pierce5, Blaine Ditto1, Deborah Da Costa1, 3, Francine Demontigny4, Jean-Benoît Deville-Stoetzel3

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Perinatal psychological distress adversely impacts the well-being and social adjustment of parents and their children. Limited studies have examined the perceived determinants of perinatal distress in immigrant parents, particularly men. This study explored Canadian-born and immigrant parents lived experiences of social stress during the perinatal period and perceptions between these stressors and psychological distress. Semi-structured interviews were conducted with Canadian-born and 1st generation immigrant women (N=21, age=34.1±3.6 yrs) and men (N=13, age=34.9±5.0 yrs) at 7.4±0.81 months postpartum in Quebec, Canada. Through thematic analysis, 6 themes were identified: *parental adjustment (*pregnancy intention, priority changes, parenting difficulties and enjoyments*)*, *couple adjustment (*partner’s mental health, conjugal conflicts, work-life imbalance*)*, *social support (*partner, friend, family, social media, employer*)*, *health care support (*access to care, mental health care, shared decision making*)*, *cultural pressure (*Canadian and heritage culture, e.g. parental leave, gender role division of labor, parenting style, openness to mental health care*)*, and *discrimination (*maternity-related, physical, gender, ethnicity*)*. Among men, barriers include difficulties establishing their role within the family and not receiving consideration by health care, and immigrant men reported gender inequality in parenting issues. Perinatal father engagement was beneficial for adjustment in men. Women reported health care delivery issues and partner’s return to work as barriers. Immigrant parents reported more social (family absence, difficulties making friends, professional difficulties), cultural (clash of cultures, gender role attitudes), discrimination (ethnic), and health care concerns related to their distress. Our results highlight different social determinants of perinatal well-being perceived by men and women from various ethnic and immigration backgrounds during the perinatal period. Understanding what parents perceive to facilitate or hinder their psychological well-being can help inform the development of tailored evidence-based programs and policies to better meet the mental health needs of Canadians and reduce gender disparities in the treatment of perinatal distress.

MSc Candidate

**25 - Evidence-based Cerebral Palsy Rehabilitation: Co-development of a Multimodal Knowledge Translation Initiative for Healthcare Professionals**

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**Background:** Cerebral palsy (CP) is the most common childhood physical disability. Early and evidence-based rehabilitation is essential for improving functional outcomes in children with CP. However, rehabilitation clinicians face many barriers to the adoption of evidence-based practices (EBP)s. The objective of this project is to co-design a comprehensive, user-friendly, and effective online knowledge translation (KT) strategy to support and improve CP-EBP among pediatric clinicians.

**Methods:** We adopted an integrated KT approach by collaborating with clinical- and parent-partners. The initiative comprises two components: an early intervention electronic (e)-KT toolkit and an online multimodal KT training program for clinicians. The e-KT toolkit incorporates summarized evidence from a literature review of randomized clinical trials on early CP rehabilitation. The online KT training program was developed with guidance from a scoping review exploring effective KT strategies, emphasizing a multifaceted and tailored approach to foster EBP for rehabilitation professionals.

**Results:** The e-KT toolkit includes nineteen (n=19) modules overviewing twenty-four (n=24) early rehabilitation interventions for children with or at risk of CP aged 0-5 years. Each module features a brief introduction, resources (e.g., training), a co-designed parent/family section (typical questions), and a clinician information section outlining studied outcomes, intervention effectiveness, and evidence level based on rigorous quality assessment using the PEDro scale. Over 50% of high-quality studies generated a moderate-strong level of evidence, demonstrating effectiveness comparable to or better than usual care in improving 22.4 and 43.9% of studied outcomes, respectively. The online KT program consists of three 15-minute video-based training modules accompanied by text summaries, quizzes, and two case studies. Informed parent and clinical perspectives were incorporated to enhance relevance and engagement. Clinical partners (n=2) found the program highly satisfactory and appropriate during the program trial run and completed it in under 1.5 hours. Site champions, identified as qualified rehabilitation professionals, were onboarded to support and empower practical toolkit applications. A champion-training booklet and a 1-hour session were designed to equip them with the necessary knowledge and resources.

**Conclusion:** Practical and accessible KT strategies can support EBP among rehabilitation professionals. The tailored, multifaceted, and co-designed KT strategy will be implemented to optimize early CP-EBPs. Lessons learned from its development, providing ready-to-use evidence and convenient EBP learning, hold potential for broader applications in rehabilitation.

Research/Medical Fellow

**26 - Lipid-laden macrophage index to detect aspiration. Is it worth it?**

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**Introduction.** A diagnostic pathway to detect aspiration is challenging and usually requires a multidisciplinary approach and a variety of tests. Lipid-laden macrophage index (LLMI) was first described in 1985 by Corwin and Irwin as a promising tool to detect aspiration. Information in the literature as well as physicians’ opinions about the clinical value of the LLMI remains controversial.

**Objectives.** To assess the clinical value and possible limitations of LLMI as a diagnostic marker to detect aspiration in children.

**Methods.** Based on the available literature we thought to answer the following questions: 1. Is there a reliable cutoff value of LLMI to detect aspiration? 2. What are the limitations of LLMI? We queried the database of PubMed using search terms “pulmonary aspiration” and “macrophages”. Search was limited to publications in English language including human and animal studies. Authors identified and reviewed 384 articles and identified 80 relevant to the studied subject.

**Results.** Research reveals different proposed cutoff values for aspirators ranging from 85 to 200 macrophages. LLMI reliability has several limitations including: inter- and intraobserver variability among pathologists scores, inability to differentiate between exogenous and endogenous lipid content, inconsistency in the definition of the term “aspiration” in publications. Also, studies in animal models have shown that the nature of the disease, frequency of aspiration, and the time frame when bronchoalveolar lavage (BAL) is performed, could all contribute to the overlap in LLMI in aspirators versus non-aspirators.

PhD Candidate

**27 - Use of zebrafish to study novel monogenic causes of congenital anomalies of the kidney and urinary tract (CAKUT) in humans**

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**Background:** CAKUT constitute the most common cause of chronic kidney disease in children. Despite evidence for significant heritability, identification of novel monogenic causes has proven difficult. Proof of causality for novel CAKUT candidate genes requires development of elaborate animal models, such as mice. However, given the rate of identification of candidate genes, more efficient strategies are needed. Zebrafish offer an attractive alternative, but have not routinely been validated to study CAKUT. The early zebrafish kidney, or pronephros, provides a relevant model to mammalian kidney development with highly conserved developmental signaling pathways.

**Methods:** Morpholino oligonucleotide knockdown in zebrafish and *in situ hybridization* (ISH)for expression studies.

**Results:** First, we confirmed knockdown of *gata3*, a transcription factor with an established human CAKUT phenotype (OMIM #131320) and expressed in the zebrafish pronephric duct (PD), results in a CAKUT-specific readout with malformation of the distal PD. The PD corresponds to the human renal collecting duct from which renal pelvis and ureters derive and is where most CAKUT phenotypes present in humans. Next, we studied a novel CAKUT candidate gene. Rare heterozygous *COL4A1* variants were recently identified in a large cohort of patients with CAKUT (Kitzler *Hum Genet* 2019). COL4A1 is a secreted protein with structural and signaling roles relevant to renal collecting duct development (Tai *Biol Open* 2013). We demonstrate *col4a1* expression along the pronephros at 24 hours post fertilization (hpf). Knockdown of *col4a1* revealed abnormal PD widening at 24hpf compared to control (11.53µm vs. 9.79µm, 95% CI: 0.87-2.6µm, p<0.0001). PD sections revealed abnormal tissue organization with cell bundling at the distal PD in *col4a1* morphants.

**Conclusion:** We demonstrated a CAKUT-specific readout for an already established (*GATA3*) and a novel CAKUT candidate gene (*COL4A1*). Our data support using zebrafish as a powerful tool to assess impact of novel CAKUT candidate genes on kidney development.

Employee

**28 - A novel 61 base-pair intronic deletion of Snrpb is crucial for Snrpb regulation and normal development in mouse.**

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Mutations in a common core spliceosomal factor called SNRPB causes cerebrocostomandibular syndrome (CCMS). Most CCMS patients have point mutations that increase levels of transcripts containing a pre-termination codon (PTC) containing alternative exon 2 (AE2). Herein, we generated a mouse line with a 61-base pair intronic deletion upstream of AE2 (D61) and show that 10% of heterozygous and homozygous embryos (*Snrpb D61/+;* *Snrpb D61/D61*) had abnormalities similar to those found in CCMS patients. *D61* mutants had microcephaly, defects in the bones of the craniofacial region and ribs and 18/47 die from 4 weeks of age onwards. These embryos also had a significant increase in expression of the AE2 and a reduction in *Snrpb* levels. In parallel, we generated a conditional mutant mouse carrying loxp sequences flanking exons 2-3 of *Snrpb*. We used mesoderm-specific *Mesp1-Cre* to delete *Snrpb,* and showed that a fraction (5/40) of heterozygous *Snrpb* *loxp/+*; *Mesp1-Cre+/-* embryos are abnormal at E9.5. They have a narrow frontonasal prominence, a smaller 2nd pharyngeal arch, an enlarged heart, and begin to die at E12.5 where 50% are found alive. In these mutants, *Snrpb* expression was not changed whereas expression of the AE2 was half of controls, suggesting a potential compensatory increase of the *Snrpb* wild-type allele. To test if the *D61* mutation fails to complement *Mesp1-Cre* mediated deletion of *Snrpb*, we generated *Snrpbloxp/D61*; *Mesp1-Cre+/-* mutant embryos. At E9.5, all of the recovered double heterozygous embryos showed an unlooped heart, misshapen somites and failed to turn. These embryos had a significant reduction in *Snrpb* levels without a change in AE2 expression. Our findings suggest that the 61-bp intronic region regulates AE2 inclusion and plays an important role in *Snrpb* regulation. Thus, these sequences should be investigated in CCMS patients that do not carry mutations in *SNRPB* codingexons.

MSc Candidate

**29 - The requirement of TMED2 in placenta formation**

Cassandra Millet-Boureima1, Talia Marc1, Hannah Gordon1, 2, Loydie Jerome-Majewska1,2

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Exchange between the maternal and fetal compartments occurs via the placental labyrinth layer, which is formed by attachment and fusion of two extraembryonic mesoderm derived tissues, the allantois and the chorion. TMED2 is expressed in the allantois and chorion and is required for placental labyrinth layer formation. To examine the role of TMED2 in extraembryonic mesoderm we used *Mesp1-cre* and mutant mice with LoxP sequences flanking exons 2 and 3 of *Tmed2*. We postulate that TMED2 is required in extraembryonic mesoderm for labyrinth layer formation. To test our hypothesis, we collected *Tmed2*LoxP/LoxP; *Mesp1*Cre/+ embryos from Embryonic day (E) 9.5 - E12.5, for histological and morphological analysis. We used immunohistochemistry and *in situ* hybridization to examine expression of proteins and genes essential for placenta formation and function. *Tmed2*LoxP/LoxP; *Mesp1*Cre/+ embryos have placental labyrinth layer formation, but arrest at E12.5. At E9.5, expression of genes important for placental development are comparable in mutant and controls, while the spongiotrophoblast marker, *Tpbpa,* was decreased in mutants. The area of the placental labyrinth layer was reduced in a subset of E9.5 and E10.5 embryos, and this difference was significant at E11.5. The fetal and maternal compartments of the placental labyrinth layer had ectopic cells and were disorganized in E11.5 mutants. In addition, expression of fibronectin, a TMED2 cargo, was increased and the monocarboxylate transporter, MCT1, was decreased. Our data indicates an essential role for TMED2 in the extraembryonic mesoderm for proper formation and development of the placenta.

MSc Candidate

**30 - Otologic Safety of Ciprofloxacin, Trimethoprim/Sulfamethoxazole, and Amphotericin B Powder**

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**Introduction:** Chronic otorrhea in patients with a long-standing history of otitis media poses a significant challenge for otolaryngologists. It can exacerbate hearing loss, impede the use of hearing aids, and cause ongoing discomfort. Clinicians use mastoid powders to battle resistant bacteria and reduce excess moisture in the ear. However, there are concerns about the safety of powders for the exposed middle ear in chronic otitis media cases. In this animal study, we assess the potential ototoxicity of mastoid powder, a combination of Ciprofloxacin, Trimethoprim, Sulfamethoxazole, and Amphotericin B, applied topically to the animal’s middle ear.

**Methods:** 15 male guinea pigs were used in this experiment. The assessment of ototoxicity involved conducting auditory brainstem responses (ABRs) measurements at frequencies of 8, 12, 16, 20, and 24 kHz, along with microscopic examinations and scanning electron microscopy (SEM) of the cochlea. After baseline measurements were taken, a hole was created in the tympanic membrane to deliver the medication. The guinea pigs acted as their own control, with one ear receiving medication and the other receiving boric acid, a proven non-ototoxic powder. ABRs were measured at baseline, immediately after surgery, 2 weeks post-application, and 4 weeks post-application. SEM was performed 2 months post-application.

**Results:** Inflammation was noted in all experimental ears at 2 weeks post-application, but it seemed to resolve after 4 weeks. A paired samples t-test was performed for ABR measurements and significant differences were found in the experimental ears at 4 weeks post-application compared to baseline (8 kHz: *p*=.006, 12 kHz: *p*<.001, 16 kHz: *p*=.016, 20 kHz: *p*<.001). Significant differences between control and experimental ears were also noted (12 kHz: *p*=.002, 16 kHz: *p*=.002, 20 kHz: *p*<.001).

**Discussion:** The tested powder showed signs of ototoxicity, but further studies should be performed to define the exact component and/or concentration that causes ototoxicity.

PhD Candidate

**31 - Neighbourhood Environments and changes in Obesity and Lifestyle Behaviours among Children Enrolled in Obesity Management Interventions: A Systematic Review**

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**Introduction:** Little is known on how neighbourhood environments impact the effects of pediatric obesity treatment. We conducted a systematic review of longitudinal studies examining associations between neighbourhood environment features and change in adiposity and lifestyle behaviour among children participating in obesity management interventions.

**Methods:** Searches were conducted in Medline, Embase, CINAHL and Web of Science for peer-reviewed articles published in English from database inception until October 2023, using terms for children/adolescents, overweight/obesity, and neighbourhood built or social environments. We included studies of children with overweight/obesity at baseline, participating in multicomponent obesity management interventions, and with at least one pre- and one post-intervention measurement of obesity or lifestyle behaviours (physical activity, diet, sedentary behaviour, sleep).

**Results:** Of the 23555 records screened, 30 full-text studies were assessed for eligibility, and 6 met inclusion criteria. Studies were conducted in the USA (n=5) and UK (n=1), with participants’ age ranging from 6 to18 years. Studies examined availability of parks (n=3), supermarkets (n=2), green spaces (n=1), walkability (n=1), recreational facilities (n=1) and composite neighbourhood indicators (n=1). Residing in neighbourhoods with more parks was associated with greater reductions in post-intervention body mass index (BMI) in two studies. Two studies reported inconsistent findings relating availability of supermarkets to changes in fruit and vegetable intake. Availability of green space was not associated with changes in BMI. Only one study examined changes in movement behaviours: residing in neighbourhoods with more recreational facilities was associated with increases in physical activity but not with screen time.

**Conclusion:** This systematic review highlights inconsistent findings among the few studies that examined neighbourhood determinants of obesity management outcomes among children and adolescents. Results suggest that neighbourhood resources that support physical activity (parks, recreational facilities) may be associated with better outcomes.

Employee

**32 - Arsenic exposure causes nephrogenesis defects in human kidney organoids**

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Chronic kidney disease (CKD) affects approximately 1 in 10 Canadians and low nephron endowment at birth is associated with increased CKD risk later in life. Emerging epidemiological data suggest that long-term exposure to high doses of inorganic arsenic (iAs) is also associated with increased CKD risk. Here, we hypothesise that *in utero* exposure to iAs increases susceptibility to CKD by perturbing nephron endowment. We are testing this hypothesis as part of our new interdisciplinary research program called **DERIVE**: **DE**velopmental **R**esearch on **I**nteractions between **V**ariants and the **E**nvironment. To assess iAs nephrotoxicity, we exposed human induced pluripotent stem cell (hiPSC)-derived developing kidney organoids to a range of low-dose iAs concentrations, similar to real-life exposures in the Canadian environment. In hiPSCs exposed to iAs, we saw a dose-dependent increase in cell death from 50 ppb to 200 ppb, with almost half of the cells dead after four consecutive days of 50 ppb iAs exposure. Interestingly, 5, 10 and 20 ppb of iAs had no effect on hiPSC viability. In hiPSC-derived kidney organoids exposed to iAs during nephrogenesis, we found that kidney organoids treated with 20 ppb iAs were morphologically indistinguishable to control organoids. In contrast, kidney organoids treated with 50 ppb had a reduced number of nephron-like structures and a lack of glomerular cells compared to control organoids. Using machine learning to analyse images of organoid development, principal component analyses revealed significant morphological changes in the 50 ppb iAs organoids compared to the 20 ppb and control organoids. Our study highlights that exposure to iAs during development perturbs nephrogenesis in a human kidney organoid model. We are currently modelling genetic polymorphisms in the arsenic metabolising gene, *AS3MT*, using CRISPR/Cas9 to understand interindividual genetic variability in the biological response to arsenic and CKD susceptibility.

Employee

**33 - The role of TMED2 in craniofacial development**

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TMED2 is a member of the transmembrane emp24 domain protein family required for cargo transport between the ER and Golgi. We identified a mutant mouse line with a loss of function point mutation in the *Tmed2* signal sequence (*Tmed299J*) in a screen for genes required for proper morphogenesis. We found that *Tmed299J* homozygous mutant embryos die at E11.5 due to placental defects. These mutants display developmental delay, failure to turn, posterior truncations, abnormal heart looping, and abnormal head development. Recently, we generated mutant mouse lines with LoxP sequences flanking exons 2 and 3 of *Tmed2* to investigate its tissue-specific requirements during embryogenesis. Using beta-actin Cre, we generated mice with heterozygous deletion of *Tmed2* and confirmed that the two mutant alleles failed to complement. While *Tmed2* heterozygous mice (*Tmed2+/-*) resembled controls, *Tmed2* homozygous mice (*Tmed2-/-*) arrested at E8.5 and showed developmental delay with a significant decrease in mRNA levels. Furthermore, knockout of *Tmed2* in neural crest cells with *Wnt1-Cre2* resulted in microcephaly and micrognathia. Cartilage and skeletal preparation of E14.5 and E18.5 neural crest mutant embryos showed reduced frontonasal cartilage and poor ossification in the mandible and bones of the head derived from neural crest cells. These results indicate that TMED2 is required in the neural crest cells for normal development of the head. Future studies will focus on identifying the TMED2 cargoes important for craniofacial development.

MSc Candidate

**34 - Abstract: Modelling Axial Defects Seen in Cerebrocostomandibular Syndrome in Mouse Model**

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**C**erebro**c**osto**m**andibular **S**yndrome (CCMS) is a rare congenital disorder characterized by craniofacial and thoracic (axial) defects, namely micrognathia, cleft face/palate, posterior rib gaps, ectopic rib, bell-shaped thorax, and scoliosis. Thoracic defects seen in patients lead to respiratory insufficiencies and, in 50% of cases, will ultimately lead to death in the first month of life. Patients carry pathogenic variants in the gene *SNRPB* which is expressed in both mesoderm and neural crest cells. Neural crest cells contribute to most of the cartilage, bones, and connective tissue of the craniofacial region. Mesoderm contributes to the remaining parts of the craniofacial region and axial skeleton. The mechanism through which *SNRPB* affects the proper differentiation of these cells in CCMS is unknown. My research objective is to phenocopy patient phenotype using a mouse model. Using *Mesp1-Cre* and conditional *Snrpb* mutant mice, *Snrpb* was deleted in mesoderm cells. Histological analysis using haematoxylin and eosin staining to analyse morphology and alician blue staining to analyse cartilage formation was completed. Additionally, alcian blue and alizarin red were used to analyse cartilage and bone formation respectively. Mutant embryos are smaller than controls and the thoracic width of these embryos are almost half the size of their wildtype littermates. These mutant embryos also have delayed chest closure, delayed cranial suture formation, small and wavy jaw, poorly defined boundaries of cartilage condensations, wavy ribs, defects in the shape of cervical vertebrae as well as scoliosis.

In the future, the levels and role of *Snrpb* and other genes important for axial development will be investigated. This research is significant as it has the potential to understand the causation of this and other congenital diseases affecting axial development to reduce neonatal mortality and to identify therapeutic targets to rescue severe presentations observed.

Post-doctoral Fellow

**35 - Acceptability and Impact on Physical Activity of an E-Health Prenatal Program Promoting Healthy Behaviours and Mental Health: Results of a Pilot Randomized Clinical Trial**

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**Introduction:** Maternal psychological distress during the perinatal period adversely impacts engagement in healthy behaviours. Despite the benefits, physical activity tends to decline during the perinatal period. This pilot study examined user acceptability and preliminary impact of an e-health intervention platform (*HealthyMoms*) designed to promote healthy behaviours and mental health during the perinatal period.

**Methods:** In total 112 pregnant participants met the eligibility criteria, 56 were randomly assigned to *HealthyMoms* and 56 to usual care. Those assigned to *HealthyMoms,* were provided access to a co-developed digital intervention providing accurate and accessible evidence-based information and strategies to optimize emotional wellness and healthy behaviours during the perinatal period. Standardized questionnaires were completed at study entry and at 6 weeks follow-up. User acceptability was surveyed for those randomized to the intervention group at 6 weeks post-randomization.

**Results:** In total, 85% of women in the intervention group found the healthy behaviours information to be relevant to wellness during pregnancy/postpartum and 83% reported that the emotional health (e.g. depression, anxiety) information provided was credible followed by Parenting (78%) and Healthy Behaviours (76%). At 6 weeks follow-up, scores on total physical activity expenditure and vigorous exercise were higher for women in the intervention compared to the control group.

**Conclusion:** Digitally delivered approaches to promote physical activity and mental wellness such as *HealthyMoms* may be an acceptable mode of optimizing mental health during the perinatal period by increasing or maintaining physical activity.

PhD Candidate

**36 - High-throughput screen on primary human acute myeloid leukemia stem cells (LSCs) identifies novel anti-LSC compounds**

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Acute myeloid leukemia (AML) is a fast-progressing blood cancer characterized by abnormal myeloid cell proliferation in the bone marrow. Despite its rarity in children, around 40% experience disease recurrence post-remission, often due to leukemic stem cells (LSCs), necessitating more aggressive treatments. Targeting the unique biology of LSCs is crucial, yet technical challenges have hindered high-throughput screens for novel therapies to target these cells.

To address these challenges, we optimized conditions for large-scale expansion and purification of CD34+ LSC-enriched fractions from a primary human AML sample, OCI-AML-8227, in vitro. Through a high-throughput screen of 11,140 compounds, we identified 25 novel anti-LSC compounds with high efficacy against LSC-enriched OCI-AML-8227 cells, including the known anti-LSC compound venetoclax.

Three leading compounds were validated for toxicity on healthy hematopoietic stem and progenitor cells (HSPCs) compared to LSC-enriched OCI-AML-8227 cells, indicating a significant differential effect. Further functional validation through colony-forming unit assays demonstrated the ability of these compounds to reduce leukemic progenitors while sparing normal progenitors. These candidates also exhibited anti-LSC activity in a second poor-prognosis model, OCI-AML-20.

Finally, compound "A" emerged as a potential candidate for further development due to its apoptotic effect on LSCs with minimal toxicity to stromal and normal cord blood cells. Its classification as an indole suggests a potential mechanism for LSC elimination through apoptosis. Moving forward, the aim is to examine LSC eradication across a panel of genetically defined primary AMLs to determine the broader applicability of compound "A" and translate preliminary results into clinical practice.

PhD Candidate

**37 - Uncovering novel monogenic causes of chronic kidney disease (CKD)**

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**Background:** Renal ciliopathies are a heterogeneous group of disorders that collectively constitute the most common monogenic cause of kidney failure in individuals under 30. The primary cilium is a mechanosensory organelle critical for the development and function of various organs, including the kidney. Ciliopathies arise from biallelic pathogenic variants in genes encoding proteins of the primary cilium. Their true contribution to end-stage renal disease remains unknown, as the clinical significance of many gene variants remains uncertain, particularly in atypical cases.

**Methods:** We combined exome sequencing data with patient-specific cell models to confirm a molecular diagnosis and describe novel genotype-phenotype associations for two patients with kidney failure due to a ciliary defect.

**Results:**

Case 1: Exome sequencing identified rare autosomal-recessive missense variants in *C2CD3*, a gene associated only with a severe syndromic phenotype (Orofaciodigital syndrome IV) in a young adult with isolated CKD of unknown etiology. Patient-derived fibroblasts and urinary renal epithelial cells demonstrated impairment of ciliary signaling pathways and renal-specific defects in ciliogenesis, along with reduced localization of C2CD3 to the primary cilium.

Case 2: A rare homozygous nonsense variant in *CC2D2A* (p.Arg34\*), a gene causing various syndromic ciliopathies, was identified in an adult with isolated kidney failure. Tissue-specific expression analysis showed that this variant impacts a transcript isoform primarily found in the kidney, with no effect on isoforms prevalent in other tissues. Patient-specific cDNA indicated confirmed escape mechanisms from nonsense-mediated decay for this variant.

**Conclusion:** Rare autosomal-recessive genetic variants in syndromic ciliopathy genes are a novel cause of isolated CKD, due to hypomorphic and renal-specific effects. This work expands the phenotypic spectrum of *C2CD3* and *CC2D2A*-related ciliopathies, emphasizing the clinical importance of expanded genetic testing, variant-specific analysis, and detailed phenotyping in cases of kidney disease with an unknown etiology.

Post-doctoral Fellow

**38 - Advancing Leukodystrophy Research: From Pathophysiology to Therapeutics**

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Leukodystrophies are a heterogeneous group of more than 50 rare genetic disorders that predominantly affect children, manifesting a wide range of symptoms and complications culminating in progressive disability and mortality within months to years post-diagnosis. Despite the recent advancements in leukodystrophy research, a significant knowledge gap persists regarding the complete understanding of leukodystrophy pathophysiology, compounded by the absence of molecular diagnosis in 20-30% of cases. For the vast majority of these diseases, there are no available curative or disease-modifying treatments and the lack of natural history data and surrogate markers to evaluate therapeutic efficacy jeopardize the design of future clinical trials. In addition, the data on patient- and family-reported outcomes of disease impact is also limited, impeding the identification of modifiable factors to alleviate disease burden. Through our collaborative and comprehensive initiatives, we aim to address these challenges. We will identify and validate novel leukodystrophy-causing genes and investigate the pathogenesis of the disorders. By characterizing the natural history of the disease, we will identify appropriate endpoints for clinical trials. Leveraging logistic support, we aim to develop therapeutics for the disorders through small molecules, gene therapy, and antisense oligonucleotides (ASO) approaches using patient-derived induced pluripotent stem cells (iPSCs) and *in vivo* models. Through strategic industry partnerships, our goal is to expedite the translation of these potential therapeutics into investigator-initiated clinical trials. Together, our approach holds promises to address the unmet needs of leukodystrophy from diagnosis to developing therapeutics to ameliorate disease outcomes and, ultimately, find cures.

MSc Candidate

**39 - Coupled in-silico-in-vitro drug discovery strategy targeting RUNX/ETO fusion protein driving AML**

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Acute myeloid leukemia (AML) is characterized by the uncontrolled proliferation of myeloid blasts leading to ineffective hematopoiesis1. AML accounts for over 80,000 annual deaths worldwide2. Oncogenic fusion proteins resulting from chromosomal rearrangements are drivers of AML and represent therapeutic targets3. Specifically, the chromosomal translocation t(8;21) results in an oncogenic homo-tetrameric fusion protein (RUNX/ETO) that is common in AML4.

The goal of the study is to utilize state-of-the-art *in silico* protein structure prediction for the RUNX/ETO fusion protein and identify interfering novel small molecules, considering toxicity and pharmacokinetics filters. Promising compounds will undergo validation in RUNX/ETO AML cellular models.

For the initial stage, the small-molecule inhibitor 7.44 was selected, as well as two other compounds that may bind similarly. The 7.44 molecule can interfere with RUNX/ETO tetramerization, restore gene expression, and increase survival in AML models4,5. The molecule affinity and binding site in RUNX/ETO homo-tetrameric fusion protein was predicted with the *in silico* tool and the LC50 of 7.44 in two AML model cell lines with the fusion protein (Kasumi-1, SKNO-1) was established. A specific RUNX/ETO interaction for 7.44 was suggested after comparing the results against a different AML model (ME-1, which does not harbor the RUNX/ETO fusion).

This represents the first step in an iterative process: the *in-silico* tool will be refined based on the *in vitro* assay results to improve the efficacy in predicting potential anti-RUNX/ETO compounds. Follow-up *in vitro* studies will include dose-response assays and toxicity evaluations against hematopoietic stem cells and critical organ models to refine the toxicity prediction.

The expected outcomes include identifying an efficient inhibitor of the RUNX/ETO fusion protein with minimal toxicity. The iterative process will optimize the *in-silico* algorithm, which can then be applied to other fusion proteins driving neogenesis.

MSc Candidate

**40 - Survey on physicians' knowledge and practice in opioids prescription at the MUHC (POST-OP)**

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Understanding physicians' practices regarding opioid prescriptions post-obstetrical and gynecological surgeries is crucial. Our study evaluated the practice of prescribing opioids among McGill-affiliated physicians (staff, fellows, and residents) in Obstetrics and Gynecology department.

We surveyed 87 physicians and obtained 36 responses (41.4% participation rate) using a REDCap, assessing demographics, post-surgery pain management, patient selection, opioid prescription knowledge, and training. Most respondents (61.1%) were from Royal Victoria Hospital, with 52.8% being female. Obstetrics was the primary practice area for 47.2%, and experience ranged from less than 5 years (30.6%) to over 20 years (36.1%). Knowledge and opioid prescription practices post-discharge were evaluated; and Mann-Whitney tests were used to determine their associations.

Amongst the 36 respondents, 30 regularly prescribe opioids. Of 30, only 26.7% were familiar with the CDC-recommended maximum daily opioid dose. Knowledge gaps in post-surgery pain management were evident. Significant differences (P<0.001) existed in opioid doses prescribed for laparoscopic hysterectomies and cesarians between physicians aware of CDC recommendations and those who were not. Additionally, 46.7% rarely assessed patients for opioid addiction history, and none used standardized screening tools. The use of pre-printed exit prescriptions for opioids varied, with 33.3% never using them and 23.3% always using them. Most (93.3%) physicians did not write prescription expiration date, 56.7% never split dispensation, and 76.7% did not teach patients on how to manage leftover opioid medications. Only 6.7% received internal training at the MUHC on pain management and 6.7% on opioid prescription, whereas many received external training (43.3% for pain management, 33.3% for opioid prescription).

In conclusion, our study underscores the vital need for improved training on opioid prescriptions following obstetrical and gynecological surgeries among McGill-affiliated physicians. Patient guidance on prescription expiration and managing leftover medications is also crucial. Implementing a targeted educational module is crucial to address deficiencies and enhance patient care outcomes.

PhD Candidate

**41 - Characterization of Focal Cortical Dysplasias Using Single Nucleus RNA Sequencing**

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**Background:** Somatic and germline pathogenic variants in genes related to the mTOR signaling pathway are the primary causes of Focal Cortical Dysplasia (FCD) II, the most common cause of drug-resistant focal epilepsy. The mechanisms by which a small subset of cells harboring mTOR hyperactivating mutations can instigatewidespread epilepsy remain poorly understood.

**Objective:**. We aim to characterize the transcription profile of various cell types within the FCDII lesion and compare to non-FCD2 epilepsy controls to unravel the impact of somatic mTOR pathway mutations on FCDII pathophysiology.

**Methods:** We utilized ultra-deep next-generation sequencing to screen somatic mutations in mTOR pathway genes in DNA extracted from 37 patients with histologically confirmed FCDII. Somatic variants were mapped across multiple FFPE blocks for each patient. Single nucleus RNA sequencing (snRNA-seq) was conducted on brain specimens from 3 non-FCDII epilepsy controls and in 4 patients with FCDII with identified causal somatic mutation. In one patient, we have samples from within and distant from the FCDII lesion.

**Results:** We identified pathogenic or likely pathogenic variants in 23 of 37 patients (62.1%). Using snRNA seq., we successfully gathered high-quality data from an average of 11,894 nuclei per specimen, which allowed us to confidently determine cell types in the samples. We will determine the differential gene expression between cell clusters of the same subtype within and distant from the FCDII lesions and compare with non-FCD II epilepsy controls. This will help us identify genes which are dysregulated in FCDII and describe distinctive cellular transcriptome profiles of various cell types within the FCDII. We will validate the top differentially expressed genes in situ using RNA-scope in tissue brain sections.

**Conclusion:** By analyzing the diversity of cell types and gene expression in cortical lesions of FCD II using snRNA seq, this study will provide insights into the cellular and molecular pathologic mechanisms that underlie epilepsy in FCDII, potentially guiding more targeted and effective treatments.

MSc Candidate

**42 - Characterizing the Claudin-3 interactome to understand Claudin-3's role in neural tube closure.**

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1Department of Human Genetics , 2Faculty of Medicine and Health Sciences, 3Department of Pediatrics

Neural tube defects are birth abnormalities that result from the improper closure of the neural tube, the precursor to the brain and spinal cord. Improper closure can lead to openings in the brain or spinal cord, such as spina bifida. Our lab showed that Claudin-3, a tight junction protein, plays a significant role in spinal neural tube closure. When Claudin-3 is depleted in chick embryos, they develop a defect that is like spina bifida. However, the interactions of Claudin-3 with other proteins in embryonic development are largely unknown. The purpose of this project is to adapt a BioID method to identify Claudin-3 interaction partners in chick embryos. We created a fusion protein where biotin ligase is fused in-frame to the N-terminus of Claudin-3. We will express this protein in the non-neural ectoderm cells of the embryo. When biotin, a small, natural molecule is added to the embryo the biotin ligase will add biotin to proteins that are near Claudin-3. We will use the ‘biotin tag’ to isolate the proteins and then identify them using mass spec. We will first confirm that the BioID::Claudin-3 fusion protein will localize to the cell membrane. Next, we will express the BioID::Cldn3 fusion protein in chick embryos and optimize conditions for *in* *vivo* biotinylation. Although this technique has been done in other species, to our knowledge this will be the first time it is being used in live chicken embryos. Finally, this project will allow us to better understand the molecular mechanisms of neural tube closure that depend on Claudin-3 and its interaction partners.

MSc Candidate

**43 - Assessing the Effects of Anti-Claudin Peptides on Mouse Blood-Testis Barrier for Stem Cell Delivery**

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Chemotherapy or radiation therapy treatments often cause infertility in male cancer survivors. Thus, researchers have proposed post-therapy transplantation of spermatogonial stem cells (SSCs) to restore patient fertility. A significant challenge to this approach is the limited number of SSCs that successfully reach their niche due to the blood-testis barrier (BTB). Various studies have shown that individual claudin family members, notably Claudin-11, contribute uniquely in respect to the paracellular properties of the BTB. Thus, targeting claudins to disrupt the BTB structure holds promise for enhancing SSC engraftment. Anti-claudin-11 peptide NT11 is designed to transiently remove Claudin-11 from the BTB. Using an air-liquid interface model to culture mouse testis tissue with NT11, I observed the removal of Claudin-11 after 16 and 72 hours. Furthermore, following removal of NT11 there was a gradual restoration of Claudin-11 expression in the BTB over the course of 72 hours. These studies will be expanded to observe the effect of NT11 on human testis biopsies. Ultimately, my study demonstrates the effectiveness of NT11 peptide in disrupting the human BTB in vitro, suggesting that it may be used clinically for SSC-based fertility restoration.

Medical Student

MSc Candidate

MSc Candidate

**44 - The Prevalence of Ovarian Torsion Among Patients with Ovarian or Para-ovarian Cysts: A Systematic Review and Meta-analysis**

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Patients presenting with ovarian cysts are at risk of developing ovarian torsion. This systematic review and meta-analysis evaluated the prevalence of ovarian torsion among patients with ovarian and para-ovarian cysts. As secondary objectives, we evaluated the prevalence of surgical repair, recurrence, ovarian preservation, pregnancy status, miscarriages, and complications in the same population. We systematically searched PubMed, Medline, and Embase databases from inception to November 2020. Inclusion criteria were patients with ovarian or para-ovarian cysts aged 13 years and older and the articles were written in English. We excluded studies with undefined cysts. Eleven studies were included in the final analysis. In the first group, 3 studies were done in 426 patients with ovarian or para-ovarian cysts who allhad torsions, 27.7% had surgical repair, 6.1% had recurrence of torsion, 44.6% had ovarian preservation, 25.4% of the patients were pregnant, 95.8% had abdominal pain, no studies reported hemorrhagic cysts, and 46.2% had nausea or vomiting. In this group, the cyst types were functional cysts, para-ovarian cysts, serous cysts, corpus luteum cysts, mature cystic teratomas, mucinous cysts, and ovarian cysts. In the second group, 8 studies of 2964 patients with ovarian or para-ovarian cysts, 7.0% of them had ovarian torsion, 29.5% had surgical repair, no studies reported recurrence of torsion, no studies reported ovarian preservation, 17.7% of the patients were pregnant, 55.6% had abdominal pain, 36.8% had hemorrhagic cysts, and 20.4% had nausea or vomiting. Quality assessment in all studies showed fair/good quality. In conclusion, our findings revealed a much higher prevalence of ovarian torsion particularly among women with ovarian and para ovarian cysts than in the general population. Therefore, in the future when treating patients with abdominal pain and a known presence of ovarian or para-ovarian cyst, it is important to consider the possibility of ovarian torsion.

MSc Candidate

**45 - Using Tissues from SEEG Electrodes for Presurgical Molecular Diagnosis of Focal Malformations of Cortical Development**

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Focal malformations of cortical development (FMCD) are prevalent causes of drug-resistant epilepsy. Surgical resection offers a treatment with seizure-free outcomes in roughly 50%-65% of cases. Recent breakthroughs have revealed that somatic mutations in mTOR pathway genes or *SLC35A2* underlie a large subset (~50%) of FMCDs, yet genetic screening typically requires surgical tissue, restricting its preoperative application. This study pioneers the use of tissue from stereoelectroencephalography (SEEG) electrodes, used as an integral part of presurgical investigations, facilitating presurgical genetic diagnosis in FMCD patients.

We aim to detect somatic mutations in cells from SEEG electrodes in epilepsy patients, including known mutation carriers and those ineligible for surgery. We hypothesize that adherent tissue surrounding SEEG electrodes in the epileptogenic zone carries somatic pathogenic variants and will allow presurgical-specific molecular diagnosis.

We've collected SEEG electrodes and brain tissue from 16 epilepsy surgery patients. For somatic mutation screening, DNA is extracted from resected brain tissue and then analyzed using our in-house FMCD gene panel, which specifically targets 20 significant genes involved in the mTOR pathway, including SLC35A2. In parallel, DNA is extracted from SEEG electrode adherent cells using REPLI-g Single Cell DNA extraction. Bioanalyzer High Sensitivity DNA Kit is used for quantification. DNA extracted from electrodes both within and at the margins of the epileptogenic zone will be analyzed. Initially, our study focuses on validating the ability to detect somatic mutations in the DNA retrieved from SEEG electrodes using droplet digital PCR (ddPCR) in patients with known mutations. Subsequently, we aim to assess our Next-Generation FMCD panel’s ability to detect somatic mutations in DNA from SEEG electrodes.

The impact of this research is that it will demonstrate SEEG's utility for FMCD's presurgical molecular diagnosis. This research also extends genetic screening to non-surgical FMCD patients, offering a broader diagnostic approach to this challenging medical condition.

Post-doctoral Fellow

**46 - Mapping subpopulations of single extracellular vesicles to develop new generation biomarkers for drug-resistance in brain tumours**

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1Bioengineering department of McGill University, 2Research Institute of McGill University Health Center

High-grade brain tumors, such as glioblastoma multiforme (GBM) present substantial diagnostic challenges due to their rapid evolution which is impossible to track due to unavailability of longitudinal molecular biomarker capabilities. Conventional diagnostic techniques provide limited insights into this dynamic and the emerging disease heterogeneity in both pediatric and adult patients with GBM. This limitation is particularly problematic when monitoring the effectiveness of treatments like chemotherapy.

GBM progression originates from within tumour initiating glioma stem cell (GSC) populations, which acquire repertoires of oncogenic changes, impacting the biology of the disease including treatment resistance. This can be modelled in mice using xenografts of patient derived GSCs that develop resistance to the alkylating agent, temozolomide (TMZ).

The systemic representation of GBM progression is reflected in the surface protein repertoire of extracellular vesicles (EVs), small membraneous particles that cancer cells, and tumour stroma release continually into the bloodstream. Complex landscapes of such individual EVs contain valuable molecular information about the state and diversity of their parental cells, as hardwired in the composition of EV-associated lipids, proteins, and nucleic acids. However, extracting actionable clues from this rich resource remains challenging due to dearth of adequate technologies and analytical tools. Thus, to resolve the multidimensional EV panorama of GBM lesions progressing toward drug resistance, we adapted an EV detection approach based on single EV confinement on a sapphire nano cavity microchip for recording the Raman spectral fingerprint coupled with a super-resolution single EV nanoimaging of the surface proteins. These inputs were processed by deep-learning algorithm to generate the respective EV population profiles.

We observed that individual EVs produced unique Raman spectra, but the integrated EV landscapes differed dramatically between treatment naïve and TMZ-treated GSC populations. We also detected a time-dependent changes in EV landscape as a function TMZ-induced stress followed by cell death occurring during drug treatment for over 24, 72, and 168 hours. Using bulk mass spectroscopy for each cell line, we identified specific markers to differentiate features of resistance via high-resolution single EV microscopy and deep learning classification of spectral analysis as a measure of probability using a convolutional neural network (CNN) method. Integrating single EV nanoimaging, we demonstrated the potential of multimodal single EV molecular profiling to detect changes in EV landscapes during different phases of chemotherapy response. These results suggest that Raman spectroscopy of changing EV populations may be adapted as liquid biopsy technique to detect the impending transition from therapeutic responsiveness to resistance in the course of brain tumour progression, thereby enabling adequate and timely intervention.

Medical Student

**47 - Free Sugar Intake in Children and Associations with Obesity, Cardiometabolic Risk Factors, and Sleep**

Patricia Li 1, Elena Comelli 2, Catherine Birken 3, Zainab Ahmed4, Jonathon Maguire 5, Evelyn Constantin1, Elise Mok 6

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**Background**: Free sugar includes sugar added to food or beverages, and sugar naturally present in honey, syrups, fruit juices and concentrates. The WHO recommends free sugar to be below 10% of daily energy consumption. Although free sugars are highly pervasive in the Canadian food supply, few studies have examined associations of free sugar with childhood cardiometabolic or sleep health.

**Objective:** Determine the association between free sugar intake and cardiometabolic risk factors, obesity, and sleep in children and youth.

**Design/Methods:** Cross-sectional study among children participating in the TARGetKids! primary care practice-based research network in Toronto. We included healthy children (1 to 15 years old) with at least one 24-hour diet recall (ASA-24), anthropometric measures, and survey data. We used a previously developed algorithm to calculate free sugar intake (main exposure) based on ASA24 data. Outcomes included BMI z-score, waist circumference, blood pressure, lipids, glucose, and sleep (duration, quality). We used multivariable regression (linear, logistic, negative binomial regression) to estimate the associations between free sugar intake and outcomes. REB approval from Unity Health Toronto, Sick Kids, and MUHC.

**Results:** We included 287 children ages 1 to 15 years. Mean free sugar intake was 43.4 g/day (174 kcal/day). Average consumption of free sugar in was 10.4% of total energy. 128 participants (44.5%) consumed above 10% of their total energy from free sugar. Univariate linear regression showed that increased free sugar intake (kcal) was associated with shortened sleep duration and increased systolic blood pressure. Associations did not remain in multivariate analyses or when free sugar was expressed in terms of % of total energy intake.

**Conclusion:** Free sugar consumption is elevated and 45% of children consuming above daily recommendations. Although we observed no evidence of associations between free sugar intake and cardiometabolic risk or sleep, it is important to improve consumption habits.

MSc Candidate

**48 - Characterization of protein interactions with the Claudin8 C-terminus during neural tube closure**

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Neural tube defects (NTDs) affect around 1 in 2500 births in Canada and can result in severe disability and infant death. They arise when the neural tube, the embryonic precursor to the brain and spinal cord, fails to close during early development. Our lab has found that claudins, a family of tight junction proteins, are required during neural tube closure. Depletion of Cldn3, -4, and -8, induces NTDs in chick embryos. Within the claudin protein, the cytoplasmic C-terminus is of particular relevance since overexpression of multiple Cldn8 C-terminal tail variants has also induced NTDs in chick embryos. My project intends to uncover the role of the Cldn8 C-terminus by investigating its interactions with cytoplasmic proteins. I hypothesize that Cldn8, through its C-terminal domain, participates in protein interactions occurring during neural tube closure. Previously, candidate interactors were collected by pulldowns with multiple GST::Claudin C-terminus fusion proteins (wildtype claudins and Cldn8 C-terminal variants) and identified via mass spectrometry. From this data I applied a pipeline of selection criteria and chose Mupp1 and Afadin as candidate proteins for further study. I am using immunofluorescence microscopy to quantify changes in localization of Mupp1 and Afadin when Cldn3, -4, and -8 are depleted from chick embryos. I expect that claudin depletion will induce mislocalization of Mupp1 and Afadin, which would provide evidence that claudins’ role in neural tube closure is to anchor Mupp1 and/or Afadin to the tight junction so they can participate in cellular events of neural tube morphogenesis. I will also transfect HEK293 cells with wildtype Cldn8 and its C-terminal tail variants (S198A, S216A, S216I, ΔYV) and conduct immunofluorescence on Mupp1 and Afadin to test if their localization at the tight junction is dependent on specific residues in the C-terminal tail to narrow down the domain of interaction.

MSc Candidate

**49 - Claudin Variants in Kidney Stone Pathogenesis**

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Kidney stones are hard, calcium-based deposits, affecting 1 in 10 adults and >1 in 10,000 children. The acute pain is severe, and 50% of first-time stone formers face a risk of recurrence, which can lead to progressive loss of kidney function via protracted inflammation. There is strong genetic and biological evidence claudins (CLDNs) are associated with kidney stone formation: 1) CLDNs are pivotal for regulating ion and solute transport in the renal epithelium 2) rare mutations in *CLDN16*, and *CLDN19* are known to cause a lethal form of kidney stone with kidney failure in young adulthood, 3) novel rare variants in 11 CLDN genes were previously discovered in our kidney stone cohort.

We hypothesize that other coding genetic variants in CLDN genes can also increase the risk of kidney stones.

Methods: Using whole-exome sequencing data from the UKBiobank (final 470K release), we examined loss-of-function (LoF) and missense variants in a targeted association study of all 24 human CLDN genes. These variants were tested for association with kidney stone formation in the largest available genetic-ancestry subset with complete data (European-ancestry individuals, n=415,237).

Results: Following false discovery rate correction (α=0.05, N=460 variants), 45 missense or LoF variants (minor-allele frequency < 0.02) across 23 CLDN genes were significantly associated with kidney stone presence. Among them, 28 variants had CADD scores >20, predicting high deleteriousness. We also confirmed the significant association of two common *CLDN14* synonymous variants, rs219780[C] (OR=1.12, *P*=1.97E-09)and rs219779[C] (OR=1.13, *P*=7.89E-12), originally reported in a previous kidney stone genome-wide association study by deCODE genetics.

Conclusions: 45 rare CLDN variants showed significant associations with kidney stone formation in our targeted-association study, suggesting that additional coding variants in claudins may contribute to kidney stone pathogenesis. Future work will validate these variants in more cohorts and conduct functional experiments with cell and animal models.

Post-doctoral Fellow

**50 - Identification of novel mechanisms and potential targets in pre-leukemic HSCs and AML within early events in leukemogenesis**

Manon Saby1

1CHHD - Eppert's lab

Despite significant advancements in recent decades, acute myeloid leukemia (AML) persists with dismal survival rates in adults (~25%) and children (~60%).

Leukemogenesis is a multistep process unfolding within the normal hematopoietic stem and progenitor cell populations (HSPCs). The malignant transformation of HSPCs ensues from acquired genetic abnormalities, leading from normal HSCs to pre-leukemic stem/progenitor clones, ultimately to AML. These pre-leukemic HSCs perpetuate disease progression, exhibiting resilience to treatment and contributing to relapse. Mutations in epigenetic modifier genes in these pre-leukemic HSCs result in abnormalities in epigenetic marks, including histone modifications and DNA methylation.

Within our laboratory, a histone H3-K27M mutation in AML and pre-leukemic HSCs has been identified. Indeed, our functional and expression data underscore the potential of H3-K27M to instigate a leukemogenic phenotype in human HSCs, conceivably mediated by a few key genes within the fetal hematopoiesis program like PLAG1 and LIN28B.

We postulate that these genes play a significant role over leukemia initiation and progression.The project aims to explore the role of fetal gene programs in pre-leukemic stem cell expansion, asses if abnormal fetal-associated gene expression in H3-K27M HSCs contribute to pre-leukemic HSC expansion, and gain insights into the mechanisms of their altered differentiation.

With targeted focus on PLAG1 and LIN28B, the aim is to ascertain whether intervention targeting these genes can rescue the H3-K27M phenotype. To examine their potential role in HSC proliferation and self-renewal, two shRNAs have been synthesized for each gene to facilitate both in vitro and in vivo experiments. Phenotypic characterization, quantification, cell cycle analysis, and apoptosis assessments will be conducted via flow cytometry, while mRNA-seq and Western blotting will scrutinize protein and RNA levels.

The overall objective is to obtain a detailed understanding of the molecular mechanisms of HSC transformation, which would provide new prognostic markers and new targets for the development of new therapies.

Post-doctoral Fellow

**51 - Strategies of mapping vascular cell populations driving brain tumour progression**

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Glioblastoma multiforme (GBM) is one of the most aggressive and lethal brain cancers affecting children and adults and characterized by aberrant vascularization and predictable repertoire of oncogenic mutations. To understand the link between oncogenic lesions and vascular anomalies, we chose a series of well characterized adult GBM-derived glioma stem cells (GSCs) and studied their vascular alterations in xenograft models. We recently reported that human glioma stem cells (GSC) with different molecular subtypes elicit specific endothelial responses, with mesenchymal GSCs inducing circumferential vascular growth, a process known as vasectesia, through the transfer of EGFR/EGFRvIII transcript *via* a subset of extracellular vesicles (EVs). This process precipitated the shift in composition of tumour-associated endothelial cell populations with diminution of the angiogenic subtype and preponderance of proliferative cells, the migratory and permeable endothelium exhibiting intermediate responses. Although EV- mediated transfer of EGFR between mesenchymal tumour cells and endothelial cells has been suggested, the detailed mechanisms underlying the complex and regional vascular patterns involved in vasectasia remain to be further investigated. The key question we are focusing on is the identity, features, subtypes and molecular vulnerabilities of endothelial and perivascular cells associated with critical spatial sites that switch on either vasectasia or angiogenesis. Hence, this project explores innovative strategies for mapping vascular cell populations driving GBM progression, integrating global and spatial multiomics approaches. An initial global approach by 3D reconstruction of a whole murine brain xenografted with mesenchymal GSCs will be performed to visualize the distinct vascular patterns in the tumour region compared to the counterpart normal region. Statistical quantification of the blood vessels using artificial intelligence techniques will be used to provide a comprehensive analysis of vascular alterations within the tumour microenvironment. To simultaneously map and characterize the molecular landscape of vascular cell populations involved in the vasectesia, spatial multiomics approaches such as Xenium and MALDI-HiPLEX-IHC can help to elucidate the transcriptomic and proteomic fingerprint of individual cells, leading to the identification of key molecular signatures defining the increased circumferential vascular growth in GBM. In addition to the mouse brain panel, a custom gene panel comprising 50 markers specific to endothelial cells including different subtypes, i.e. proliferative, migratory, angiogenic and permeable, as well as perivascular cells, will be employed in the Xenium spatial transcriptomic analysis to examine the differential expression levels of endothelial and perivascular cells associated with either vasectasia or angiogenesis. Subsequently, post-Xenium immunofluorescence labelling tumour cells with CD44, endothelial cells with CD31 and pericytes with alpha smooth muscle actin (αSMA) will be conducted on the same tissue slide for segmentation purpose when overlaying the results from Xenium spatial transcriptomics. To establish an *in vitro* model for studying the different subtypes of endothelial cells involved in angiogenesis and vasectasia, CRISPR activation libraries coupled with high throughput 3D screening will be performed to identify downstream targets of EGFR/EGFRvIII contributing to specific vascular response. Collectively, it is hoped that this multi-modal approach of dissecting the mechanisms of vasectasia could be an added newly knowledge to the understanding of the tumour vasculature in patients with GBM for improved outcome and therapeutic strategy.

PhD Candidate

**52 - Defective oligodendrocyte maturation and myelination in EPRS1-related leukodystrophy**

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*EPRS1*-related leukodystrophy (*EPRS1*-HLD) is a rare white matter disease associated with prominent hypomyelination and subsequent neurodegeneration. It arises from biallelic variants in the gene encoding the ubiquitously expressed glutamyl-prolyl-tRNA synthetase, EPRS1, an indispensable enzyme responsible for the charging of proline and glutamate tRNA species. It has been previously shown that disease-causing variants in *EPRS1*-HLD reduce enzymatic activity, resulting in decreased charging of tRNA-Pro, however, the selective vulnerability of the central nervous system (CNS) is unclear. Pathogenic variants in several other aminoacyl tRNA synthetases have been associated with hypomyelinating disorders, supporting a link between proper protein production and myelin development. To address this, we generated induced pluripotent stem cells (iPSCs) from two patients with *EPRS1*-HLD and differentiated them into oligodendrocytes, along with their isogenic controls. Shared defects in oligodendrocyte maturation, morphology, and myelination were identified in the *EPRS1*-HLD lines. This data provides first insight into specific vulnerabilities of OLs to perturbations in protein synthesis and sheds light on the pathogenesis of *EPRS1*-HLD.