

MEDICAL GENETICS RESEARCH DAY 2022

POSTER SESSION I

- 1 Genome-Wide Association Study of vincristine-induced neurotoxicity in pediatric oncology patients
- 3 Viral gene therapy successfully delivers PAX6 protein to the retina of blind PAX6-mutant aniridic mice
- 5 Optimizing a lipid nanoparticle platform for delivery of a CRISPR treatment to the cornea of mice with the blindness aniridia
- 7 Targeting Aminoadipate-Semialdehyde Synthase (AASS) using Antisense Oligonucleotide (ASO) as a novel therapeutic approach for Pyridoxine-Dependent Epilepsy (PDE)
- 9 Efficient generation of oligodendrocytes and oligocortical spheroids from isogenic Huntington disease human pluripotent stem cells
- 11 Genetic variation and pesticide exposure influence sex-specific blood DNA methylation signatures of early-stage Parkinson's disease
- 13 Understanding the role of human XIST in maintenance of human inactive X chromosome
- 15 Role of UHRF1 binding to H3K9me3 in DNA methylation maintenance at heterochromatic regions in naïve embryonic stem cells
- 17 Examination of the histone acetyltransferase p300 in β 3-adrenergic induced remodelling of thermogenic adipose tissues
- 19 Delineating the role of DPP4+ mesenchymal stromal cells in skeletal muscle regeneration
- 21 Characterizing the hypoxia an oxidative stress response pathway in *C. elegans*
- 23 Role of Mediator subunit MED15 in human lung cancer cell stress response
- 25 The genomic structures, diversity, and regulation of extrachromosomal DNAs (ecDNAs) in cervical cancer
- 27 Use of long read whole genome sequencing for precision diagnosis and treatment of individuals with Autism Spectrum Disorder



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MEDICAL GENETICS RESEARCH DAY 2022

POSTER SESSION I

- 29 Scrambler: Simulating complex genomic rearrangements in chromothripsis
- 31 Studying the life course to unravel the complexity of psychiatric disorders
- 33 Adolescents' perspectives of genetic testing & its personal utility: developing the adolescent-reported genetic testing utility InDEx
- 35 Utility of a genomic results e-booklet for parents of pediatric neurology patients who have undergone genomic sequencing
- 37 Exploring genetic counsellors' experiences and perspectives with discussing Medical Assistance in Dying (MAiD) in Canada

POSTER SESSION II

- 2 Modelling and understanding the pathogenic Mechanisms Underlying CLDN11-linked Hypomyelinating Leukodystrophy
- 4 Adenine base editor corrects 75% of aniridic blindness pathogenic base pair in a humanized mouse embryonic stem cell model
- 6 CRISPR-mediated gene therapy of GNAO1 in patient iPSCs
- 8 Design and evaluation of an epigenetic therapeutic strategy for Huntington's disease
- 10 Investigating the impact of a neurodevelopmental disease-linked mutation in TOP2B on early neural brain development
- 12 Shortening the silencer: combination of human and mouse XIST domains generated a functional XIST
- 14 The Mest DMR regulates Klf14 imprinting via allele-specific sub-TAD structures
- 16 Methylation in the developing embryo



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MEDICAL GENETICS RESEARCH DAY 2022

POSTER SESSION II

- 18** Drosophila assays for variant interpretation in the polycomb repressive complex 2 related syndromes
- 20** Med15 role in maintaining function of mature beta cells
- 22** Telomerase Reverse Transcriptase and its non-canonical participation in DNA damage response
- 24** Characterizing the biological effects of a novel G-quadruplex destabilizing ligand
- 26** Haploinsufficiency drives metastatic growth of mouse uveal melanoma
- 28** Identification of novel genomic structures and regulation patterns at HPV integration events in cervical cancer
- 30** Transcriptomic and cellular signatures of chronic addictive substance exposure in 3D human-derived neural organoids.
- 32** Lessons from the past: genetic ancestry and demographic history of the Nicoyan Peninsula, a longevity hot-spot
- 34** Adolescents' implicit and explicit attitudes towards their peers with genetic conditions
- 36** Evaluating family-centered care in Pediatric Neurology: parents'/caregivers' perspectives
- 38** Exploring healthcare providers' experiences and perspectives of rapid genome-wide sequencing for critically ill infants in British Columbia NICU settings
- 39** Exploring Diversity, Equity, and Inclusion initiatives in the admissions processes of genetic counselling programs

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ABSTRACT BOOKLET

1 Genome-Wide Association Study of vincristine-induced neurotoxicity in pediatric oncology patients

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Background: Vincristine-induced peripheral neuropathy (VIPN) is a relatively common, yet highly debilitating toxicity that is caused by vincristine chemotherapy in up to 78% of cancer patients. VIPN is particularly severe in children, often requiring dose reduction and affecting both survival and quality of life. Although previous studies have demonstrated several genetic variants are associated with VIPN risk, the clinical relevance has been limited by small sample sizes, weak associations with VIPN, and lack of independent replication. **Objective:** To identify and validate pharmacogenomic risk factors of VIPN in two independent cohorts of pediatric cancer patients. **Methods:** A total of 2,037 pediatric patients were recruited from nine Canadian pediatric oncology centers. VIPN diagnosis was defined according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. Using phenotypic case-control matching of recruiting sites and power calculations, a discovery cohort consisted of 390 cases [CTCAE grade ≥ 2] and 348 controls [CTCAE grade 0], and a replication cohort (279 cases and 245 controls) were identified. Genome-wide genotyping (Illumina Global Screening Array v2.0 with multi-disease drop-in panel) and imputation were conducted for all patients, and genome-wide analysis was conducted using logistic regression (PLINK v1.9) adjusted for the relevant clinical and demographic variables along with genetic ancestry. **Results:** These analyses are ongoing, the genome-wide analysis revealed novel genetic associations of common variant in STXBP5-AS1 gene ($p=2.4 \times 10^{-6}$) with reduced risk of VIPN, and an intergenic variant ~54kb downstream the TUBB4A gene with increased VIPN risk ($p=5.1 \times 10^{-6}$). **Conclusion:** These preliminary results suggest that genetic variations in TUBB4A, the major constituent of microtubules; a key component of the cellular cytoskeleton and the main target of vincristine, and STXBP5-AS1 (regulator of neurotransmitter release) contribute to the overall susceptibility of VIPN in patients with pediatric cancers.

2 Modelling and understanding the pathogenic Mechanisms Underlying CLDN11-linked Hypomyelinating Leukodystrophy

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Claudin-11, encoded by the CLDN11, is a tight junction protein enriched in the central nervous system (CNS) myelin and the testes. In the CNS, claudin-11 is found in radial components, a specialized ultrastructural feature of brain myelin, stabilizing the apposition of membranes in the internode. It mediates adhesion between myelin membranes and potentiates the insulative properties of myelin. De novo stoploss mutations in CLDN11 were identified as novel causes of Pelizaeus-Merzbacher disease (PMD), a hypomyelinating leukodystrophy. How the stoploss mutations in CLDN11 cause PMD is unclear. I aim to investigate how mutant claudin-11 (mClaudin-11) leads to PMD. Towards this, our lab has established several model systems to study mutant claudin-11. These include a) stable HEK293 cell lines with ectopic expression of control and mutant CLDN11, b) patient-derived and isogenic knock-in human induced pluripotent stem cells (hiPSCs), and c) a humanized CLDN11 mouse model carrying the stoploss mutation. Using these models, I will characterize the impact of the mClaudin-11 on the molecular, cellular, neuropathological and behavioural levels. Findings to date suggest that the stability of mClaudin-11 is compromised, with lower overall protein levels in mutant lines compared to control. Preliminary experiments suggest motor function deficits and impaired male fertility in mClaudin-11 mice. We anticipate that the planned studies will clarify the mechanisms underlying the pathogenesis of the disease and highlight potential therapeutic strategies.

3

Viral gene therapy successfully delivers PAX6 protein to the retina of blind PAX6-mutant aniridic mice

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Background: Aniridia is a rare eye disorder caused by mutations in the PAX6 gene that results in cells expressing half the normal amount of PAX6 protein. Typically, children born with aniridia progress to blindness by early adulthood. Recently, there have been clinical successes with recombinant adeno-associated virus (rAAV) gene therapies and there are now two such FDA-approved therapies available. We hypothesize that by increasing PAX6 expression via an intravenous gene-augmentation rAAV therapy, we can improve eye function in aniridia. Here we test if rAAV can deliver PAX6 to the endogenously PAX6-expressing cells of the mutant retina in a mouse model of aniridia. **Methods:** We evaluated two different therapeutic viruses, rAAV9-PHP.eB smCBA-3xFLAG/PAX6-WPRE, in which PAX6 expression was driven by a ubiquitous promoter; and rAAV9-PHP.eB Ple331-3xFLAG/PAX6-WPRE, in which PAX6 expression was driven by a restricted promoter (“MiniPromoter” Ple331). In both viruses, PAX6 contains a FLAG-tag which allowed us to distinguish between viral and endogenously-expressed PAX6. In addition, we delivered a “spike” of a fluorescent control virus, rAAV-PHP.eB smCBA-EmGFP-WPRE, that allowed us to track the expression pattern in-vivo via fundus imaging. **Results:** Fundus imaging at 1, 3, and 5 months post-injection (PI) revealed widespread viral expression in the retina of mice that received the fluorescent control virus. Furthermore, immunofluorescence analysis at 1-month PI showed that the fluorescent control virus is able to target all four cell types that naturally express PAX6 in the retina (ganglion, amacrine, horizontal, and Müller glia). Excitingly, we were also able to detect viral PAX6 expression in ganglion, amacrine, and horizontal cells in the retina of wild-type and aniridic mice. **Conclusion:** We demonstrated the ability of our therapeutic virus to deliver PAX6 to the retina of aniridic mice. Further steps will investigate whether levels of viral PAX6 are therapeutic and will involve further immunofluorescence and molecular characterization.

4

Adenine base editor corrects 75% of aniridic blindness pathogenic base pair in a humanized mouse embryonic stem cell model

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CRISPR/Cas9 systems, including the adenine base editor, have revolutionized the field of molecular medicine, enabling targeted editing of the genome for the treatment of genetic diseases. These therapeutic approaches can be applied to treat incurable disease such as the congenital blindness aniridia, a disease caused by heterozygous variants resulting in dominant haploinsufficiency of the transcription factor PAX6. Here, we developed CRISPR Humanized Minimally Mouse Models (CHuMMMs) designed to enable the translation of a CRISPR therapy to treat aniridia. We hypothesize that a therapy optimized in minimally humanized mouse embryonic stem cells (ESCs) will be able to differentiate between non-variant and variant chromosomes, and thus be a potential therapy for aniridia patients. We have generated minimally humanized mouse ESC lines and tested therapeutic conditions by transfection of CRISPR reagents into ESCs by electroporation. In preparation for testing the therapy in vivo, we have generated and characterized a control non-variant CHuMMM mouse. To date, we have found our most successful CRISPR strategy corrected the variant on an average of 75% variant chromosomes, and impacted <1% of non-variant chromosomes. The phenotyping results were not significantly different in the humanized CHuMMMs compared to wild-type mice. These results demonstrate that humanization alone does not result in an ocular phenotype, making it a suitable model for CRISPR therapy development. The next steps for our in vitro-optimized CRISPR therapy will be to test it in vivo in a CHuMMM of aniridia to determine if it can restore expression of Pax6 and prevent blindness in mouse.

5

Optimizing a lipid nanoparticle platform for delivery of a CRISPR treatment to the cornea of mice with the blindness aniridia

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Background: Aniridia is a rare congenital blindness with unmet therapeutic needs. This genetic blindness is caused by dominant mutations in the PAX6 gene. Here we aim to optimize a non-viral-delivery platform that can facilitate the translation of CRISPR gene editing into a viable approach for treating aniridia. Lipid nanoparticles (LNPs) are the most popular non-viral vector for delivery of nucleic acids. However, LNPs have no known success for Cas9 ribonucleoprotein complex (RNP) delivery to the eye. We hypothesize that an optimized LNP platform can deliver Cas9 RNPs and DNA template (CRISPR treatment) to therapeutically important cells of the cornea. **Methods:** A reporter system was used to evaluate ex vivo and in vivo delivery of our LNP-RNP CRISPR treatment. Our novel LNP platform was initially tested by delivering multiple LNP-RNP CRISPR treatments to cultured mouse cortical neurons and the outcome was evaluated by histological analysis. Next, the best LNP-RNP CRISPR treatment was injected into normal and aniridic mouse corneas and the transfection efficiency was assessed by histological analysis 21 days later. **Results:** On average, $7\% \pm 4.7\%$ SD of ex vivo cortical neurons were edited after a single LNP-RNP CRISPR treatment, and this number increased to $30\% \pm 11\%$ SD following the second treatment. Also, excitingly, we demonstrated for the first time, wide-spread gene editing in two out of three cell layers of the cornea using an LNP-RNP CRISPR strategy. On average, $6.2\% \pm 2.9\%$ SD and $13.1\% \pm 2.2\%$ SD of in vivo stromal and endothelial cells were edited in aniridic and normal corneas, respectively. **Conclusion:** We obtained successful ex vivo and in vivo gene editing with our LNP-RNP CRISPR treatments and the ability of our LNPs to transfect the cornea highlights the potential of our novel delivery platform to be used in CRISPR-based gene editing therapies of corneal diseases.

6

CRISPR-mediated gene therapy of GNAO1 in patient iPSCs

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Background: De novo autosomal dominant mutations in GNAO1 result in a rare disorder called GNAO1-associated encephalopathy. GNAO1 codes for Gao that plays important roles in signal transduction and other cellular pathways that are currently under investigation. More than forty pathological variants of GNAO1 have been reported, but there is still no effective and permanent treatment for this disorder. Here, we propose CRISPR-mediated gene therapy as a therapeutic approach. To efficiently deliver CRISPR into cells, we use lipid nanoparticles (LNPs) which have gained global attention for their use in the COVID-19 vaccines. **Objectives and Methods:** The objective of this project is CRISPR-mediated knockdown of R209H – one of the common GNAO1 gain-of-function variants causing a Movement Disorder. Given the important functions of Gao, we hypothesize that reduction of mutant mRNA will not be sufficient and correction of the gene may be essential in mitigating disorder-associated phenotypes. CRISPR/Cas9 gene editing will be optimized for delivery and knockdown efficiency in HEK293 cells. Knockdown efficiency is assessed at the DNA (DNA sequencing), RNA (quantitative PCR), and protein (Elisa) levels. We have also received iPSCs from a patient carrying the R209H mutation. GNAO1 knockdown will be further optimized in patient cells differentiated into neurons. **Results:** CRISPR knockdown of GNAO1 has been established using HEK293 cells. Future experiments will focus on characterization, differentiation, and gene editing of patient iPSCs. **Future Perspectives:** This study increases our understanding of GNAO1 knockdown in patient iPSCs. It will also provide proof-of-principle to support gene correction of GNAO1 as the first step towards an effective treatment. In vivo gene editing in a mouse model carrying the desired variant will be an appropriate future step.

7

Targeting Aminoacidic Semialdehyde Synthase (AASS) using Antisense Oligonucleotide (ASO) as a novel therapeutic approach for Pyridoxine-Dependent Epilepsy (PDE)

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Pyridoxine-dependent Epilepsy (PDE) is caused by mutations in the ALDH7A1 gene that encodes for the α -Aminoacidic Semialdehyde Dehydrogenase enzyme (Antiquitin). Antiquitin is involved in the multi-step pathway of cerebral lysine catabolism. In turn, antiquitin deficiency leads to the accumulation of metabolic intermediates that lower the level of pyridoxine (vitamin B6), causing interference with the production of neurotransmitters in the brain. This is thought to cause the characteristic seizures seen in the disease phenotype. The current state-of-art treatment to control seizures is vitamin B6 supplementation. However, more than 75% of patients still suffer from neurodevelopmental disabilities, which are believed to be caused by the accumulation of neurotoxic metabolic intermediates. Therefore, we hypothesize that lowering the levels of these metabolites by blocking an upstream enzyme in the lysine catabolic pathway, α -Aminoacidic Semialdehyde Synthase (AASS), using antisense oligonucleotides (ASOs), will diminish the lysine intermediate metabolites; thus correcting the phenotype of the disease. To test this hypothesis, we aim to evaluate the effectiveness of ASO-based therapy in decreasing the levels of AASS and the levels of toxic metabolites in PDE neuronal cells and PDE mouse models. To do so, several ASOs were designed with different nucleotide chemical modifications to target AASS with high specificity and minimal toxicity. AASS expression, protein levels, and metabolite levels were measured in primary astrocytes using qPCR, immunoblotting, and mass spectrometry, respectively. The best candidate ASOs will then be injected into PDE mice via intracerebroventricular injection to investigate their efficacy in vivo. As a preliminary outcome, we were able to see a significant knockdown in AASS expression was produced by two of the AASS-targeting ASOs in primary-cultured astrocytes from wild-type C57BL/6J mice.

8

Design and evaluation of an epigenetic therapeutic strategy for Huntington's disease

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Huntington's disease (HD) is an autosomal dominant, progressive neurodegenerative disorder caused by a CAG triplet repeat expansion in exon 1 of the huntingtin (HTT) gene. The mutation produces a mutant huntingtin (HTT) protein with a toxic gain-of-function. Mutant HTT expression causes selective loss of medium spiny neurons in the striatum and the development of a triad of motor, cognitive, and psychiatric symptoms. As a result, many therapeutic approaches for HD aim to reduce mutant HTT abundance. Because wild-type HTT has regulatory activity, is involved in synaptic vesicle trafficking and intracellular transport, and protects against mutant HTT-associated toxicity, the most favoured approaches for HD treatment will selectively reduce mutant HTT while preserving wild-type HTT levels. Post-mortem studies suggest that HD pathogenesis is associated with large-scale changes in DNA methylation (DNAm) patterns, and that specific changes in DNAm at the HTT locus affect transcription factor binding and HTT expression. In HD patients, unique patterns of DNAm are measurable adjacent to HTT and near the HTT CAG repeat expansion. Importantly, increased DNAm at these sites is also associated with slower progression of motor deficits. We sought to further investigate the impact of DNAm on HTT expression in the context of the exon 1 CAG repeat expansion, and to explore the effect of manipulating DNAm at the HTT locus. In vitro, we demonstrate that DNAm has a significant and measurable effect on HTT regulatory region activity that is dependent on CAG repeat tract size. We also outline a strategy to evaluate our approach in vivo in the Q175FDN mouse model of HD using CRISPR-Cas9 and lipid nanoparticle (LNP) technology. This approach will improve our understanding of the role of epigenetics in HD pathophysiology, and represents a novel therapeutic strategy for HD treatment targeting the most proximal cause of HD.

Efficient generation of oligodendrocytes and oligocortical spheroids from isogenic Huntington disease human pluripotent stem cells

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Recent studies using Huntington disease (HD) animal models have revealed that myelin abnormalities precede neuronal loss and are primarily driven by intrinsic mutant huntingtin (mHTT)-mediated defects in oligodendrocytes, the myelinating cells of the central nervous system. While these findings have implicated oligodendrocyte dysfunction in HD, an understanding of oligodendroglia pathology and the mechanisms involved in the context of human physiology remains lacking. The objective of this research is to delineate abnormalities in human HD oligodendroglia in order to develop approaches to reverse them. Here, using an isogenic HD (IsoHD) human pluripotent stem cell (hPSC) allelic panel we have previously developed representing a range of CAG lengths (30, 45, and 81 repeats), we show the successful generation and isolation of highly ramified cells expressing O4, a cell surface oligodendrocyte progenitor marker, by day 75 of differentiation. Cell frequencies calculated by flow cytometry showed that 40-60% of the total population were O4+ cells, with no differences between IsoHD lines harbouring different CAG repeat lengths. To investigate whether specific oligodendroglial populations are affected in HD, we performed single-cell RNA sequencing at Day 81 of oligodendroglia differentiation. Clustering analysis revealed seven oligodendrocyte lineage clusters, with no unique sub-populations across different CAG lengths. To further investigate cell type-specific differences across genotypes we are now analysing differential gene expression for each of the clusters. In addition, we successfully generated oligocortical spheroids, which show expression of the transcription factor MYRF, a key myelin regulatory factor necessary for oligodendrocyte maturation, at week 16 of differentiation. The efficient generation of oligodendrocytes and oligocortical spheroids from an isogenic HD hPSC panel provides an opportunity to investigate oligodendroglia pathology and the influence of CAG repeat length in HD in the context of human physiology.

Investigating the impact of a neurodevelopmental disease-linked mutation in TOP2B on early neural brain development

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As many as 17% of Canadians live with neurodevelopmental disabilities (NDDs), with monogenic mutations being implicated in the etiology of 40% of NDDs. The aim of this project is to investigate the impact of one such mutation in TOP2B, encoding type II topoisomerase beta, on brain development. A de novo mutation in TOP2B has been identified in several patients with intellectual disability, autism spectrum disorder, seizures and microcephaly. How this mutation, which results in a histone-to-tyrosine (p.His58Tyr) substitution, causes disease is currently unknown. Topoisomerases have important functions in DNA replication and RNA transcription. TOP2B is highly expressed in the brain and plays a key role in regulating the transcription of many genes important for nervous system development. Here we employ isogenic pluripotent stem cell (hPSC) lines harboring the TOP2B p.His58Tyr variant or null mutations to examine its impact on several aspects of early brain development in the context 2D monolayer neural and 3D cerebral organoid systems. These include measures of neural progenitor proliferation, efficiency of differentiation, neurite outgrowth, synapse formation and cerebral organoid cortical layering and lamination. This work will contribute knowledge on the role of TOP2B in general and the impact of the p.His58Tyr variant specifically on early brain development as well as the disease mechanisms involved, with potential relevance for therapeutic development.

11

Genetic variation and pesticide exposure influence sex-specific blood DNA methylation signatures of early-stage Parkinson's disease

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Sporadic Parkinson's disease (PD) is influenced by sex differences, genetic susceptibility (G), and environmental (E) factors, making it challenging to identify individuals at risk. Early-stage PD biomarkers would be useful for identifying those at risk at an actionable stage to slow the onset and/or progression of the disease. DNA methylation (DNAm) may be a suitable PD biomarker as it is both genetically regulated and embeds environmental exposures. However, the extent to which sex, genetic variation, and environmental exposures individually or collectively influence PD-associated DNA methylation is an important, yet unresolved issue. We assessed genome-wide blood DNAm, SNP genotype, and pesticide exposure in a case-control study of French agricultural workers and their spouses with early-stage PD (TERRE: 71 cases, 147 controls). DNAm at > 850,000 CpGs was analyzed with EPIC BeadChip arrays, while genotyping at > 300,000 SNPs was performed with NeuroChip arrays. We assessed PD-associated differential DNA methylation in a sex-stratified manner across co-methylated regions (CMRs). Additionally, we compared the fits of the base model against G, E, G+E, and G×E (G representing SNPs within 75kb, E representing pesticide exposure) models for each CMR using the Akaike information criterion (AIC). We found that differential DNA methylation associated with early-stage PD was unique within each sex, and that DNAm variation at the majority of PD-associated CMRs was best explained by including SNP genotype in the models. These observations will be useful for future study design and epigenetic PD biomarker development, particularly highlighting sex differences in PD and the necessity of including genotype into such analyses.

12

Shortening the silencer: combination of human and mouse XIST domains generated a functional XIST

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X-chromosome inactivation (XCI) is a crucial process in eutherian mammals to compensate dosage between females and males. XCI is initiated by a 17-19 kb long non-coding RNA called XIST, which is composed of different repeat domains that recruit diverse proteins and heterochromatic changes resulting in the silencing of one of the X chromosomes. Repeat A is highly conserved and essential for silencing. Repeat E is critical for XIST localization through matrix proteins, such as CIZ1. Repeats B to D are involved in recruiting the Polycomb Repressive Complex (PRC) and in mouse, a 600-nucleotide region, the PID, is part of the B and C and is bound by HNRNPK a key protein for recruitment of non-canonical PRC1, that in turn recruits PRC2. To find a minimal functional XIST that can be used for chromosome therapy and to identify critical domains we tested two XIST inducible transgenes integrated into an autosome in a differentiated cell line. We hypothesized that the first transgene with the A, F regulatory and E human domains would induce silencing and PRC2 recruitment, whereas the second one with the same domains plus the PID mouse region would additionally recruit PRC1. We measured silencing of two nearby and four distal genes by pyrosequencing and qPCR, and recruitment of heterochromatic marks by IF-FISH. The AFE transgene did not silence distal genes nor recruit heterochromatic changes, with the exception of CIZ1. The second transgene with the PID addition was able to silence and recruit all marks, even though it was not equivalent to full XIST levels. By chemical inhibition of PRC1 we found that H3K27me3 was not dependent on UbH2A. By RIP we confirmed the recruitment of HNRNPK by the PID region. The results suggest that the addition of PID improved silencing and heterochromatic recruitment, demonstrating the feasibility of finding a minimal functional XIST.

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X-chromosome inactivation (XCI) overcomes the dosage imbalance between 46,XX females and 46,XY males. The lncRNA XIST induces XCI by binding of heterochromatin-inducing proteins via multiple tandem repeats. While much is known about the establishment of XCI, less is known about the maintenance of the inactive X (Xi). To understand the role that XIST plays in XCI maintenance, I am generating deletions of XIST using CRISPR/Cas9 in a clonal, diploid female cell line with a phased genome, hTERT RPE1. The impact of these deletions on maintenance will be assessed by changes in allelic X-linked gene expression by pyrosequencing and impact on epigenetic marks by immunofluorescence combined with RNA FISH. I hypothesized that the broad acetyl peak within XIST would be essential for expression and have generated three independent XIST knock-out clones all of which showed no XIST expression, indicating that the deletions were on the Xi, and the importance of this region for XIST expression. To observe and quantify the level of reactivation of genes from the Xi, I have generated pyrosequencing SNP assays, which suggest most genes retain silencing but some reactivate in my system.. In addition, to study the synergistic effects contributing to the process of Xi maintenance, I have generated an HPRT1 deletion construct on the active X chromosome, allowing quantification of the reactivation frequency from the Xi, by colony counting in HAT or q-RT-PCR. I am currently establishing the dynamic range for these assays, but somewhat surprisingly I see reactivation in my XIST-intact cells. Understanding the role that XIST plays in the maintenance of the Xi is crucial as aberrant XIST expression impacts the differentiation of stem cells and is observed in multiple cancer types. Information from my research may help develop therapeutic tools for treating breast cancer and other X-linked disorders.

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The imprinted gene *Mest* is regulated by a gametic differentially-methylated region (gDMR) at its promoter CpG island (CGI), retaining oocyte-derived DNA methylation (DNAm) throughout development. Consequently, *Mest* is a paternally-expressed gene. The maternally-expressed gene *Klf14* is located ~200 kb downstream of *Mest* on mouse chromosome 6. Although its promoter CGI is kept unmethylated in most tissues, maternally-inherited DNAm is paradoxically required for *Klf14* expression. Although imprinting at *Mest* is well understood, the mechanism regulating *Klf14* imprinting is still unknown. Here, we show that *Mest* and *Klf14* reside within the same topologically associating domain (TAD) in mouse embryonic stem cells (ESCs), defined by sites of biallelic CTCF binding at the boundaries. To study imprinting aspects in cell-based assays, we established a new panel of F1 hybrid ESC lines from reciprocal crosses between C57BL/6J and CAST mice. Upon differentiation of these ESCs into FLK1+ vascular progenitor cells, we confirmed maternal allele expression of *Klf14*. Using allele-specific 4C-seq in our undifferentiated F1 hybrid ESCs, we show that CTCF binding to the unmethylated *Mest* gDMR generates a paternal allele-specific sub-TAD required for *Klf14* silencing. Using CRISPR-Cas9, we deleted part of the *Mest* gDMR containing this CTCF binding site and obtained F1 ESCs with paternal, maternal, or homozygous deletions. Whereas deletion of the DNA methylated maternal allele has no effect, removing the CTCF binding from the unmethylated paternal allele leads to biallelic *Klf14* expression in FLK1+ cells and loss of the paternal sub-TAD in ESCs. These results provide a mechanistic model for the imprinting of *Klf14* and establish the *Mest*-CpG2-*Klf14* locus as a new imprinting cluster regulated by a single imprinting control region (ICR), the *Mest* gDMR. Our observations also define a new role for maternally methylated gDMRs, which are all associated with promoter-proximal functions: our results show they can also exert long-range effects via allele-specific modulation of TAD structures.

15

Role of UHRF1 binding to H3K9me3 in DNA methylation maintenance at heterochromatic regions in naïve embryonic stem cells

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In mammals, DNA methylation (DNAm) primarily occurs on the 5th carbon of cytosine at CpG dinucleotides and is deposited by different DNA methyltransferases, with DNMT3A/3B responsible for de novo DNAm in early embryonic development. Once established, DNAm is maintained by DNMT1, which is recruited to hemi-methylated DNA during replication. Interestingly, prior to de novo methylation of the embryo, the genome undergoes a wave of DNAm erasure to confer totipotency on the embryonic stem cells (ESCs). This demethylation is the result of reduced DNMT1 recruitment to chromatin over successive rounds of cell division. However, not all regions of the genome are demethylated during this epigenetic reprogramming, with young transposable elements and imprinted loci resisting DNAm erasure in the embryo. Interestingly, these genomic regions tend to be enriched for H3K9me3, which has high affinity for the TTD domain of UHRF1. UHRF1 functions to recruit DNMT1 to replication foci through its UBL domain and its deposition of the H3K18/23-ubiquitin chromatin modification, which are both recognized by the RFTS domain of DNMT1. Thus, we propose that UHRF1 is necessary for maintaining DNAm at H3K9me3-marked genomic regions during DNAm erasure. To test this hypothesis, we will use mouse naïve ESCs with a knock-in construct encoding a Uhrf1 with a non-functional TTD domain on one allele and a floxed WT Uhrf1 on the other allele. Excision of the floxed Uhrf1 will be induced using 4-hydroxytamoxifen in the experimental ESCs. We will then determine whether disruption of the TTD domain results in loss of DNAm at H3K9me3-marked regions using targeted and whole genome bisulfite sequencing. Additionally, the recruitment of mutant UHRF1 to H3K9me3-marked regions will be assessed using ChIP-sequencing. Ultimately, our study will further elucidate the mechanism by which DNAm is retained at critical regions of the genome during its erasure in early embryonic development.

16 Methylation in the developing embryo

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Primordial germ cells (PGCs) are specialised gamete precursors, which following meiosis, develop into the mature eggs and sperm. Prior to their maturation, these cells must undergo genome-wide erasure of DNA methylation so that the establishment of sex-specific marks on the developing gonads can occur on a clean epigenetic slate. This process is fundamental in reconstituting cellular pluripotency, and faulty can lead to aberrant effects in fertility. Despite these facts, mechanisms surrounding epigenetic reprogramming remains highly uncharacterized, with questions surrounding what facilitates cellular demethylation. We hypothesize that extrinsic signals are provided by the somatic gonad cells that allow PGCs to undergo parental imprint erasure following colonization of the germline. We will address this hypothesis by looking at methylation erasure in ectopic PGCs that do not reach their site of function within the developing gonads. These cells are typically eliminated by apoptosis, but using Bax mutant mice, we can bypass cell death within ectopic PGCs. We can then visualize whether these cells undergo epigenetic reprogramming by using a Tel7KI-GFP reporter. Tel7KI-GFP is an insertion that is epigenetically silenced if paternally inherited. By generating Bax^{-/-} mice carrying a paternally inherited Tel7KI-GFP transgene, we can assess whether methylation within ectopic PGCs are erased, by detecting GFP. If our hypothesis is correct, ectopic PGCs should fail to reactivate the GFP in the Tel7KI allele because they require extrinsic signals provided by the gonads. Regardless of the results of this experiment, it will establish whether the genital ridge plays an important role in epigenetic erasure and lay the foundation for future work in understanding chromatin remodelling.

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Brown and beige adipose tissues are specialized for burning calories due to the presence of uncoupling protein (UCP)-1. When activated by β 3-adrenergic signalling, UCP-1 dissipates the mitochondrial H⁺ gradient, producing heat instead of ATP. In the context of obesity, enhancing thermogenic fat activity increases metabolic rate, thereby mitigating weight gain and related metabolic complications. Various histone modifying proteins control thermogenic fat development and function. Histone 3 lysine 27 (H3K27) is significant because acetylation (H3K27ac) activates gene transcription, while methylation (H3K27me1-3) causes gene silencing. Addition of acetyl moieties to H3K27 requires the homologous histone acetyltransferases (HATs) p300 and CBP. While HAT binding and H3K27 acetylation increases on thermogenic genes with β 3-adrenergic receptor (AR) agonism, it is unclear if p300 is necessary for β 3-AR induced remodelling of thermogenic adipose tissues. We hypothesized that mice lacking p300 in their brown and beige fat would be unable to activate the thermogenic gene program when given a β 3-AR agonist. Selective deletion of p300 in the thermogenic adipocytes was achieved by crossing mice with loxP sites flanking exon 9 of p300 with mice carrying Ucp1-Cre. All experiments were conducted using sexually mature (8-12 week-old) male and female floxed controls and Cre-positive littermates. Mice were weighed daily and given an intraperitoneal injection of the highly-specific β 3-AR agonist CL-316,243 (1 mg/kg body mass) each day for 1 week, after which adipose depots were collected for qPCR analysis. Despite loss of p300, thermogenic gene expression profiles of knockout adipose tissues responded similarly to control mice when given the β 3-AR agonist. Our data suggests that p300 is dispensable for the stereotyped remodelling of thermogenic adipose tissues in response to β 3-AR agonism, possibly due to functional compensation by the homologous histone acetyltransferase, CBP. Future studies will examine the combined role of p300 and CBP in β 3-AR-induced remodelling of thermogenic fats.

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The polycomb repressive complex 2 (PRC2) is an epigenetic reader/writer that catalyzes mono-, di-, and trimethylation of H3K27. Mutations in PRC2 members EZH2, EED and SUZ12 cause overgrowth and intellectual disability syndromes, namely Weaver syndrome, Cohen-Gibson syndrome, and Imagawa-Matsumoto syndrome respectively. Pathogenic PRC2 mutations manifest variable expressivity, conferring a wide range of phenotypic effects. Notably, 2.4% of the general population will have a rare missense or nonsense variant in one of these 3 genes. Therefore, attributing or excluding disease causality for rare coding variants may pose a challenge for clinical laboratories and, physicians. The creation of well-calibrated assays, that accurately predict functional impact of variants, will help resolve these challenges. We have taken advantage of genetic strategies in *Drosophila* to develop assays that can screen hundreds of naturally-occurring variants seen in human populations. Our combination of strategies includes CRISPR/CAS9-based genome editing, Recombination Mediated Cassette Exchange and phiC31 integrase-mediated transgenesis to develop reagents that can test variants in EZH2, EED and SUZ12, including: (i) Mimics of the human variant in the fly's orthologous gene (ii) Direct expression of the variant human protein in the fly. Our assays test the ability of human PRC2 variants to rescue null phenotypes, or to cause known PRC2 phenotypes in *Drosophila*. We show that pathogenic EED mimetic mutations can be distinguished from benign EED mimetic variants by interrogating classical esc phenotypes such as extra sex combs in males. Additionally, direct expression of an EZH2 pathogenic patient variant showed reduced ability of the variant to cause transcriptional silencing compared to wildtype EZH2 expression, in the position effect variegation assay of the white gene in *Drosophila*. Our work to date demonstrates the utility of numerous approaches to functional characterization of EED and EZH2 variants, and in the future SUZ12 variants, using the *Drosophila* assay platform.

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Fibro/adipogenic progenitors (FAPs) are a type of mesenchymal stromal cells (MSCs) residing in skeletal muscle. Recent studies utilizing single cell technologies have unveiled the heterogeneity of MSCs and revealed dipeptidyl peptidase 4 (DPP4) as a marker of a MSC subset. DPP4+ MSCs in adipose tissue are shown to be highly proliferative progenitors that give rise to mature adipocytes in physiological condition but also retain osteogenic potential. This prompted us to investigate if muscle FAPs bear similar characteristics. In this study, we initially showed that in skeletal muscle approximately 45% of FAPs expressed DPP4 at steady state. We further showed that DPP4+ FAPs were more capable of forming colonies in vitro compared to DPP4- FAPs. Using in vivo EDU labeling, we found that DPP4+ FAPs expanded rapidly within 24 hours of acute injury, suggesting that they are one of the first responders to injury. We are planning to further explore the function of DPP4+ FAPs by coculturing them with myofibers to determine if they can accelerate myogenic differentiation of satellite cells and promote regeneration. Additionally, DPP4+ FAPs will be specifically ablated during muscle injury using a DPP4CreERT2; DTAflox mouse strain to evaluate if they play a role in regeneration. Collectively, this study will contribute to our understanding of the hierarchical relationship of different FAP subpopulations and their specific roles in muscle regeneration. Understanding how they response to muscle injury in healthy and pathological conditions, such as muscular dystrophies, would provide an avenue for therapeutic interventions in patients with impaired muscle regeneration.

20 Med15 role in maintaining function of mature beta cells

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Type 1 diabetes (T1D) is an autoimmune destruction of the insulin secreting beta cells, resulting in hyperglycemia. Current treatments for T1D involves insulin injections, which is not without risks, or transplantation of islets from cadaver donors. There is, however, a severe lack of islet donors available; therefore, in vitro production of the beta-cells from stem cells would provide a valuable source of transplantation material. However, currently, not enough is known about how beta cells mature or maintain function throughout life to generate successful transplants. Both pre-natal development and post-natal maturation of beta cells require appropriate transcription of specific gene programs. Transcriptional coregulators, such as Mediator (MED), partner with transcription factors to regulate gene transcription. Interestingly, Mediator subunit MED15 plays important roles in lipid and glucose metabolism across species, suggesting it may be a conserved metabolic regulator. To study MED15 in beta cells, our lab generated mice with beta cell specific Med15 knockout (KO) and found that Med15 is required for pre-natal development and post-natal maturation. However, we do not yet know if Med15 plays a role in beta cells after the maturation period. To study the function of Med15 in adult beta cells we are using an inducible beta cell specific KO mouse model, allowing mice to develop into adults and then inducing Med15 deletion. In these mice, glucose tolerance tests revealed impaired glucose tolerance as early as two weeks after KO. Expression of maturation markers and beta cell functional genes were downregulated following KO, suggesting that the adult beta cells can lose their mature state after Med15 loss. This suggests that Med15 is required to maintain functional maturity and accurate glucose stimulated insulin secretion in adult beta cells. This knowledge could be used to produce longer lasting beta cells in vitro for transplantation.

21

Characterizing the hypoxia and oxidative stress response pathway in *C. elegans*

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Aberrant stress response activation allows cancer cells to grow and proliferate in harsh microenvironments featuring hypoxia and oxidative stress. Uncovering the mechanism behind stress response activation can reveal potential new cancer drug targets. The pathways driving stress resistance are conserved, so I use the model organism *Caenorhabditis elegans*, a nematode roundworm to study these pathways. Mammalian peroxisome proliferator-activated receptor alpha (PPAR) is a transcription factor (TF) required for inflammation inhibition and the formation of reactive oxygen species (ROS) during tumorigenesis. Our lab showed that the *C. elegans* PPAR homolog, nuclear hormone receptor NHR-49, is required for animal survival in starvation, hypoxia, and oxidative stress; in hypoxia, it is as important as the master regulator hypoxia inducible factor 1 (HIF-1), a cancer drug target. However, in contrast to HIF-1 signaling, the NHR-49 hypoxia and oxidative stress response pathways are poorly characterized. Excitingly, a reverse genetic RNA interference (RNAi) screen in our lab recently identified multiple TFs which may act within the NHR-49 controlled stress response pathways. Of these, I aim to validate eight of the most promising and least studied candidates, *nhr-38*, *nhr-81*, *nhr-174*, *hmg-11*, *ceh-43*, *tab-1*, *madf-5*, and *ztf-11* to determine whether they affect starvation survival, hypoxia, and oxidative stress resistance via NHR-49. Mapping the *C. elegans* NHR-49 stress response pathways will provide us with a better understanding of hypoxia and oxidative stress responses.

22

Telomerase Reverse Transcriptase and its non-canonical participation in DNA damage response

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BACKGROUND: Telomerase is a specialized DNA polymerase that maintains the length of telomeres at chromosomal ends by synthesizing telomeric repeats de novo from an internal RNA template. Without telomerase activity, telomeres shorten with each cell division, eventually reaching a length that would prompt either senescence or apoptosis. Telomerase reverse transcriptase (TERT), the catalytic component of telomerase, is upregulated in the majority of human cancers, often at a level that is more than sufficient to keep telomere length above the critical threshold. Previously, our laboratory reported that recombinant TERT expression protects transformed fibroblasts that utilize an alternative telomerase-free telomere lengthening mechanism against drug-induced DNA damage, indicating that TERT has a role in DNA damage response which is independent from its canonical telomeric function. In the present study, we treated recombinant TERT-expressing ovarian cancer cells with genotoxic agents of distinct mechanisms of action to identify possible DNA damage response pathways that TERT participates in. **METHODS:** We conducted dose-response cytotoxicity assays with recombinant TERT-expressing PEO1 and PEO6 – isogenic ovarian cancer cell lines that are deficient and proficient, respectively, in the error-free DNA repair pathway homologous recombination (HR). Genotoxic drugs used include CX5461 (a G-quadruplex stabilizer currently in clinical trials for HR-deficient cancers) and the following FDA-approved chemotherapeutic agents: bleocin (direct double-strand break inducer); etoposide (topoisomerase II inhibitor); niraparib and olaparib (poly-ADP ribose polymerase (PARP) inhibitors); and cisplatin, oxaliplatin, and mitomycin C (cross-linking agents). **RESULTS:** Recombinant TERT confers significant protection against bleocin, CX5461, and etoposide, and insignificant protection against PARP inhibitors and cross-linking agents, on HR-deficient PEO1. On the other hand, HR-proficient PEO6 with and without recombinant TERT expression respond similarly to all drugs tested. **CONCLUSIONS:** TERT participates in error-prone DNA repair pathways which are more prominently used by ovarian cancer cells lacking error-free repair capability, and as such is a potential therapeutic target for telomerase-positive HR-deficient cancers.

23 Role of Mediator subunit MED15 in human lung cancer cell stress response

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The immune system has many mechanisms to protect the body from diseases. However, cancer cells can manipulate themselves to be resistant to these mechanisms. To survive and proliferate, cancer cells activate transcription factors and rewire the transcriptome to promote resistance to oxidative stress. Oxidative stress worsens prognosis and treatment responses in cancer, and therefore, inhibiting this pathway can provide a therapeutic entry point to combating cancer. Preliminary research at our lab has shown that MED15, which is a subunit in the Mediator complex, is upregulated in numerous human cancers and is associated with poor overall survival. We explored the role of MED15 in immune response and oxidative stress response in A549 human lung cancer cells by knocking down (KD) and knocking out (KO) exon 5 of MED15 using RNA interference and CRISPR prime editing, respectively. RNA-seq results showed decreased expression of inflammatory markers, such as prostaglandin-endoperoxide synthase-2 (PTGS2, encoding cyclooxygenase-2 [COX-2]), and the chemokine ligands CXCL1, -2, -3 and -5. We validated these findings using real-time quantitative polymerase chain reaction and western blots. Additionally, we treated A549 cells with optimized levels of signaling molecules tumor necrosis factor α (TNF- α) and interleukin 1 β (IL-1 β) which stimulate the production of proinflammatory PTGS2 and CXCLs, finding significant downregulation of PTGS2 after treatment with MED15 loss. Furthermore, transcriptome analysis and qPCR data showed that MED15 KO and KD cell lines had downregulated oxidative stress responses. We will validate these findings by treating MED15 KO A549 cells with oxidative stressors to determine if MED15 plays a role in cell resistance to exogenous oxidative stress and if gene expression is altered. These results and future findings can help determine the role of MED15 in mammalian cells and could become relevant in future cancer treatment strategies.

24 Characterizing the biological effects of a novel G-quadruplex destabilizing ligand

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Guanine-rich sequences of DNA can form transient four-stranded structures called G-quadruplexes (G4s), and are often enriched in guanine-rich regions, including gene promoters and repetitive sequences of genomic DNA such as telomeres. G4 formation can regulate DNA metabolic processes such as transcription and replication, and may also induce replication stress and promote the generation of fragile single-stranded DNA. This has led to the extensive investigation of the potential DNA-damaging effects of G4-stabilizing small molecule ligands in cancer treatment. On the other hand, mutations promoting G4 formation can lead to neurodegenerative disorders such as amyotrophic lateral sclerosis and frontotemporal dementia. At the same time, 15% of human cancers rely on a telomerase-independent mechanism of telomere maintenance, known as alternative lengthening of telomere (ALT), which is initiated by DNA damage induced through telomeric G4-formation. This prompts the question of whether G4-destabilizing small molecules could be therapeutically valuable in treating these diseases. Recently, our collaborators Robert Hudson and David Monchaud reported the G4-destabilizing properties of a small molecule called Phenylpyrrolocytosine-based G-clamp (PhpC). PhpC destabilizes G4s in vitro, but to be therapeutically relevant, its biological effects must first be investigated in cellular models. Here we show the biological and G4-resolving effects of PhpC in the well-characterized human breast cancer cell line MCF7.

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Introduction: Human papillomavirus (HPV) drives cervical cancer. Despite available vaccines, cervical cancer remains the fourth most deadly cancer of females, with ~311,000 deaths annually. Extrachromosomal DNAs (ecDNAs) are large circular DNAs that drive cancer progression. Importantly, ecDNAs are large enough to contain entire genes or regulatory elements, making them suitable vehicles for oncogenes and enhancers. How ecDNAs promote cancer, how they form, and how they are regulated are all poorly understood. The extent to which HPV contributes to ecDNA formation has not been studied. **Methods:** We used existing short-read whole-genome and transcriptome sequencing to profile 118 cervical cancer tumour samples from the Human Immunodeficiency Virus (HIV)+ Tumour Characterization Project (HTMCP). We analysed these samples using an ecDNA detection tool (AmpliconArchitect). **Results:** We identified putative ecDNAs in 35/118 (30%) cervical cancer samples. In almost half of samples with ecDNA (16/35; 46%), we detected ecDNAs containing HPV DNA. Out of the 50 detected ecDNAs, most contained partial or entire human genes (43/50; 86%), including 33 unique oncogenes such as MYCN, ERBB2 (2 samples), ERBB4, JUN (2 samples), YAP1, FGFR1/2, and MET. Among these genes, ERBB family members were enriched (adjusted p-value = 0.00325), as were cellular processes relating to metastasis (adjusted p-value = 1.8×10^{-5}), consistent with the poorer prognosis of ecDNA+ cancer samples reported previously. The oncogenes on ecDNAs tended to be expressed at high levels compared to non-ecDNA-bearing samples, indicating ecDNA-mediated transcriptional up-regulation as an oncogenic mechanism. **Conclusions:** We noted increased expression of oncogenes on ecDNAs among cervical cancer samples, and recurrence of ERBB-containing ecDNAs in a subset of cases. Although preliminary, our observations are consistent with a role for ERBB receptors in some cervical cancers and raise the possibility that ERBB inhibitors might have a role in such cases.

26 Haploinsufficiency drives metastatic growth of mouse uveal melanoma

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Uveal melanoma (UM) is the most common intraocular cancer; in Canada, 130 individuals are diagnosed every year. Half of these patients will develop fatal metastases. Nearly 90% of UMs are driven by hyperactivation of the Gq/11 signaling pathway due to a somatic oncogenic mutation in one of two genes encoding heterotrimeric G-protein alpha subunits, GNAQ or GNA11. Most metastatic cases are characterized by the loss of the Breast Cancer Associated Protein 1 gene (BAP1), which encodes a deubiquitinase enzyme that regulates global gene expression. Loss of BAP1 is strongly associated with poor prognosis and reduced survival. However, the effects of BAP1 loss are complex, and attempts to model the effects of BAP1 have produced unexpected results, wherein knockdown resulted in fewer metastatic tumors. In this study, we studied the effects of Bap1 loss in our GNAQQ209L UM mouse model, which drives rapid UM and CNS melanoma with metastasis to the lungs. Homozygous Bap1 loss (Bap1^{flox}/KO) reduced the number of lung lesions, like some previous attempts to model Bap1 loss. However, in combination with GNAQQ209L, Bap1 haploinsufficiency (Bap1^{flox}/+ or Bap1^{KO}/+) increased the fraction of lung lesions that became macroscopic in size. Bap1 haploinsufficiency, but not homozygous knockout, also led to the formation of macroscopic liver lesions. Hence, BAP1 haploinsufficiency can drive UM metastasis. We next compared the effects of Bap1 loss on the transcriptome of primary mouse and human UM. This analysis identified 270 genes that are differentially expressed in the same direction (up or down) in both species. Epithelial-to-mesenchymal transition (EMT) was the most significantly affected gene ontology Hallmark and was driven by gene up-regulation.

27

Use of long read whole genome sequencing for precision diagnosis and treatment of individuals with Autism Spectrum Disorder

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Autism Spectrum Disorder (ASD) is the most common childhood developmental disability, affecting 1 in 58 Canadian school-aged children. ASD is defined by deficits in social communication and interactions, as well as restricted and repetitive behaviours. ASD diagnosis is complex with a highly variable pattern of behavioural symptoms, including co-morbidities (e.g. seizures). ASD is heterogeneous and can be caused genetically by both inherited and de novo mutations. Structural Variants (SV) represent substantial genomic diversity. Their role in ASD is undetermined, largely due to limitations in the commonly used short read whole genome sequencing (WGS). Similarly, aberrant DNA methylation is known to be associated with ASD. Long read genome sequencing offers previously unseen insight into the genome of individuals with ASD at a reasonable cost, while deriving small variants, SV, and methylation patterns. My project will derive and integrate previously unseen genomic changes identified from long read genome sequencing with phenotypic (symptom-based) data, to improve diagnosis and treatments in participants with ASD. From a cohort of 500 participants with childhood ASD I will identify participants who have no definitive genetic abnormalities from pre-existing short read WGS. Using long read WGS I will analyze and determine the impact of participants' structural variants, their methylome and imprinting, and their small variants. Upon discovering variants of interest, I will look for variant association within the original cohort and publicly available data of the variant of interest, and integrate the genomic data with the participants' clinical phenotype to resolve the behavioural impacts of the genetic variants. This project will increase the clinical utility genomic data by providing a stable and defined ASD view of the patient, which will allow us to provide an individualized and cost-effective treatment in an anticipatory, rather than a reactive way.

28

Identification of novel genomic structures and regulation patterns at HPV integration events in cervical cancer

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Integration of human papillomavirus (HPV) into the human genome has been implicated in the transformation of HPV-infected cervical cells into invasive cancer. We used Oxford Nanopore Technology's (ONT) PromethION to sequence the whole genomes of 59 cervical tumours (17 TCGA and 42 HTMCP cases) and characterize the structural changes that occur at HPV integration events. Using long-read data, we detected HPV integration sites, identified the integrated viral genes, assembled HPV-associated structural rearrangements, and resolved haplotype-specific DNA methylation patterns. Integration sites denote chimeric breakpoints between the HPV and human genomes, and these sites were grouped into integration events by phasing the sites together on the long reads. We detected 496 HPV integration sites that were grouped into 126 integration events across 53 tumours. Integration events displayed variable patterns, including the number of integration sites, copy number change between the breakpoints, and structural variants flanking the integration event. We thus classified the events into 6 HPV integration types: amplification integration, deletion integration, extrachromosomal DNA integration, repeat region integration, translocation integration, and multi-breakpoint integration. ONT DNA methylation data showed dysregulation of human enhancers proximal to and on the same allele as HPV integration events. We also assayed the methylation status of CpGs within the HPV genome at individual integration events to reveal which are epigenetically active within each sample and compared the regulation of integrated HPV to unintegrated samples. Our application of Nanopore sequencing has thus elucidated previously cryptic viral and human genomic dysregulation resulting from HPV integration in cervical cancer. Identifying the genomic consequences of HPV integration will enhance our understanding of HPV-induced carcinogenesis and may thus lead to new therapeutic strategies.

29 Scrambler: Simulating complex genomic rearrangements in chromothripsis

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Chromothripsis is a catastrophic localized rearrangement of a chromosome. Due to the occurrence of many genomic rearrangements in a single region, describing a chromothriptic event completely is quite difficult. The currently used genomic technologies lack the resolution to define the precise architecture of chromothripsis, which is crucial to understand the genes they impact and their clinical relevance. Compared to conventional approaches, long-read sequencing and optical genome mapping technologies have greater potential in resolving these highly complex rearrangements. However, because chromothriptic events vary greatly from one case to another, generating large experimental long-read sequencing and optical genome mapping datasets to validate the computational methods and pipelines for chromothripsis detection is not highly cost-efficient or feasible. I present a chromothripsis simulator, scrambler, that I have developed to enable users to generate custom chromothriptic events that can be used downstream with the simulators of short/long sequence reads as well optical genome maps for a detailed comparison of these technologies in detecting and resolving chromothriptic events. Scrambler will enable program developers to design and implement more sophisticated algorithms to map chromothripsis in sequencing and optical genome mapping datasets, and further research that will ultimately better our understandings of the mechanism and contribution of chromothripsis to disease.

30 Transcriptomic and cellular signatures of chronic addictive substance exposure in 3D human-derived neural organoids.

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Neural organoids, "mini-brains," are small bundles of interconnected brain cells grown from human stem cells that can recapitulate precise brain regions associated with neurological disease. Although neural organoids have been used to identify important new biological mechanisms of various neuropsychiatric disorders over the past two decades, they have yet to be fully explored in the context of Substance Use Disorder (SUD). To understand the genetic mechanisms underlying SUD, I plan to experimentally replicate recreational drug use in neural organoids by exposing them to various addictive substances (such as alcohol, opioids, and nicotine) over several days. Next, I will use state-of-the-art genetic sequencing technology to pinpoint precisely which genes are impacted by the addictive drugs. This study is among the first to investigate the direct effects of addictive compounds on living human brain tissue. It may reveal promising new genetic targets for SUD research that may be used to justify further clinical trials for the treatment of SUD and related disorders, or to advance the development of medicines to treat SUD.

31 Studying the life course to unravel the complexity of psychiatric disorders

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Psychiatric conditions are becoming more common in children and adolescents. These conditions often remain into adulthood. Our DNA and genes contribute to development of these conditions alongside environmental stressors. Current measures of genes contributing to risk of psychiatric conditions do not capture natural changes in gene behavior over time. These measures are limited to groups not represented in genetically informative studies, including children. With children at risk for psychiatric conditions such as anxiety and depression, they are ideal candidates for improved risk prediction for these conditions. Using genotype data from 3,400 children from the Canadian Healthy Infant Longitudinal Development (CHILD) study, this project aims to optimize the genetic measures of risk of psychiatric conditions in children. We will incorporate measures of gene behavior, particularly those involved in brain and immune development in childhood, to strengthen these prediction tools. Optimized predictions will allow for future clinical application of these scores to underrepresented populations, including identification of at risk children who might need earlier support.

32 Lessons from the past: genetic ancestry and demographic history of the Nicoyan Peninsula, a longevity hot-spot

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Little is known about the genetics of Costa Rica and its Nicoyan Peninsula, a longevity hotspot worldwide. The reasons behind the Nicoyans' increased health and life span remain unclear. European and African migrations following colonization, brought together previously separated populations. We performed global ancestry analysis on 500 Costa Rican genomes, including 100 from the Nicoyan Peninsula. Our results revealed an average increment of 12% African and 27% Indigenous American (NAT) ancestry in Nicoyans compared to non-Nicoyans. To explain if these differences could be the result of differing demographic histories, we used genetic-based migration modeling. Earlier and singular migrations of European and African ancestry were seen in Nicoya, whereas smaller but constant African migrations were observed in the rest of Costa Rica, matching demographic and historical records of the country. Lastly, given the different genetic histories observed in Costa Rica, we assessed the relationship between genetic ancestry and the unique health-span observed in Nicoyans. A total of five genes passed suggestive significance thresholds ($p < 0.001$), having an origin bias of African or European ancestry in Nicoyans. These have been associated to a variety of phenotypes such as, bone-density, blood-pressure and cell-counts. Ongoing assessments aim to provide further insight into the contribution of genetics to the increased health-span in the Nicoyan population. Health-span and longevity will be predicted using individual genetic data using ancestry-aware polygenic score (PGS) methods. The introduction of population stratification into the PGS will yield information about the relationship of genetic ancestry to environmental factors contributing to these complex phenotypes. Our analyses show the importance of treating populations with a holistic perspective, where the present-day environment and genetics are a snapshot of human diversity. Population genetics allow us to piece together a population's history through their genetics, helping to deepen our understanding of health-related traits.

Adolescents' perspectives of genetic testing & its personal utility: developing the adolescent-reported genetic testing utility InDEx

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Background: The utility of a diagnostic genetic test broadly refers to its value for patients, families, clinicians, healthcare systems and societies. Personal utility describes the value of a genetic test from the perspective of the patient. Decision-makers seek evidence of value from the patient perspective to inform funding decisions and clinicians desire tools to guide the provision of patient-centred care for complex technologies. **Objective:** This study aims to understand adolescents' experiences and perspectives of genetic testing and its personal utility. Ultimately, this work will advance development of the Adolescent-reported Genetic testing Utility InDEx (A-GUIDE), a novel, patient-reported outcome measure tool for genomic medicine. **Methods:** Informed by a literature review, we developed a preliminary list of A-GUIDE items which will be refined through qualitative interviews with ~15 adolescents between 14-19 years, who have received their genetic testing results within the past year. Parents/caregivers will be invited for separate interviews to understand their perception of the utility of the genetic test results their child received. Participants will be recruited from BC Children's Hospital, Vancouver, The Hospital for Sick Children (SickKids), Toronto, or can self-refer. Participants will be asked to reflect on their experience with genetic testing and on the meaning of personal utility. They will also be asked for feedback on the relevance, comprehensibility, and comprehensiveness of preliminary A-GUIDE items. A short demographic and genetic testing history survey will be administered at the end of the interview. **Thematic analysis of interviews will be guided by an interpretive description approach.** **Outcomes & Relevance:** Evidence from this study will be used to inform patient-centred care by genetic counsellors and other healthcare providers who order genetic/genomic testing and the development of an outcome measure to support evidence-informed genetic/genomic testing policy. This work will also contribute to the growing body of research related to optimizing genetic counselling practice for adolescents diagnosed with genetic conditions.

Adolescents' implicit and explicit attitudes towards their peers with genetic conditions

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There is increasing access to and uptake of genetic technologies by younger people, including those affected with genetic conditions and those curious about precision health. Efforts to improve literacy of adolescents to understand genetic ancestry test results, clinical genetic testing, and participation in genomics research have been previously undertaken. Improving attitudes and perceptions towards individuals with genetic conditions as a motivation for improved genetics education has not been pursued. Research has demonstrated that children who lack knowledge about genetic disorders may have harmful attitudes towards people with disabilities, but also that disability awareness can successfully modify these attitudes. We aimed to determine how adolescents perceive their peers with genetic conditions by examining their implicit and explicit attitudes. English speaking adolescents (10-18 years) were invited to complete a Disability Attitudes Implicit Association Test (DA-IAT), a validated measure of attitudes people may be unwilling or unable to express. We also conducted focus groups; a semi-structured interview centring on a fictionalized vignette about a young girl with Down syndrome helped address explicit attitudes. We used pragmatism as an analytical paradigm for our mixed methods study. Descriptive and inferential statistics were used to analyze DA-IAT and sociodemographic data, phronetic iterative analysis with constant comparison as a coding strategy for transcripts, and an interpretive description approach to develop a conceptual model. We used information power and theoretical sufficiency as measures of data completeness and quality. A total of 22 adolescents (7 identified as female; 14 as male; 1 as non-binary) completed the DA-IAT and participated in one of 4 focus groups. Participants had a mean DA-IAT score of 0.73 (SD=0.45); a one-tailed t-test ($t=6.80$, $p<0.00001$) indicated a statistically significant implicit preference for non-disabled people. Despite most participants being implicitly prejudiced against disabled people, they demonstrated more neutral explicit attitudes during the focus groups. One factor that appeared to result in truly low prejudice (i.e., low implicit and low explicit attitudes) was whether an adolescent had a close relationship with someone who had a genetic condition. Our results demonstrate that although genetics education may be a useful intervention, social engagement would be especially beneficial in shifting adolescents' perceptions of their peers who have genetic conditions. Findings from this study will be used to inform the design of interventions that address biased perceptions of people with genetic conditions, reduce prejudices, improve social interactions, and better prepare adolescents for the world beyond school.

35

Utility of a genomic results e-booklet for parents of pediatric neurology patients who have undergone genomic sequencing

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Genomic testing, including whole exome and whole genome sequencing, has become increasingly common in clinical settings such as pediatric neurology where the high diagnostic yield has potential clinical value. However, in many specialty clinics, there may be no genetic counsellor to help families navigate the complexities following testing. Families have identified the need for a support tool to help them to understand and adapt to the genomic results, and to find suitable resources to aid their post-testing journey, whether or not their genomic result is diagnostic. In response to this need, a customizable, multilanguage, Genomic Results e-Booklet (GRB) was co-developed with parents to guide and support families. The goal of this qualitative study is to determine the long-term utility of the GRB. We propose to interview about 15 parents of children who pursued genomic testing through the Pediatric Neurology Program at BC Children's Hospital and who received a customized GRB for their child. The interviews will be about 15-20 minutes duration, and will be completed by January 2023. Interviews will occur 10 weeks after the families receive the GRB from their pediatric neurologist. The recorded interviews will be transcribed and coded using NVivo. NVivo codes will be analyzed for patterns, shared experiences and themes. We hope to obtain insight into the long-term utility of GRB for families by identifying the ways in which the GRB is used. We anticipate that if the GRB is useful to families undergoing testing in Pediatric Neurology, it is likely to be useful to families who pursue genomic testing in other settings, particularly those without genetic counselling resources, which, in our hospital includes many specialty clinics. This study may demonstrate that the GRB can help fill the void many families feel after undergoing genomic testing while also helping to stretch limited genetic counselling resources.

36

Evaluating family-centered care in Pediatric Neurology: parents'/caregivers' perspectives

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Family centered care (FCC) is a method of service delivery that prioritizes the partnership between families and healthcare providers. A family-centered approach is of significant benefit to both children and families. FCC results in improvements in parental satisfaction and coping, child health status, communication between family and health care providers, family functioning and transition to health care in an adulthood setting. FCC has also been shown to better meet child and family needs with increased access to care such as receiving more genetic counselling, greater access to specialty care, and receiving timely access to services. Although it is well recognized that FCC is associated with improved outcomes for parents and children diagnosed with chronic conditions, there is often a disconnect between the principles of FCC that are supported in theory and the realities of clinical practice. The purpose of this study is to evaluate the perspective of parents/caregivers in the pediatric neurology clinic where there are no genetic counselors (GCs) within the multidisciplinary teams to gain a better understanding of the families' experience and provide opportunities to discuss how care can be improved. We will be measuring the perceptions of parents'/caregivers' of FCC using the Measure of Processes of Care (MPOC-20) and parents' perception of their child's quality of life using the Pediatric Quality of Life (PedsQL) survey. MPOC-20 is a clinically validated and reliable tool which can be used with parents of children with a broad range of medical conditions across children's ages. The PedsQL is a clinically validated and reliable tool to measure severity of chronic health conditions and impact of these conditions on pediatric health-related quality of life. Conducting an evaluation of FCC will allow for targeted interventions (GC integration, educational resources) to be developed that will meet the specific needs of patients and their families.

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Background: Genetic counsellors are health care professionals who help patients and their families understand and cope with genetic conditions, while also discussing applicable resources and options for genetic testing. Genetic counsellors discuss many difficult topics with their patients, but little is known about the discussions that they are having with their patients about Medical Assistance in Dying (MAiD). **Aims:** The purpose of our study is to determine Canadian genetic counsellors' perceived knowledge about MAiD, the types of discussions that they are having about MAiD with their patients, and the contexts in which these discussions arise. A secondary aim is to provide learning objectives for genetic counsellors who would like to be prepared to discuss assisted death with their patients. **Methods:** We are using a mixed methods approach involving a short survey, which elicits the participants' perspectives, experiences, and perceived knowledge about MAiD, followed by semi-structured interviews with 10-15 genetic counsellors who have discussed MAiD with patients in various settings. Descriptive statistics will be used to analyze quantitative data. Transcripts will be analyzed using an interpretive description approach. This methodology aims to describe and understand participant experiences and insights to inform clinical practice. **Preliminary Results:** Preliminary data show that genetic counsellors have discussed MAiD with patients and/or their family members affected by motor neuron disease, dementia, cancer, congenital anomalies, and connective tissue disorders. Though most participants have indicated that it is important for genetic counsellors to be knowledgeable of and prepared to discuss MAiD, the majority have indicated that they do not know about the eligibility criteria and process for accessing MAiD in Canada. As data continues to be collected and analyzed, we anticipate that this study will reveal themes that may help genetic counsellors to better understand how they can prepare for conversations about assisted death with their patients.

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Background: Genetic diseases are a significant cause of infant mortality, morbidities and admission to neonatal intensive care units (NICUs). Rapid genome- and exome-wide sequencing (rGWS) has a faster turn-around-time compared to conventional genetic testing and a diagnostic rate of up to 60% when employed in NICU patients. rGWS has been found to benefit the health of neonates and improve parental experiences in the NICU. Yet, healthcare providers (HCPs) working in NICUs have been reported to experience barriers when using rGWS to care for critically ill infants. Further, little is known about the experiences of many non-genetic HCPs working with rGWS in Canadian NICUs. As Canadian NICUs are highly multidisciplinary environments, it is important to understand the experiences of all NICU stakeholders working with rGWS so that support and education of NICU healthcare teams can be optimized to deliver an efficient, evidence-informed, and appropriate clinical service. **Research Aim:** The aim of this study is to explore the perspectives and experiences of non-genetic HCPs with rGWS in two Canadian NICUs to (1) understand the barriers and challenges encountered when using rGWS; and (2) gain insight into the benefits associated with using rGWS in Canadian NICUs. **Methods:** Non-genetic HCPs of various specialties will be recruited from the BC Women's Hospital and Victoria General Hospital NICUs. Participants will complete a demographic survey and a one-on-one semi-structured interview. We plan to recruit 15 participants across various specialties. We will use an interpretive descriptive approach to code and analyze the interview transcripts to identify patterns, themes, and variation in non-genetic HCPs' experiences with rGWS. **Relevance:** The results from this study will help inform the development of guidelines on the use of rGWS in Canadian NICUs to facilitate the effective implementation of this technology. These guidelines may provide support to non-genetic HCPs working with rGWS, thereby improving the care and support provided to NICU families.

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As of 2021, 91.5% of genetic counsellors self-identify as being White and 84.7% identify as being female. Increasing the diversity of the healthcare workforce is one way in which racial/ethnic disparities in healthcare delivery can be addressed. As admissions committees (AC) are directly responsible for deciding who the next generation of genetic counsellors will be, it is essential that we understand the ways in which they are (or are not) considering diversity when selecting their future cohorts. This study aims to explore genetic counselling program admissions committees approaches to addressing issues related to the lack of diversity in the genetic counselling (GC) profession. This will be done through a survey completed by an AC member of GC programs exploring the diversity (racial/ethnic, gender, sexual orientation, disability status, neurodiversity, rural/low socioeconomic status background) of admissions committees and student cohorts over the past 3 years. From these data we will purposively recruit approximately 15 individuals to conduct semi-structured interviews to (1) understand how admissions committees view diversity in the context of their admissions processes; (2) to explore DEI initiatives employed by programs in their admission processes and their perspectives on these initiatives; and (3) to understand facilitators and barriers to diversification. Coding and analysis of the interviews will be done using an interpretive description approach through a framework of intersectionality. Considering the sensitive issues inherent to GC sessions, a genetic workforce lacking in diversity can be particularly impactful on the experience of minority patients. By developing an understanding of the current landscape of DEI initiatives in GC programs, this data could be used to inform new strategies that could be employed by all selection committees with the goal of increasing diversity within their programs and consequentially, the profession as a whole.