9:00 am - 9:10 am	Welcome and Opening	Peter Williamson *Rautaki Hononga / Kaitakawaenga (Māori Strategic Framework Facilitator)* Louise Bicknell *Co-Director Genetics Otago*
Session One		Chair: Megan Leask
9:10 am – 9:30 am	A long non-coding RNA differentially regulates neuronal health	Indranil Basak *Department of Biochemistry*
9:30 am – 9:45 am	CYP2D6*71 is a poor metaboliser allele common in Polynesian and Māori people and absent from Europeans	Martin Kennedy *Department of Pathology and Biomedical Science, Christchurch*
9:45 am – 10:00 am	What Really Drives Bioinformatic Tool Accuracy?	Paul Gardner *Department of Biochemistry*
10:00 am – 10:15 am	ATP13A2-inhibited astrocytes model early-onset Parkinson's pathologies in vitro	Luke Geddes *Department of Biochemistry*

SciCom Award Presentations		Chair: Louise Bicknell
10:15 am – 10:30 am	What fraction of the genome is functional? *Illustration*	Daniela Schiavinato, DJ Champion, Paul P. Gardner
	The pāua that clings to the sea': a new species of abalone found only in waters off a remote NZ island chain *Article*	Kerry Walton, Hamish Spencer, Nic Rawlence
	Are kiwi and moa recent immigrants from Australia? Neither fossils nor genetic evidence support the story *Article*	Nic Rawlence, Alan Tennyson, Pascale Lubbe
	Predicting 'male-time' with the Androgen Clock *Video*	Victoria Sugrue
	Why study the rarest form of Parkinson's disease? *Video*	Luke Geddes, Indranil Basak and Eden Li
10:30 am – 11:00 am Morning Tea Break (SciCom Award People's Choice Voting)		

Session Two		Chair: Sheri Johnson
11:00 am – 11:20 pm	Bacterial lipopolysaccharide modulates methylation in colorectal cancer	Rachel Purcell *Department of Surgery, Christchurch*
11:20 pm – 12:00 pm	**Lightning Talks** (6 mins each including Q+A) 3D Perspectives on Spatiotemporal HOX Gene Expression from the Native Onychophoran, *Peripatoides Novaezealandiae*	Taylor Gallagher *Department of Biochemistry*
	A Hot New Biomarker Has Entered the Villa: Modelling the effect of DNA amount in CRC Detection Assays on Downstream Sequencing Metrics	Ruby Werry *Department of Biochemistry*
	Outcomes from a Nationwide Bioinformatics Training Programme: Reflecting and Looking Ahead	Tyler McInnes *Genomics Aotearoa*
	Unravelling the role of large genome deletions (LGD) in drug-resistant *Mycobacterium tuberculosis* isolates	Abraham Siaw *Department of Microbiology and Immunology*
	Building the Androgen Clock: An Epigenetic Predictor of Long-term Male Hormone Exposure	Victoria Sugrue *Department of Anatomy*
	A Comparison of Progeny of Selected and Unselected Sires within the Setting of a Community-Based Breeding Programme Catering to Women Smallholder Goat Rearers in Bihar, India	Shalini Abeykoon *AbacusBio Ltd.*

12:00 pm – 12:15 pm	Unravelling the Secrets of Chordate Whole-Body Regeneration using single-cell sequencing	Megan Wilson *Department of Anatomy*
12:15 pm – 12:30 pm	Understanding the genetics and epigenetics of the post-viral fatigue syndromes, Myalgic Encephalomyelitis / Chronic Fatigue Syndrome, and Long COVID	Warren Tate *Department of Pathology*
12:30 pm – 1:30 pm Lunch (poster judging)		

Session Three		Chair: Matt McNeil
1:30 pm – 2:30 pm	Genomics Aotearoa and Taonga species	Peter Dearden *Department of Biochemistry and Genomics Aotearoa*
2:30 pm – 2:45 pm	Loss of aminoarabinose lipid modification leads to cephalosporin antibiotic resistance in *Pseudomonas aeruginosa*	Maddie Hardie Boys *Department of Microbiology and Immunology*
2:45 pm – 3:00 pm	Thermal resilience in the endemic triplefins of New Zealand	Sheri Johnson *Department of Zoology*
3:00 pm – 3:30 pm Afternoon Tea Break		

Session Four		Chair: Megan Wilson
3:30 pm – 3:50 pm	Roll-over dogma: female specific ribosomes and sex determination	Tim Hore *Department of Anatomy*
3:50 pm – 4:05 pm	Missing Heritability in Heart Failure: Don't Sweat the Small Stuff?	Gemma Moir-Meyer (via Zoom) *Department of Medicine, Christchurch*
4:05 pm – 4:20 pm	Defining therapeutic vulnerabilities in drug-resistant strains of *Mycobacterium tuberculosis*	Matt McNeil *Department of Microbiology and Immunology*
4:20 pm – 4:35 pm	Evolving vertebral counts without evolving the segmentation clock	Shannon Taylor *University of Oxford*
4:35 pm – 4:45 pm	Awards Presentation	Louise Bicknell *Co-Director Genetics Otago*
4:45 pm Close		

Poster Presentations

Poster Number	Title	Presenting Author
1	Understanding drivers of metastasis to develop better treatments for oestrogen receptor positive breast cancer	Sophie Tunnicliffe
2	Liquid biopsy based DNA methylation biomarker panel for facilitating minimally invasive prostate cancer detection	Atreyi Dutta
3	Development of a Prognostic Tool for Identifying High-Risk Prostate Cancer Patients	Zahra Shafaei Pishabad
4	Exploring the link between Infertility and Ovarian Cancer	Bridget Fellows
5	Role of the TP53 Splice Mutations in cancer progression	Apeksha Bhandarkar
6	Predicting Functional Genomic Features	Daniela Schiavinato
7	Exploring the disease mechanism caused by variants in histone H4	Tira McLachlan
8	Exploring Mendelian-informed genetic variants for their potential to alter cancer risk using the UK Biobank	Emily Nielsen Dandoroff
9	Pathogenicity and Interactions of ORC3	Mykilah O'Sullivan
10	A Genetic Characterisation of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome Predisposition	Bryn Griffiths
11	Targeting oncogenic lncRNAs in colorectal cancer-associated macrophages	Brooke Morrison
12	Molecular characterisation of novel *de novo* variants in *ELAVL2*	Meghan Mulligan
13	Developing a blood-based epigenomic signature for ME/CFS and Long COVID using Peripheral Blood Mononuclear Cells (PBMCs)	Sayan Sharma
14	Prioritisation of Māori and Pacific Gout-Associated Non-Coding Genetic Variants Using Enhancer Assays	Selina Williams

Abstracts Oral Presentations:

CYP2D6*71 is a poor metaboliser allele common in Polynesian and Māori people and absent from Europeans

Leonie M. Hitchman[1], Nicholas J. Magon[1], Allison L. Miller[1], Campbell R. Sheen[5], Elizabeth Dunn[5], Stephanie M. Bozonet[1], John F. Pearson[2], Masahiro Hiratsuka[6], Allamanda Faatoese[2], Tony R. Merriman[3,4], Anthony J. Kettle[1], Martin A. Kennedy[1]

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CYP2D6 is one of the most important pharmacogenes responsible for metabolising a wide range of medications. The gene is extremely polymorphic, which can lead to variable activity of the CYP2D6 enzyme, and elevated risks of adverse drug reactions and treatment failure. However, the full extent of variability in *CYP2D6* is not yet known, particularly for understudied populations. We employed nanopore sequencing of 6.6kb amplicons encompassing *CYP2D6* (Liau et al., 2019) to detect all genetic variants within a group of Māori volunteers, largely affiliated with the Ngāti Porou iwi, in Aotearoa New Zealand. We confirmed the prevalence of an allele called CYP2D6*71 that was initially discovered in Han Chinese people and not observed in Europeans, which constitutes close to 10% of alleles in this Māori cohort. This confirmed and extended our earlier findings in a cohort of Pacific people (Hitchman et al., 2022). Understanding the functional impact of the CYP2D6*71 allele is crucial to allow accurate inference of metabolizer phenotypes, and to determine whether it should be included in routine *CYP2D6* tests. The key CYP2D6*71 variant (rs118203758) causes a G42E substitution in the N-terminal membrane insertion region of CYP2D6, and it has been previously suggested that this may inactivate the enzyme. In order to establish the functional relevance of CYP2D6*71, we employed two distinct approaches. First, we used recombinant expression system in mammalian cells, with preparation of microsomes and incubation with the CYP2D6 substrates metoprolol and solanidine, followed by mass spectrometric analysis. This work in two different cell lines failed to yield interpretable data. Second, we identified several individuals with CYP2D6*71 in combination with known non-functional *CYP2D6* alleles, then used mass spectrometry to analyse the metabolic products of solanidine, a recently described biomarker for CYP2D6 activity, in stored plasma samples. In these cases, evidence for negligible metabolism of solanidine could be seen, in contrast to plasma from people with functional *CYP2D6*. This preliminary, but fairly compelling, observation indicates that CYP2D6*71 is most probably a non-functional, poor metabolizer allele. Given the prevalence of this allele in Polynesian and Māori people, *CYP2D6* testing in these populations must include the key CYP2D6*71 variant (rs118203758) to ensure phenotypes are correctly inferred. Furthermore, CYP2D6*71 occurs at an appreciable frequency in Han Chinese people, so these findings may also be relevant to this population.

References
Hitchman et al. (2022) Front Genet 13:1016416.
Liau et al. (2019) Pharmacogenomics 20:1033-1047.

What Really Drives Bioinformatic Tool Accuracy?

Paul P Gardner
University of Otago, Dunedin, New Zealand.

In bioinformatics, multiple tools often exist for the same task, yet their accuracy can vary significantly. I have led several projects evaluating the accuracy of bioinformatic software. One of which is focused on detecting protein-coding sequences in nucleotide sequences, which are commonly used in long non-

coding RNA pipelines [1]. Benchmarks like this prompted my team to explore what factors are linked to software accuracy [2]. We found that citation-based metrics (H-index, impact factors, citations) had no correlation with accuracy. Instead, indicators of long-term software support, such as GitHub activity, were strongly associated with better performance. This suggests that sustained support for bioinformatics tools is more beneficial than pursuing citation-based reputation. I will conclude with a grudge-based investigation into the link between academic department affiliation and software accuracy [3]. These studies highlight the crucial role of continuous development and interdisciplinary collaboration in producing reliable bioinformatic software.

1. Champion DJ, Chen TH, Thomson S, Black MA, Gardner PP (2024) Flawed machine-learning confounds coding sequence annotation. bioRxiv. https://doi.org/10.1101/2024.05.16.594598
2. Gardner PP et al. (2022) Sustained software development, not number of citations or journal choice, is indicative of accurate bioinformatic software. https://doi.org/10.1186/s13059-022-02625-x
3. Gardner PP (2024) A Bioinformatician, Computer Scientist, and Geneticist lead bioinformatic tool development - which one is better? https://doi.org/10.1101/2024.08.25.609622

ATP13A2-inhibited astrocytes model early-onset Parkinson's pathologies *in vitro*

Luke P.R. Geddes, Luca K.C. Gray, Katie Peppercorn and Indranil Basak

Kufor-Rakeb Syndrome is a rare autosomal recessive disorder causing early-onset Parkinson's disease in teenage youth. Associated with mutations in the *ATP13A2* gene, the condition results in ATPase dysfunction and reduced metal cation transport, leading to toxic effects during neural development. Here, using CRISPR technology, we inhibited the *ATP13A2* gene to generate juvenile Parkinson's disease-mimicking astrocyte and dopaminergic neuron co-cultures. In a blinded study, we observed a 49.88% decrease in neuronal counts when cultured with *ATP13A2*-deficient astrocytes, in comparison to neurons cultured with healthy astrocytes. Both direct and indirect contact between dopaminergic neurons and CRISPR-inhibited astrocytes compromised neuronal mitochondrial stability with a 73.39% increase in fluorescence detected using a MitoTracker assay. These data suggest that *ATP13A2*-deficient astrocytes may contribute to the neuronal pathology of juvenile Parkinson's disease. Current neurodegenerative literature indicates astrocyte defects have strong effects on neuronal health, but our group research is uncovering new insights into the variations occurring in juvenile Parkinson's disorders. These ongoing experiments are indicating that Kufor-Rakeb neuronal death is more strongly associated with astrocyte mutations than mutations in the neurons themselves.

Bacterial lipopolysaccharide modulates methylation in colorectal cancer

Rachel Purcell

Hypermethylation is a hallmark of colorectal cancer (CRC) arising from the serrated pathway and often involves methylation of tumour suppressor genes, such as *MLH1*. We have previously identified lipopolysaccharide (LPS) from *Fusobacterium* and *Bacteroides* spp. as a functional link between the microbiome and tumour microenvironment. As LPS has been reported to influence DNA methylation in epithelial cells, we aimed to investigate the ability of LPS from these species to influence DNA methylation in CRC cell lines. The human CRC cell line, HT29, was treated with LPS from *Fusobacterium spp.* and/or *Bacteroides spp*., and RT-qPCR was used to determine *MLH1* expression in LPS-treated cells compared to LPS free to determine possible hypermethylation. Reduced representation bisulfite sequencing (RRBS) was performed on DNA extracted from LPS-treated and control cells. Sequences were aligned with the human genome (GRCh38) and methylation profiles obtained. RNA from patient CRC samples and cell lines were used to validated methylation data. *MLH1* RNA expression was decreased post LPS treatment compared to untreated controls, suggesting possible methylation of its promoter. RRBS identified 25 differentially hypermethylated gene promoter sites in the LPS treated group compared to control, including *TRIM67* and *EDIL3,* with known tumour suppressor properties. Methylation data was validated in a CRC cohort of ~300 cancer samples, showing

significantly decreased expression of these genes in tumours with high levels of *Fusobacterium spp.* This preliminary data shows that bacterial LPS found within the colorectal tumour microenvironment can induce hypermethylation in CRC cell lines, which may lead to tumour suppressor inhibition and development of CRC through the serrated pathway.

3D Perspectives On Spatiotemporal HOX Gene Expression From The Native Onychophoran, *Peripatoides Novaezealandiae*.

Taylor Gallagher[1], *Peter Dearden*[1]
[1]*Department of Biochemistry, University of Otago, Dunedin, New Zealand*

The native Dunedin velvet worm *Peripatoides novaezealandiae* occupies an early branching position in *Panarthropoda*, a super-phylum containing the classical *Arthropoda* (insects, crustaceans, and myriapods), and the subphyla *Onychophora* and *Tardigrada*. *Panarthropoda* displays remarkable diversity in animal form, and yet, modern *Onychophora* share morphological similarities to their distant ancestor *Hallucigenia sparsa*, an extinct Cambrian lobopodian, despite approximately 550 million years of evolution. These similarities ultimately raise a fundamental question: how did diversity in animal form rise in *Panarthropoda*? Recent sequencing of the *P. novaezealandiae* genome has revealed that the highly conserved Hox genes, usually ordered and expressed colinearly, are unconventionally organised. Separated by megabases of DNA containing thousands of other genes, *P. novaezealandiae* Hox genes are segregated in two-dimensional space. This challenges our current understanding of spatiotemporal Hox gene expression, which based on knowledge in *Drosophila*, has a requirement for co-regulatory interactions. We predict that Hox gene co-regulatory interactions are maintained in this unique organisation by dynamic changes to three-dimensional genome organisation during development. For the first time in this species, we will validate the boundaries of Hox gene expression using fluorescent *in-situ* hybridization and assess changes to genome organisation during development using Omni-C Proximity ligation. Ultimately, this research will not only begin to highlight the role (if any) played by dynamic genome organisation in the development of a non-model organism, but will also provide fundamental insight into the rise of diversity in *Panarthropoda* from ancestral Cambrian roots.

A Hot New Biomarker Has Entered the Villa: Modeling the effect of DNA amount in CRC Detection Assays on Downstream Sequencing Metrics

Ruby Werry, Michael Dunnet, Parry Guilford

As colorectal cancer (CRC) diagnoses increase globally, so does the necessity of accessible, effective diagnostic tools - particularly since early diagnosis is the strongest predictor of long term survival. The Guildford Lab has recently developed an equitable blood test for CRC that employs rolling circle amplification (RCA) and Nanopore sequencing technologies to detect circulating tumor DNA (ctDNA) as a biomarker. The assay enlists unique molecular identifiers (UMI) which are ligated to DNA molecules before PCR amplification, and enable individual molecules to be tracked through PCR cycles. Consensus calling reads within the same UMI family improves read accuracy and somatic variant calling; however, this requires DNA samples to be over-sequenced and has diminishing returns. In this work, I aimed to determine the appropriate sequencing depth required to achieve specific UMI-family sizes based on input DNA amount. Differing amounts of DNA were introduced into the RCA assay, then following Nanopore sequencing each amount was processed through a bioinformatic pipeline to determine the relationship between UMI-family size, total sequencing depth, and DNA input amount. While the results of this study are specific to the current RCA assay, the methodology employed here serves as a template for other sequencing-based somatic variant calling pipelines.

Outcomes from a Nationwide Bioinformatics Training Programme: Reflecting and Looking Ahead

Tyler McInnes
Genomics Aotearoa

Since 2017 Genomics Aotearoa and NeSI (New Zealand eScience Infrastructure) have collaborated to provide bioinformatics and genomics training free of charge to researchers in Aotearoa New Zealand. Following the format of The Carpentries (an internationally recognised training institution), we have run more than 100 workshops in-person and online, reaching more than 2,000 total attendees. The training programme includes a portfolio of beginner workshops covering R programming, the command line, and working on servers, as well as specialized genomics topics such as RNA-seq data analysis, long-read genome assembly, single-cell RNA-seq, and pangenome graphs. The workshop portfolio was developed by over 30 experts from 15 institutions, including both international collaborators and local contributors who have taught internationally. The training programme is itself a valuable resource which has provided support to community initiatives (*e.g.,* IndigiData Aotearoa), fosters relationships with the private sector (*e.g.,* collaborative conferences), and offers teaching and training opportunities to Postdocs and PhD students. This talk will showcase some of the key outputs of the training programme, highlight opportunities for researchers in Aotearoa who want to engage in bioinformatics training, and invite discussion on how we best serve the community going forward.

Unravelling the role of large genome deletions (LGD) in drug-resistant *Mycobacterium tuberculosis* isolates

Abraham Siaw, Htin Lin Aung

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), claims 1.2 million lives annually. The rise of drug-resistant TB (DR-TB) strains further exacerbates the situation. Previous studies using Illumina short-read sequencing identified large genomic deletions (LGDs) of more than 5kb, comprising phospholipases gene cluster (*plcABC*) and PPE38 genes in DR-TB strains, but its limitations in resolving long repetitive regions leave gaps in our knowledge. This study aims to utilize long-read Oxford Nanopore Technology (ONT) for whole-genome sequencing to confirm the genome structural variant of this LGD, not a result of limitations of short-read Illumina sequencing. ONT results confirmed the presence of this LGD. These genes are critical for Mtb virulence. The *plc* genes support pathogenesis, intracellular survival, and lipid metabolism, while PPE38, a substrate of the ESX-5 secretion system, plays roles in immune evasion and host-pathogen interactions. Interestingly, this study identified these deletions in DR-TB, which raised questions about their contribution to DR-TB. These findings suggest a potential link between deletions of genes encoding virulence factors and drug resistance, opposing current understanding. Further research is needed to revisit the functional impact of these deletions, which may lead to a novel insight into DR-TB and introduce new therapeutic approaches.

Building The Androgen Clock: An Epigenetic Predictor Of Long-Term Male Hormone Exposure

Sugrue, V.J.[1]; Prescott, M.[2]; Glendining, K.A.[2]; Zoller, J.A.[5]; Narayan, P.[6]; Lu, A.T.[4]; Bond, D.M.[1]; Ortega-Recalde, O.J.[1]; Grant, M.J.[6]; Bawden, C.S.[5]; Rudiger, S.R.[5]; Haghani, A.[4]; Hore, R.R.[10]; Sears, K.E.[8]; Wang, N.[9]; Yang, X.W.[9]; Snell, R.G.[6]; Anderson, G.M.[1]; Garratt, M.[1]; Horvath, S.[3,4]; Campbell, R.E.[2]; Hore, T.A.[1]
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Aging is a complex process characterised by biological decline and a wide range of molecular alterations. Epigenetic clocks leverage age-associated changes in DNA methylation to estimate chronological age and identify factors influencing aging. Here, we utilise epigenetic clocks to explore sex differences in biological aging and the female-specific lifespan advantage commonly observed among mammals. We observe an accelerated aging rate in adult male sheep compared to females. Notably, the removal of androgens by male castration decelerates the aging rate, suggesting a causal role of androgens in the sex difference in longevity. We identify several androgen-sensitive CpG dinucleotides that progressively lose methylation throughout the lifespan in intact males but remain stable in castrated males and females. Using these sites, we develop a novel epigenetic predictor – the *Androgen Clock* – capable of estimating the period of androgen exposure. Our results show that the clock's 'ticking rate' can be accelerated in female mice by dihydrotestosterone supplementation, or halted entirely in males by castration. Finally, we explore potential applications of this tool in medicine and agriculture. Beyond this, the Androgen Clock offers a valuable model for understanding age-associated DNA methylation changes by its capacity for manipulation through small molecule intervention without compromising cell survival.

A Comparison of Progeny of Selected and Unselected Sires within the Setting of a Community-Based Breeding Programme Catering to Women Smallholder Goat Rearers in Bihar, India

Shalini Abeykoon, *Dr Peter Amer (Supervisor), Associate Professor Phil Wilcox (Supervisor), Dr Chanda Nimbkar*

Research on the Black Bengal goat breed is limited for studies conducted specifically on smallholder community-based breeding programmes (CBBPs). The primary objective of this study was to establish whether the intervention of a CBBP to select breeding sires at the age of 100 days according to a score derived by combining several selection criteria resulted in progeny with higher average daily gain (ADG). A secondary objective was to determine the temporal effect of the project intervention on ADG. Analysis of the data using multiple linear regression models revealed an overall statistically significant result ($P < 0.001$) that progeny of selected sires had a superior ADG 13.6% higher than the raw mean ADG for kids of unselected sires. Further models created to show the temporal effect on ADG by analysing records of only kids of unselected sires show that there has been average improvement of 2.4g per year ($P < 0.001$) from 2018 to 2023. Overall, these results support the wide popularity of selected sires in a rapidly growing number of villages and show substantial gains in kid growth rate throughout an intervention project that included a CBBP.

Unravelling the Secrets of Chordate Whole-Body Regeneration using single-cell sequencing

Berivan Temiz, Michael Meier and Megan J. Wilson.
Department of Anatomy, University of Otago, Dunedin, New Zealand.

Botrylloides diegensis, a marine chordate, displays a remarkable ability for whole-body regeneration (WBR) by regenerating an entire body system from its vascular network. Our research encompasses gene and pathway characterisation, transcriptome profiling, and haematological analyses during various stages of regeneration. We have recently used single-cell RNA sequencing (sc-RNA-seq) of mature colonies and multiple WBR stages to gain new insights into this chordate model of WBR.

Notably, we observed the emergence of large transient cell populations exclusively during the early stages of WBR. Although lacking distinct highly expressed markers, sub-clustering revealed shared molecular signatures with committed cell clusters, suggesting orchestrated differentiation processes. We identified *SoxC* as a pivotal stem cell marker, exhibiting robust expression within aggregates of stem-like cells, regeneration vesicle-forming cells, and cells initiating organogenesis. Our cell trajectory analyses consistently depict a trajectory from *SoxC*+ cell populations through transient states towards more specialised cell lineages. Our findings collectively highlight the remarkable plasticity inherent in *B. diegensis* WBR.

Understanding the genetics and epigenetics of the post-viral fatigue syndromes, Myalgic Encephalomyelitis / Chronic Fatigue Syndrome, and Long COVID

Warren P. Tate[1], Katie Peppercorn[2], Bryn W.C. Griffiths[2], Sayan Sharma[1], Euan Rodger[1], Aniruddha Chatterjee[1]

[1]Department of Pathology, Dunedin School of Medicine, [2]Department of Biochemistry, School of Biomedical Sciences, University of Otago, Dunedin, New Zealand

Establishing specific molecular diagnostic tests for those at risk, or those suspected of having the complex post viral syndromes, Myalgic Encephalomyelitis / Chronic Fatigue Syndrome (ME/CFS), and Long COVID (LC) is hugely challenging. Each affected patient has a subselection of >200 reported symptoms. The syndromes arise in susceptible people (~5% of the population) after a viral/stressor assault. No gene linkages had been found until recently when Precision Life (Oxford) added a combinatorial platform to GWAS so multiple SNPs could be evaluated together. SNP clusters (15) linked to 14 genes accounted for 91% of the ME/CFS samples in the UK biobank. We are investigating whether SNP molecular signatures can be developed for affected families to indicate *those at risk*. Major differences in the DNA methylation patterns with both LC and ME/CFS patients compared with healthy controls has indicated that epigenetics has great promise for developing a molecular signature for *early diagnosis*. A longitudinal relapse /recovery study in ME/CFS patients revealed methylation on CpG sites changed during relapse but was restored to pre-relapse state on recovery. Collectively, our DNA methylome, transcriptome and proteome studies account for dysfunctional pathways in immune/inflammatory modulation, mitochondrial/energy production, lowered metabolism, and circadian clock function.

Loss of aminoarabinose lipid modification leads to cephalosporin antibiotic resistance in *Pseudomonas aeruginosa*

Hardie Boys, M.T.,[1], Taylor-Wardell, S.,[1] Pletzer, D.[1]

[1]Department of Microbiology and Immunology, University of Otago, Dunedin, NZ.

Pseudomonas aeruginosa is a multi-drug resistant pathogen, frequently causing respiratory tract infections in cystic fibrosis patients, urinary tract and burn wound infections in immunocompetent patients. The hypervirulent Liverpool Epidemic Strain (LESB58), utilises resistance mechanisms including an impermeable outer membrane and β-lactamase enzymes which hydrolyse cephalosporin antibiotics. The *arnBCADTEF-ugd* operon (*arn*; 9,380-bp) in *P. aeruginosa* encodes a lipid modification system that synthesizes and incorporates an aminoarabinose sugar into the lipopolysaccharide of the outer membrane. We hypothesized that deletion of *arn* could result in resistance to antibiotics targeting the bacterial cell wall. Antimicrobial susceptibility assays demonstrated that deletion of *arn* (Δ*arn*) increased resistance to the cephalosporin antibiotics ceftazidime (32-fold), cefepime (8-fold) and cefotaxime (8-fold). RNA-sequencing Δ*arn* and wild-type strains following exposure to ceftazidime revealed significant upregulation of the β-lactamase enzyme *ampC* within Δ*arn*. Generating a double mutant (Δ*arn*/Δ*ampC*) reduced resistance to ceftazidime (8-fold), indicating AmpC to be the main cause of cephalosporin resistance observed in Δ*arn*. Interestingly, the double mutant Δ*arn*/Δ*ampC* did

not rescue resistance completely to WT (2-fold difference), and bacterial enumeration demonstrated antibiotic tolerance, indicating additional undiscovered resistance mechanisms. Our findings provide novel evidence of a previously undiscovered link between the lipid modification system and cephalosporin resistance in *P. aeruginosa*.

Thermal resilience in the endemic triplefins of New Zealand

Riordan, Breana[1]; Ludo Dutoit[1]; King, Tania[1], Beheregaray, Luciano[2], Gemmell, Neil[1], Hickey, Tony[3]; Johnson, Sheri[1]
[1]Department of Zoology, University of Otago
[2]Molecular Ecology Lab, Flinders University
[3]School of Biological Sciences, University of Auckland

The capacity to adjust to shifting thermal conditions, such as those in the context of climate-mediated ocean warming, is crucial for the persistence of marine ectothermic species. Studies are increasingly showing that fish populations displaying high innate thermal plasticity can undergo molecular, metabolic, and structural remodelling at all biological levels of organisation in an integrated response against heat stress. We investigated the thermal resilience and capacity for thermal acclimation of two New Zealand | Aotearoa endemic intertidal fish species, the common triplefin (*Forsterygion lapillum*) and the estuarine triplefin (*Forsterygion nigripenne*), highlighting the roles mitochondria and differential gene expression play in thermal resilience. The fish were exposed to four temperatures (10, 14, 18 and 22°C) for 4-weeks before using a novel fluorescent approach to assess the performance of membrane potential and ATP production in brain mitochondria under thermal ramping scenarios. After 8 weeks of acclimation, RNA-Seq analyses were performed on brain and heart tissue from fish subject to the control (10°C) and 22°C temperatures. Both the common and estuarine triplefin from warm acclimated treatments showed enhanced maintenance of mitochondrial function at higher temperatures compared to fish from cooler treatments, though only ATP production was significantly enhanced in both species. Differential gene expression and gene ontology highlighted an induction of stress response pathways, heat shock protein genes and oxidoreductase activity in warm acclimated tissues, alongside a rearrangement of metabolic functions facilitating increased carbohydrate metabolism. In conclusion, both species display plasticity in mitochondrial performance, enhancing upper thermal tolerance. Furthermore, transcriptional alterations indicate that both species undergo thermal compensation and homeostatic adjustments under warming conditions. Overall, these results support strong resilience for these triplefin species against future warming. Future work will compare these intertidal species to closely related subtidal species, which are predicted to show greater sensitivity to climate change.

Missing heritability in heart failure: don't sweat the small stuff?

Moir-Meyer, G.L. [1,2], Pearson, J.F.[3] , Walker, L.C.[2], Earle, N.J.[4], Poppe, K.K.[4], Rolleston, A.K.[5], Doughty, R.N.[4], Troughton, R.[1], Richards, A.M.[1], Wiggins, G.A.R. [2], Pilbrow, A.P.[1*]*

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Heart failure (HF) is a leading cause of hospitalisation in adults over 65 years in Aotearoa, and one in four patients die within one year of diagnosis [1]. HF disproportionately affects Māori, who are diagnosed 17 years earlier than New Zealand Europeans on average and have worse outcomes [2]. It is estimated that over 25% of susceptibility to HF is inherited [3]. However, studies to date demonstrate

that the disease's genetic risk is poorly understood, leaving the observed heritability of HF largely unexplained. Considerable efforts have been made to explore the role of single nucleotide polymorphisms (SNPs) in HF, but only a small proportion of HF cases are attributable to monogenic cardiomyopathies and genome wide association studies (GWAS) have yielded few insights [3]. Compared with SNPs, which make up ~0.05% of the human genome by coverage, copy number variants (CNVs) cover 100 times (5-10%) more of the genome [4]. These DNA deletions and duplications are associated with numerous genetic syndromes including congenital heart disease [5], cancer [6], and developmental and neuropsychiatric disorders [7]. We hypothesise that CNVs may disrupt genes or regulatory elements that are associated with development and progression of HF and explain some of the "missing heritability". To explore whether CNVs are associated with HF, we genotyped participants in the Coronary Disease Cohort Study [8] on the Affymetrix Precision Medicine Diversity Array across 850,000 markers. A total of 37,591 CNVs were detected across 1,848 participants including 408 that had reported a diagnosis or hospital admission for HF. Of these, 2,714 deletions encompassed genes in participants with HF, while 9,036 deletions overlapped genes in participants without HF. Since deletions are more likely to be associated with pathogenicity, we focused on 151 genes that were overlapped by deletions in individuals with HF, but were copy number normal in all other participants. Five of these genes had been previously implicated in HF, including *ITGA10*, which is associated with dilated cardiomyopathy [9]. The remaining HF-specific genes will be prioritised based on their likely role in cardiac disease development before undergoing validation (to confirm CNV calling) and functional interrogation. By identifying known HF-related genes in this preliminary analysis, we have demonstrated the feasibility of our approach. Future work will involve a case-control analysis in this cohort in combination with the Multi Ethnic New Zealand Study of Acute Coronary Syndromes cohort [10], and Canterbury Healthy Volunteers Cohort [11] (including 545 Māori) to identify novel HF risk loci. Our research will contribute to improved genetic screening for HF and inform patient management strategies. Moreover, it will provide new insights on the mechanisms underlying susceptibility to HF, and progress genetic representation by expanding the number of CNVs known to occur in Māori.

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Defining therapeutic vulnerabilities in drug-resistant strains of *Mycobacterium tuberculosis*

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Drug-resistant (DR) Mycobacterium tuberculosis strains are a severe threat to public health globally. The emergence of drug resistance in pathogens is often linked to mutations in core metabolic pathways. However, such mutations are often at the cost of fitness, resulting in reduced growth, survival and virulence. We hypothesised that specific pathways become more essential for the growth and survival of DR strains to compensate for fitness costs associated with drug resistance. By targeting these pathways, new therapeutic avenues may emerge. This research aims to comprehensively define collateral vulnerabilities in DR Mycobacterium tuberculosis strains on a genome-wide scale and identify potential gene targets for the development of innovative anti-tuberculosis (TB) treatments. We used Whole Genome CRISPR interference (WG-CRISPRi) to assess gene vulnerability across the entire genome of different DR strains. This work identified both vulnerabiliites that are unique to different resistant strains, as well as some that are shared across multiple strains. This work provides fundamental insights into the mechanisms that allow DR Mycobacterium tuberculosis to adapt to the costs of mutations and how these adaptations generate druggable vulnerabilities.

Evolving vertebral counts without evolving the segmentation clock.

Shannon Taylor and Berta Verd

A major question in evolutionary developmental biology is how organismal diversity is generated. Lake Malawi cichlid fishes are a fascinating system with which to study this problem, varying in phenotype but having extremely limited genetic diversity. We have focused on the evolution of somite number in these fishes, as segmentation/somitogenesis process is very well understood in other species. We studied the cichlids fish, *Astatotilapia calliptera* and *Ramphochromis chilingali*, which form 30 and 38 somites respectively. The rate of segment production is the same in these two species, so we focused on differences in axial morphogenesis during somitogenesis. We found differences in embryonic morphology at the onset of somitogenesis but similarities in the dynamics of axial elongation between these species. At the onset of somitogenesis, *R. chillingali* embryos are longer than their *A. calliptera* counterparts, and the pre-somitic mesoderm (which will give rise to the somites) is larger and has more cells in *R.chillingali* than in *A. calliptera*. However, the Tbox genes, which are required for axial elongation in other vertebrates, are expressed identically between the two species. Altogether, our work suggests that morphogenetic differences at the onset of somitogenesis might important in evolving vertebral count, while the process of somitogenesis can remain unchanged.

Abstracts Poster Presentations:

Understanding drivers of metastasis to develop better treatments for oestrogen receptor positive breast cancer

Sophie Tunnicliffe, Anita Dunbier

Oestrogen receptor-positive breast cancer (ER+ BC) is a highly prevalent disease, that is treatable with endocrine therapies. However, ER + BC can become resistant to treatment, developing into metastatic breast cancer (mBC), of which there is a lack of immunocompetent metastatic models. Previous work has developed a syngeneic 129Sv/Ev mouse model using SSM3 cells, which displayed low metastatic capacity, consistent with their derivation from a primary breast cancer**.** Therefore, further research is required to develop a cell line with an increased metastatic rate, representative of mBC. *PTEN* is frequently mutated in breast cancer, and causes an increase in tumour cell proliferation and metastasis. It is hypothesised that knocking out *PTEN* from SSM3 cells will increase their metastatic potential. CRISPR-Cas9 was used to edit and knockout *PTEN* from SSM3 cells. Successful edits were selected for using fluorescent activated cell sorting. Cell line edits were assessed using RT-qPCR with preliminary results suggesting successful PTEN knockout in several clones. Subsequently, the metastatic ability of edited cell lines will be assessed using a wound healing assay and a cellular proliferation assay. A successful SSM3 cell line with an increased metastatic ability will contribute to developing a relevant mBC model in which to test treatments.

Liquid biopsy based DNA methylation biomarker panel for facilitating minimally invasive prostate cancer detection

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Prostate cancer (PCa) is the most common cancer in men, with 4,000 new cases and 700 deaths annually in New Zealand. Transrectal ultrasound-guided prostate biopsy, the standard diagnostic procedure for PCa detection, is invasive and poses infection risks. Liquid biopsy has the potential for minimally invasive early cancer detection but requires standardization and more analytical and clinical trial evaluation. DNA methylation is a stable and heritable genome modification whose alterations have been indicated in early stages of tumorigenesis. This project aims to establish a DNA methylation biomarker panel using cell-free DNA derived from patient plasma samples. After profiling the epigenetic landscape of cfDNA, the prominent differentially methylated regions(DMRs) will be identified by comparing the epigenetic signatures between cancer and non-cancer patients. They will be correlated with methylation patterns and gene expression levels obtained from matched prostate biopsy tissues. This integrated approach endeavours to identify robust epigenomic biomarkers unique to PCa. We have generated 9 cfRRBS libraries and are preparing additional ones. Preliminary data obtained offers substantial insights into the differential methylation patterns between the non-malignant and malignant groups. We anticipate liquid biopsy will enable non-invasive PCa screening, enhancing early detection and outcomes.

Development of a Prognostic Tool for Identifying High-Risk Prostate Cancer Patients

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Prostate cancer (PCa) is the most common cancer among men in New Zealand, causing over 700 deaths annually. While most cases are indolent, some progress to aggressive, incurable metastatic disease. Distinguishing between these forms is challenging. Mutations in the *TP53* tumour suppressor gene (~15-30%) or elevated levels of the truncated p53 isoform Δ133p53 (~30%) are linked to poor PCa patient outcomes. This study investigates whether *TP53* mutations or elevated Δ133p53 levels can better identify aggressive GS = 7 PCa. Using a cohort of 200 formalin-fixed, paraffin-embedded (FFPE) samples, we have extracted DNA and RNA from 200 samples and performed staining slides with H&E (to assess tumour cellularity), CD3 (T-cell infiltration), and CD163 (macrophage infiltration). Targeted sequencing of *TP53* has been completed for 96 samples, with mutations being identified using QIAGEN CLC Genomics Workbench. Additionally, we have optimized RT-qPCR assays to measure p53 isoforms, including FLTP53, Δ40TP53, Δ133TP53, TP53α, TP53β, and TP53γ. Probe assays are being optimized to improve specificity for FFPE samples. Once data is fully generated, we will evaluate the association of *TP53* mutations and *Δ133TP53* expression with clinical outcomes such as time to relapse, GS, and PSA levels.

Exploring the link between Infertility and Ovarian Cancer

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An estimated 1 in 6 individuals experience infertility over the course of their lifetime and the cause is unexplained in 30% of couples. Whilst 50% of infertility cases are estimated to have a genetic cause, far fewer individuals with non-syndromic infertility find a genetic answer. Women with a history of infertility also have increased risk of developing ovarian cancer. Ovarian cancer is the deadliest female reproductive cancer, with only 1 in 3 women surviving 5 years post-diagnosis. This intriguing link suggests that there may be shared biological factors at play. One such factor is LHX9, a transcription factor essential for ovarian development and expressed in stem cell populations in the adult ovarian surface epithelium (OSE) – the tissue from which many ovarian cancers develop. Cellular turnover in the OSE for wound healing post-ovulation is thought to facilitate accumulation of mutations and development of ovarian cancer. *Lhx9* null mice have complete gonadal agenesis and are sterile, whilst *Lhx9* heterozygous females display altered ovary morphology and subfertility as they age. We are exploring if subtle perturbations to mammalian gonadal development cause adult infertility and ovarian cancer. The UK Biobank cohort was used to identify human germline *LHX9* variants associated with infertility and ovarian cancer and *in silico* predictive tools to prioritise candidate variants that might contribute to altered fertility status or risk of reproductive cancer development. *In vitro* studies will be used to investigate the direct functional consequences on protein stability and DNA binding. Together, this project aims to combine population data with molecular studies using a mouse model, to better understand the role of LHX9 in infertility and ovarian cancer.

Role of the *TP53* Splice Mutations in cancer progression

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The *TP53* gene, a key tumor suppressor, frequently undergoes mutations, including splice mutations that alter RNA splicing by causing exon skipping or intron retention. These mutations are particularly important in *TP53*, which produces at least nine mRNA transcripts and 12 functional protein isoforms. Despite this significance, little is known about their impact on cancer biology. We conducted an in-silico analysis of 23,017 mutations from (18,562) somatic samples (via cBioPortal) and (4,455) germline samples (via the IARC database) to study the molecular and clinical features of *TP53* splice mutations. About 7% of all mutations occurred at splice sites, distributed across various donor and acceptor sites, with X125, X126 and X187 being the most prevalent. Tumors with *TP53* splice mutations exhibited higher mutation counts and greater genomic alterations compared to other mutation types. Analysis of individual splice sites revealed distinct molecular and clinical differences. For example, donor site X125 and X331 was associated with higher mutation counts and higher genome alteration fractions when compared to acceptor site X126 and X332. This analysis highlights the distinct molecular and clinical effects of individual *TP53* splice mutations, emphasizing the need for further studies to understand their impact on p53 isoform function and cancer biology.

Predicting Functional Genomic Features

Daniela Schiavinato, Karla E. Rojas Lopez, Helena B. Cooper, Michael A. Black, Paul P. Gardner

Advancements in sequencing technologies are enabling the identification of numerous potential proteins, non-coding RNAs, and genomic activities. However, there are conflicting conclusions about which regions of the genome are functional, as activity alone can be noisy and misleading. Stronger evidence for function comes from demonstrating evolutionary selection. However, quantifying conservation is challenging for non-coding elements like lncRNA, where interactions or structures, rather than sequence conservation, can be key. Thus, approaches integrating multiple functional features may provide a more accurate discrimination between genes and background sequences. We investigate the association between gene functionality and genomic features by comparing functional protein-coding and non-coding genes to regions of the genome expected to be largely non-functional. We evaluate the relative importance of five groups of genomic features, selected based on their potential to predict gene functionality: intrinsic sequence features, sequence conservation, transcription, genomic repeat association, protein-coding or RNA-specific features, and epigenetic signatures. We rank these features using Random Forest (RF) classification models and find that inter-species evolutionary conservation and transcription are the strongest and most consistent association between functional genes and genomic features. We also observe that, compared to short ncRNA and protein-coding sequences, lncRNAs show less defined functional signals, evidenced by relatively poor-performing classification models. To explore this further, we apply our RF model to assess current lncRNA annotations, revealing that around 60% are indistinguishable from background sequences. Our findings highlight the importance of evolutionary conservation and transcription in determining sequence functionality, underscoring the need to consider these features when distinguishing functional sequences from noise. The less distinct functionality signals observed in lncRNA and the large proportion of annotations that cannot be distinguished from background sequences suggest that current thresholds might not adequately account for experimental and biological noise. We aim to present a ranked list of high-confidence lncRNAs that are most distinguishable from background sequences.

Exploring the disease mechanism caused by variants in histone H4

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Genetic alterations in many of the 14 histone H4 genes cause Tessadori-Bicknell-vanHaaften syndrome, a rare neurodevelopmental disorder where individuals show intellectual disability, developmental delay

and reduced brain size. There is also a wide spectrum of non-neurological features including vision abnormalities and facial dysmorphism. All alterations identified are missense variants, clustered into two main protein regions, but not in the post-translational modification heavy N-terminal tail. The identified substitutions cluster in regions of protein-protein interactions, either with histone H3, which forms a dimer with H4 in the nucleosome, or with H3-H4 protein chaperones, which support the dynamic loading and unloading of histones during transcription and DNA replication. While there is strong genetic evidence to support this new disorder and zebrafish model data supported an impact on organism development, there has been little in vitro study of the variants to understand the direct impact of the missense variants. This project sought to investigate the impact of the substitutions on protein interactions, using co-immunoprecipitation techniques, with aims to first develop a robust protocol for detecting H4 in western blotting, and then to test the impact of selected variants on interaction with H3 and MCM2, a chaperone required during DNA replication.

Exploring Mendelian-informed genetic variants for their potential to alter cancer risk using the UK Biobank.

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Height is a well-established risk factor for cancer development: each 10cm increase in height correlates to a ~10% increase in cancer risk. There does not appear to be a simple explanation for this association, reflecting the genetic complexities of both height and cancer. Mendelian (single gene) syndromes have tremendous power to potentially better understand such complex associations. The relationships between Mendelian overgrowth syndromes and cancer have been studied extensively, with the majority conferring increased cancer risk. Conversely, we know far less about growth-restricting syndromes and cancer risk, however, one pituitary dwarfism, Laron syndrome, was recently identified as having a protective effect against cancer development. This observation prompted our hypothesis that other growth-restricting syndromes are also protective against cancer. Our research utilised the expansive phenotypic and genetic data in the UK Biobank to explore potential associations between variants in genes associated with growth-restricting syndromes, and cancer risk. First, we optimised *in silico* predictive algorithms to identify likely deleterious variants in short stature and microcephaly genes. The use of variant pathogenicity scoring algorithms informed the construction of UK Biobank cohorts consisting of participants with short stature and microcephaly-associated variants. These cohorts were investigated for associations with neoplasm-related data fields. Analysis of neoplasm incidence and type utilised logistic regression, while linear regression was used to investigate age at diagnosis. Through this research, we aim to understand more broadly the protective role variants in these disease-linked genes might play, enabling more precise risk prediction in an era of personalised medicine.

Pathogenicity and Interactions of ORC3

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Origin Recognition Complex subunit 3 (*ORC3*) is a fundamental component of the Pre-Replication complex (Pre-RC) and plays a crucial role in the loading of proteins to chromatin replication origins at the initiation of DNA replication. Variants in Pre-RC proteins are of interest, as previously identified variants have been associated with Rare Mendelian Genetic disorders, notably Meier-Gorlin syndrome. The associated genes encode proteins critical for cell cycle progression, coordination, DNA replication and repair. Until recently, no variants had been identified in *ORC3*. We have identified a patient cohort of individuals with either monoallelic or biallelic variants in *ORC3*, showing a wide range of clinical features, including Meier-Gorlin syndrome. Functional experiments have been conducted to explore previously unknown aspects of ORC3 like protein interactions, localisation, binding, and structure and

how these may differ in variants. Specifically, variant Val216Asp has shown a significant reduction in the proteins ability to interact with and bind chromatin-associated proteins, hindering its optimal function and potentially decreasing the efficiency of DNA replication mechanisms and downstream processes.This project seeks to identify the mechanisms and interactions of *ORC3* and to understand the phenotypic complexity observed in affected families, providing insights into the potential pathogenicity of the variants.

A Genetic Characterisation of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome Predisposition.

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Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is a debilitating disease characterised by persistent exhausting fatigue, muscle/joint pain, post-exertional malaise, and mental fatigue. ME/CFS is a post viral/stressor fatigue syndrome for which affected individuals have a genetic predisposition. Novel computational analysis from Oxford's Precision Life showed that 91% of ME/CFS cases in the UK Biobank were explained by one of 84 unique combinations of 3 to 5 SNPs from a pool of 199 SNPs. We are investigating whether a subset of the *critical SNPs* can be used to develop a test for those at risk who have ME/CFS genetic predisposition. A preliminary study using PCR amplification and sequencing of gDNA from peripheral blood mononuclear cells (PBMC) has shown NZ ME/CFS patients have most abundant SNPs identified in the Precision Life study. Now we are analysing the presence of a wider range of these critical SNPs in the gDNA from five ME/CFS patients and four controls. This study aims to test whether particular combinations of SNPs could be used to assess risk in affected ME/CFS family members, and how these genetic variations relate biologically to confer susceptibility for the significant population of individuals who suffer with ME/CFS.

Targeting oncogenic lncRNAs in colorectal cancer-associated macrophages.

Brooke Morrison, Dr Sarah Diermeier

Poor colorectal cancer (CRC) patient outcomes evidence the need for innovative and curative treatments. CRC resistance to current therapeutics is largely attributed to the tumour microenvironment (TME) – which buffers the tumour from the normal colon. Here, cell activity is influenced to promote tumorigenesis, with tumour-associated macrophages (TAMs) driving immunosuppression in CRC. TAM activity is altered through long non-coding RNAs (lncRNAs), which are regulatory molecules implicated in a range of diseases, including CRC. Upregulated lncRNAs are oncogenic across multiple cancer types, where knockdowns (KD) can generate anti-cancer effects. We hypothesise that KD of oncogenic lncRNAs in CRC TAMs with antisense oligonucleotides (ASOs) may induce an anti-cancer effect. 12 upregulated lncRNAs in TAMs were identified from a CRC single-cell RNAseq dataset. Our quantitative scoring matrix assessed each lncRNA's potential for KD to produce an anti-cancer effect by evaluating expression fold-changes, survival outcomes, normal expression, and literature evidence. Two lncRNAs '*Helios* and '*Echo'* scored highest, thus ASOs were designed to target them. In a model CRC cell line for KD, HCT116, stable *Echo* and positive control expression formed the baseline for ASO transfection. Future experiments will elucidate the transcriptomic and phenotypic effects of target knockdown, determining their potential as a novel therapeutic.

Molecular characterisation of novel *de novo* variants in *ELAVL2*

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Disruption during neurodevelopment can result in neurodevelopmental disorders (NDDs), which are characterised by a variety of clinical features, including intellectual disability and developmental delay. *ELAV-like 2* (*ELAVL2*) encodes an RNA-binding protein that is known for its crucial roles during brain development. To date, ELAVL2 has not been associated with NDDs. We have collated an international cohort of 14 patients with *de novo* variants in *ELAVL2,* all of whom present with overlapping neurodevelopmental phenotypes. Six truncating variants and a large-scale gene disruption have been identified, confirming that haploinsufficiency for *ELAVL2* is the likely disease mechanism. However, seven missense variants and a terminal exon frameshift required further molecular investigation. It was hypothesised that these variants also act in a haploinsufficient manner, by disrupting the levels or function of ELAVL2. A cycloheximide chase assay confirmed that several variants reduced the stability of ELAVL2, supporting our haploinsufficiency hypothesis and that these variants are pathogenic. Variants that did not decrease protein stability were studied for their impact on ELAVL2 homodimerisation, an interaction that is not well documented. Through co-immunoprecipitation, it was confirmed that mammalian ELAVL2 can form a homodimer and that this interaction is upheld in the presence of select patient variants. These results support that variants in *ELAVL2* cause a novel NDD, connecting this well-established neuronal protein with the neurodevelopmental phenotypes seen in patients. Future experiments to understand the specific targets and processes that are likely being disrupted would be advantageous to provide insight into ELAVL2 pathophysiology.

Developing a blood-based epigenomic signature for ME/CFS and Long COVID using Peripheral Blood Mononuclear Cells (PBMCs)

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Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) and Long COVID (LC) are debilitating, chronic conditions with complex pathophysiology, affecting millions globally and with thousands of cases in New Zealand. Due to the absence of a molecular diagnostic marker, early diagnosis remains challenging, leading to delayed treatment, highlighting its urgent need. Epigenetic modifications, particularly DNA methylation, play a critical role in the pathogenesis and progression of human diseases, and their changes have shown the potential to become routine diagnostic tools in the clinic. Preliminary studies using reduced representation bisulphite sequencing (RRBS) in 10 ME/CFS patients showed large methylation changes compared to 10 healthy controls, with these changes being associated with pathways like immune functions and mitochondrial energy production. Our recent study using 15 RRBS methylomes identified 118 common differentially methylated regions (DMRs) between Long COVID vs Healthy and ME vs Healthy ($P<0.05$, >10% methylation difference) from PBMCs, showing the similarities between the two conditions and associated with functional categories like Immune, Transcriptional and Cytoskeleton. Furthermore, we are aiming to perform a detailed mapping of whole genome methylomes from the cell-free DNA and analyse existing PBMC data to derive a robust molecular signature for LC and ME/CFS patients with high sensitivity and specificity.

Prioritisation of Māori and Pacific Gout-Associated Non-Coding Genetic Variants Using Enhancer Assays

Selina Williams, Patrick Gibbons, Milly Morice, Megan Leask.

Gout is the most prevalent form of inflammatory arthritis. It is characterised by high levels of serum urate that leads to deposition of monosodium urate crystals (MSUCs) in the joints. The presence of MSUCs causes an innate inflammatory immune response, known as a gout flare.
Genetics is known to be involved in gout, but how genetics could uncover the molecular mechanisms that lead to the inflammatory response to MSUCs has not yet been discovered. Māori and Pacific populations have a high prevalence of gout, however when looking at Polynesian GWAS it is most commonly non-coding genetic variants that are associated with gout, as opposed to traditionally studied missense variants. This study aimed to research 3 of these non-coding regions to understand their activity through zebrafish enhancer and luciferase assays. One region selected was in the regulatory region of ABCG2 (*rs45499402, rs149027545* & *rs138409370*), an ATP binding cassette involved in urate transport. The other two variants chosen: CREBRF (a metabolic regulatory region) *rs12513649* & *rs150207780* are in linkage disequilibrium with eachother and another known missense variant *rs373863828* that is protective against diabetes, adiposity and high myostatin levels.
Our results suggested that all 3 regions that were assayed showed enhancer activity, as well as the ABCG2 region and CREBRF rs12513649 indicating allelic differences. Ultimately, this research has suggested a new direction towards the non-coding genome for further study into the innate immune response of gout.