genetics otago

Genetics Otago presents

18th November 2020

Genetics Otago Zoom Symposium

Wednesday, 18th November 2020

9:30am – 9:35am	Mihi Whakatau	
9:35am – 9:45am	Welcome	*Dr Louise Bicknell & A/Prof Logan Walker* *Acting GO Directors*
Session 1		**Chair: Caitlin Harris**
9:45am – 10:15am	50 Years of Population Genetics: a Personal Reflection	*Professor Graham Wallis,* *Dept of Zoology, University of Otago, Dunedin*
10:15am – 10:30am	Sexual conflict in a shared genome	*Dr Ludovic Dutoit,* *Dept of Zoology, University of Otago, Dunedin*
10:30am – 10:45am	Evolution of the world's only alpine parrot – genomic adaptation or phenotypic plasticity, behaviour and ecology	*Dr Michael Knapp,* *Dept of Anatomy, University of Otago, Dunedin*
10:45am – 11:00am	From Genotype to Phenotype: Understanding idiopathic disease	*Dr Megan Wilson,* *Dept of Anatomy, University of Otago, Dunedin*
11:00am –11:30am	**Tea/coffee break**	
Session 2		**Chair: Connor McGuinness**
11:30am – 11:45am	Potential under-ascertainment of New Zealand women at high-risk of breast cancer in clinical care	*Dr Vanessa Lattimore,* *Dept of Pathology & Biomedical Science, University of Otago, Christchurch*
11:45am – 12:00pm	Translating the non-coding variome of the Pacific	*Dr Megan Leask,* *Dept of Biochemistry, University of Otago, Dunedin*
12:00pm – 12:15pm	Splicing defects in RARS2 deficiency	*Dr Guillem de Valles Ibáñez* *Dept of Paediatrics & Child Health, University of Otago, Wellington*
12:15pm – 12:30pm	Familial immune thrombocytopenia (ITP) and hereditary persistence of fetal haemoglobin (HPFH) associated with a pathogenic variant of MYB	*Prof Ian Morison* *Dept of Pathology, University of Otago, Dunedin*

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Bus Stop Talks (Students)		
12:30pm – 1:00pm	Investigating DNA methylation as a mechanism for paradoxical gene activation using epigenomic editing technology	*Rakesh Banerjee* *Dept of Pathology, University of Otago, Dunedin*
	Whole body regeneration and wound healing comparison in *Botrylloides leachii*	*Rebecca Clarke* *Dept of Anatomy, University of Otago, Dunedin*
	Ancient DNA reveals strong phylogeographic structure within the extinct New Zealand bush wren *(Xenicus longipes subsp.)*	*Alex Verry* *Dept of Zoology, University of Otago, Dunedin*
	Investigating the role of DNA methylation in paradoxical gene expression using a CRISPR-based system	*Reema Waly* *Dept of Pathology, University of Otago, Dunedin*
	Activity of *Bacillus sp.* as a biocontrol agent in controlling green mould disease in oyster mushrooms *(Pleurotus sp.)*	*Yung Xin Tan* *Dept of Biochemistry, University of Otago, Dunedin*
1:00pm – 2:00pm Lunch		
Session 3		**Chair: Sai Shyam**
2:00pm – 2:30pm	Whole-genome landscape of melanoma subtypes	*Dr Felicity Newell* *QIMR Berghofer Medical Research Institute, Brisbane*
2:30pm- 2:45pm	Moving to molecular markers in endometrial cancer	*Dr Claire Henry* *Dept of Obstetrics, Gynaecology & Women's Health, University of Otago, Wellington*
2:45pm – 3:00pm	Understanding Antimicrobial Tolerance in Bacterial Pathogens	*Dr Rachel Darnell,* *Dept of Microbiology & Immunology, University of Otago, Dunedin*
3:00pm – 3:15pm	The genetics of eating disorders: what do we know and where are we going?	*Prof Martin Kennedy,* *Dept of Pathology & Biomedical Science, University of Otago, Christchurch*
3:15pm – 3:30pm	Investigating microbial communities: assembly and the impact of environmental stressors	*Dr Tina Summerfield,* *Dept of Botany, University of Otago, Dunedin*
3:30pm – 4:00pm Tea/Coffee		

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Session 4		Chair: Alex Verry
4:00pm – 4:15pm	Features of Functional Human Genes	*A/Prof Paul Gardner,* *Dept of Biochemistry, University of Otago, Dunedin*
4:15pm – 4:30pm	Cancer phylogenetics using single-cell RNA-seq data	*Dr Jiří Moravec,* *Dept of Computer Science, University of Otago, Dunedin*
Bus Stop Talks (Students)		
4:30pm – 5:00pm	"The more the merrier"? Making Better Use of GBS Data	*Jie Kang* *Dept of Mathematics and Statistics, University of Otago, Dunedin*
	Circulating tumour cells as a model to identify tumour-specific epigenetic signatures of colorectal cancer metastasis	*Sai Shayam Vasantharajan* *Dept of Pathology, University of Otago, Dunedin*
	Restriction enzyme based next generation sequencing effectively recapitulates tumour mutation burden and cancer mutation signatures	*Connor McGuinness* *Dept of Biochemistry, University of Otago, Dunedin*
	Step Change for Ryegrass Breeding	*Caitlin Harris* *Dept of Biochemistry, University of Otago, Dunedin*
	Understanding pollen abortion in female kiwifruit	*Liam Le Lievre* *Dept of Biochemistry, University of Otago, Dunedin*
5:00pm – 5:30pm	**Awards and Closing**	

Abstracts

Keynote Speakers:

50 Years of Population Genetics: a Personal Reflection

Professor Graham Wallis, Department of Zoology, University of Otago, Dunedin

The field of population genetics is somewhat unusual in that its theory was well-developed nearly 50 years before good data became available to test it. A central question concerns the underlying genetic basis for fitness differences among organisms. When protein electrophoretic data became widely available in the 1970s, and DNA sequence data some time later, although we had more idea about how much variation was there, the central question remained unresolved. Many population geneticists (myself included) were seduced by the patterns of population and species-level variation revealed by increasingly powerful techniques, and the phylogenetic and phylogeographic questions that could be addressed. For these questions, neutrality was a more convenient null hypothesis, and analysis of natural selection at a molecular level became rather neglected. Now with the reality of deep sequencing of entire genomes of multiple individuals, we can return to the central problem with more hope of successful resolution, but there are still many hurdles to overcome. I give some examples of my research in this framework over 40+ years as a geneticist.

Whole-genome landscape of melanoma subtypes

Felicity Newell
QIMR Berghofer Medical Research Institute, Brisbane

Melanoma is a malignant tumour of pigment-producing melanocytes and the most common form in European-derived populations is cutaneous melanoma, which is associated with the mutagenic effects of ultraviolet radiation in sunlight. Rarer subtypes of melanoma can also occur in the eyes (uveal), internal mucosal membranes such as the mouth (mucosal) and the non-hair bearing palms, soles and nail beds (acral). In this presentation, I will discuss the use of whole genome sequencing to better understand the genomic landscape of melanoma tumours, focussing on analyses in rarer subtypes. Uveal melanomas have few genomic alterations, whereas cutaneous melanomas have the highest point mutation burden. In contrast with uveal and cutaneous subtypes, both acral and mucosal melanoma subtypes have a high number of copy number alterations and structural rearrangements. Although a some of the same genes are altered between subtypes, each subtype exhibits its own pattern of gene aberrations, highlighting different potential therapeutic options.

Invited Speakers:

Understanding Antimicrobial Tolerance in Bacterial Pathogens

Rachel L Darnell, Melanie K Knottenbelt, Francesca O Todd Rose, Gregory M Cook
Department of Microbiology and Immunology University of Otago, Dunedin

Antimicrobial resistance is a rapidly evolving global health emergency that threatens the many advancements of modern medicine. Antimicrobial tolerance is the ability of a bacterium to survive high antimicrobial exposure and is an essential precursor to the development of antimicrobial resistance. Despite this, the molecular mechanisms underpinning tolerance remain largely unknown. Teixobactin is a new antimicrobial with no reported resistance and is critically effective against multidrug-resistant bacterial pathogens. I have demonstrated that the multidrug-resistant bacterial pathogen *Enterococcus faecalis* exhibits extraordinary tolerance to teixobactin compared to *Staphylococcus aureus*, and identified the two- component regulatory system CroRS as a key regulator of this tolerance. Our aim is to now improve our understanding of the molecular mechanisms that underpin tolerance and to identify chemical inhibitors targeting tolerance to prevent resistance development.

Sexual conflict in a shared genome

Ludovic Dutoit
Department of Zoology, University of Otago, Dunedin

Males and females pursue different strategies for reproduction ultimately resulting in large phenotypic differences between sexes. Yet, males and females are almost identical at the genetic level, with the exception of sex chromosomes when they are present. I will explore genetic differences appearing between males and females on autosomes, ask whether we can identify single genes under sexual conflict and whether sex-specific selection has the potential to maintain genetic diversity.

Moving to molecular markers in endometrial cancer.

Henry C, Dore M, Danielson K, Filoche S
Department of Obstetrics, Gynaecology & Women's Health, Division of Health Sciences Wellington.

Endometrial Cancer is the most common gynaecological cancer. Unfortunately, Aotearoa New Zealand has one of the fastest rising rates of endometrial cancer, with incidence increasing particularly in young Pasifika women. As such, there is a need to update how we diagnose and treat this disease.

Firstly, the Levonorgestrel Intrauterine System (LNG-IUS), also known as Mirena®, is gaining traction as an alternative treatment for hyperplasia (precursor lesions) and early stage endometrial cancer for women who are either unable to undergo surgery, or wish to retain fertility. However, around half of women will not respond to this treatment, and the mechanisms for resistance are not understood. Predictive molecular biomarkers are needed to identify and understand who will benefit from the Mirena, and who will still need surgery as first line of care.

Secondly, the TCGA characterised the molecular subtypes of endometrial cancer in 2013, yet these classifications have not yet made it to routine clinical practice. The four subtypes (*POLE* mutated, MMR deficient, p53 mutated and non-specified) have distinct prognostic outcomes and should be incorporated into risk stratification of cancer patients. These molecular types have not been investigated in NZ women, and further research is needed to identify pathways to introduce this new system into care.

Evolution of the world's only alpine parrot – genomic adaptation or phenotypic plasticity, behaviour and ecology

Denise Martini, Bruce Robertson, Neil Gemmell, Michael Knapp
Department of Anatomy, University of Otago, Dunedin

Climate warming, in particular in island environments, where specialised habitat is reducing and opportunities for species to disperse are limited, may become a serious threat to cold adapted alpine species.

In order to understand how alpine species may respond to a warming world, we need to understand the drivers that have shaped their habitat specialisation as well as the evolutionary adaptations that allow them to utilize alpine habitats. For example, a species that has strong physiological adaptations to cold, high-altitude environments may respond less flexibly than a generalist species whose adaptations are mainly behavioural.

The endemic and threatened New Zealand kea (*Nestor notabilis*) is known as the only alpine parrot in the world. As a species restricted to New Zealand's mountainous South Island, it may be highly susceptible to climate warming. Here we use whole genome data of the kea and its close, forest adapted sister species, the kākā (*N. meridionalis*) to reconstruct the evolutionary history of both species and identify the functional genomic differences that underlie their habitat specialisations.

Our findings do not identify major functional genomic differences between kea and kākā in pathways linked to high-altitude adaptation. Rather, they provide evidence that selective pressures on adaptations commonly found in alpine species are present in both *Nestor* species, suggesting that habitat differences between the two are more likely based on behaviour and phenotypic plasticity. Strongly divergent demographic responses to past climate warming between the species nevertheless highlight potential future threats to kea survival in a warming world.

Potential under-ascertainment of New Zealand women at high-risk of breast cancer in clinical care.

Lattimore, Vanessa[1]; Parsons, Michael[2]; Spurdle, Amanda[2]; Pearson, John[3]; Northcott, Hadley[1]; Lehnert, Klaus[4]; Sullivan, Jan[5]; Lintott, Caroline[5]; Bawden, Suzannah[5]; Morrin, Helen[1,6], Robinson, Bridget[1,7] and Walker, Logan[1].

[1]Mackenzie Cancer Research Group, Department of Pathology and Biomedical Science, University of Otago, Christchurch, NZ; [2]Genetics and Computational Biology Division, QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia; [3]Department of Pathology and Biomedical Science, University of Otago, Christchurch, NZ; [4]Centre for Brain Research and School of Biological Sciences, The University of Auckland, NZ; [5]Genetic Health Service NZ, South Island Hub, Christchurch Hospital, NZ; [6]Cancer Society Tissue Bank, Department of Pathology and Biomedical Science University of Otago, , Christchurch, NZ; [7]Canterbury Regional Cancer and Haematology Service, Canterbury District Health Board, Christchurch Hospital, Christchurch, NZ.

Identification of cancer-causing variants in breast cancer susceptibility genes has well-defined and actionable implications for breast cancer prevention and treatment, critical for reducing disease incidence and mortality. Routine diagnostic screening for pathogenic variants in the breast cancer susceptibility genes, including *BRCA1* and *BRCA2*, is typically performed for individuals from suspected high risk breast (and ovarian) cancer families. However, it is unclear how many *BRCA1* and *BRCA2* pathogenic variant carriers in New Zealand fail to be referred for genetic screening despite existing triage tools and guidelines for breast (and ovarian) cancer patients. Additionally, the uptake of multi- gene panel screening is identifying an increasing number of variants of unknown clinical significance. Deciding who should receive genetic testing and interpreting the test results are two major dilemmas for health care professionals. Our aim was to evaluate the genetic variants identified in a high risk breast cancer cohort and determine the proportion of breast cancer patients with disease-causing variants that may not receive genetic screening.

We have undertaken detailed panel-gene sequencing of the entire *BRCA1* and *BRCA2* genes, and the coding regions and splice sites of *CDH1*, *PALB2*, *PTEN* and *TP53*, of 367 female breast cancer patients recruited by the Cancer Society Tissue Bank between December 2014 and May 2017. The variant classification criteria from the expert panel, ENIGMA, and the ACMG/AMP guidelines were used to evaluate a total of 1,685 variants.

Our study identified that 13 (3.5%) breast cancer patients carried a pathogenic or likely pathogenic variant in *BRCA1*, *BRCA2*, *PALB2*, or *PTEN*, while variants of unknown clinical significance were found in 35 (9.8%) women. The majority of pathogenic variant carriers had grade 3 tumours (10/13) (p- value<0.05); however, no other clinicopathological characteristics were found to be significantly different between (likely) pathogenic variant carriers and non-carriers, nor between variant of unknown significance carriers and non-carriers. Notably, 46% of the identified (likely) pathogenic variant carriers had not been referred for a genetic assessment and consideration of genetic testing. Bioinformatics tools (SPiP, SPiCE and SpliceAI) will be used to prioritise the variants of unknown clinical significance for further assessment using laboratory assays.

Our study shows a potential under-ascertainment of women genetically predisposed with an increased risk of breast cancer being offered genetic screening in New Zealand. These results suggest that further research into the prioritisation of breast cancer patients (and their relatives) for genetic screening is required to help reduce the impact of this disease.

Translating the non-coding variome of the Pacific

Megan Leask
Department of Biochemistry, University of Otago, Dunedin, New Zealand

The large burden of chronic kidney disease (CKD) and gout in Māori and Pacific people suggests that this population has a unique set of predisposing genetic variants. We know from large European GWAS that most variants that influence disease (90%) are non-coding however the non-coding genome in Māori and Pacific is completely uncharted. And because genomics databases do not contain information specific to Māori and Pacific, there is currently no way to assign function or causality to variants when they are identified. This talk will outline the novel tools we use to identify and assign function to non-coding genetic variants that are specific and disease-relevant to Māori and Pacific people.

Cancer Phylogenetics Using Single-Cell RNA-Seq Data

Jiří C. Moravec[1], Sarah Diermeier[2], David Spector[3], Rob Lanfear[4], and Alex Gavryushkin[1]

[1]*Department of Computer Science, University of Otago, New Zealand*
[2]*Department of Biochemistry, University of Otago, New Zealand*
[3]*CSH, United States of America*
[4]*Ecology, Evolution and Genetics, the Australian National University, Australia*

Phylogenetics methods are emerging as a useful tool to understand cancer evolutionary dynamics, including tumour structure, heterogeneity, and progression. Most currently used approaches utilise either bulk whole genome sequencing (WGS) or single-cell DNA sequencing (scDNA-seq) and are based on calling copy number alterations and single nucleotide variants (SNVs). Here we explore the potential of single-cell RNA sequencing (scRNA-seq) to reconstruct within-organism cancer evolutionary dynamics. scRNA-seq is commonly applied to explore differential gene expression of cancer cells throughout tumour progression. The method exacerbates the single-cell sequencing problem of low yield per cell with uneven expression levels. This accounts for low and uneven sequencing coverage and makes SNV detection and phylogenetics analysis challenging. In this paper, we demonstrate for the first time that scRNA-seq data contains sufficient evolutionary signal and can be utilised in phylogenetic analyses. We explore and compare results of such analyses based on both expression levels and SNVs called from our scRNA-seq data. Both techniques are shown to be useful for reconstructing phylogenetic relationships between cells, reflecting the clonal composition of the tumour. The approach utilising SNVs appears to be more powerful and informative overall, but require and increased computational cost or a significant data reduction as a more complex Bayesian analysis is required to get sensible results, while the approach based on the standardised expression levels performs well with a simpler maximum likelihood phylogenetics reconstruction, but shows an evidence of a convergent evolution. Our results suggest that scRNA-seq can be a competitive alternative or useful addition to conventional scDNA-seq phylogenetic reconstruction. Our results open up a new direction of somatic phylogenetics based on scRNA-seq data. Further research is required to refine and improve these approaches to capture the full picture of somatic evolutionary dynamics in cancer.

Investigating microbial communities: assembly and the impact of environmental stressors

Tina Summerfield
Department of Botany, University of Otago, Dunedin, New Zealand

The microbial community plays a critical role in primary production, carbon cycling, and nutrient cycling in many environments. We have used high throughput sequencing approaches to provide high resolution data on microbial diversity in a selection of environments including New Zealand coastal waters, grasslands and streams. We have included approaches to investigate microbial community assembly and the impact of anthropogenic activities on microbial communities. I will present our investigations of microbial communities and the potential impact of environmental stressors such as those associated with climate change and agricultural activities.

Selected Talks:

Splicing defects in *RARS2* deficiency

Guillem de Valles Ibáñez
Department of Paediatrics and Child Health, University of Otago, Wellington, New Zealand

Recessive variants in *RARS2*, the nuclear gene encoding the mitochondrial argynil t-RNA synthetase, were initially reported in pontocerebellar hypoplasia. Subsequently, a recessive *RARS2* early-infantile (<12 weeks) developmental and epileptic encephalopathy was described with hypoglycaemia and lactic acidosis. Here, we describe two unrelated patients with a novel *RARS2* phenotype of late infantile onset myoclonic developmental and epileptic encephalopathy, presenting with a progressive myoclonic movement disorder from 9 months on a background of normal development. Development plateaued and regressed thereafter, with mild to profound impairment and multiple drug-resistant seizures. Both patients had compound heterozygous *RARS2* variants with likely impact on splicing or gene expression. After an analysis of 43 patients published with 34 *RARS2* variants, we found that 30 (86%) variants are predicted to affect splicing or gene expression, by affecting directly the splicing sequence or indirectly by creating cryptic splice sites, through nonsense-mediated decay or by affecting the promoter or the start codon. Our findings show that, as for other t-RNA synthetases, a reduced expression of the protein is the main pathological mechanism for *RARS2* deficiency. This deficit in expression is driven primarily by variants that impact splicing and affects energy demanding organs such as the brain.

Features of Functional Human Genes

Helena B. Cooper[1], and Paul P. Gardner[1]
[1]*Department of Biochemistry, School of Biomedical Sciences, University of Otago.*

Proteins and non-coding RNAs are functional products of the genome that carry out the bulk of crucial cellular processes. With recent technological advances, researchers can sequence genomes in the thousands as well as probe for specific genomic activities of multiple species and conditions. These studies have identified thousands of potential proteins, RNAs and associated activities, however there are conflicting conclusions on the functional implications depending upon the burden of evidence researchers use, leading to diverse interpretations of which regions of the genome are "functional". Here we investigate the association between gene functionality and genomic features, by comparing established functional protein-coding and non-coding genes to non-genic regions of the genome. We find that the strongest and most consistent association between functional genes and any genomic feature is evolutionary conservation and transcriptional activity. Other strongly associated features include sequence alignment statistics, such as maximum between-site covariation. We have also identified some concerns with 1,000 Genomes Project and Genome Aggregation Database SNP densities, as short non-coding RNAs tend to have greater than expected SNP densities. Our results demonstrate the importance of evolutionary conservation and transcription for sequence functionality, which should both be taken into consideration when differentiating between functional sequences and noise.

The genetics of eating disorders: what do we know and where are we going?

This talk does not include any potentially triggering images

Kennedy, M.A., Jordan, J., Miller, A.L., Cleland, L., Kennedy, H.L., Bulik, C.M.
Department of Pathology and Biomedical Science, University of Otago, Christchurch, New Zealand

Most mental disorders are complex traits, influenced by both genetics and environmental factors. Identifying the genetic components that underlie risk of a mental disorder can provide leads into the molecular pathways, physiological processes and cell types that may shape the biology of each disorder. Eating disorders are highly heritable, polygenic, and severe conditions that are poorly understood and difficult to treat. We are involved in large scale, international efforts to recruit and study many thousands of people with eating disorders, in order to gain insights into the underlying biology and the relationships these conditions have with other traits. This talk will briefly review results from the initial large GWAS study on anorexia nervosa to which New Zealand researchers contributed, then describe the approaches and logistical challenges to recruiting over 3000 New Zealand participants for this work in progress.

Familial immune thrombocytopenia (ITP) and hereditary persistence of fetal haemoglobin (HPFH) associated with a pathogenic variant of *MYB*

I.M. Morison[1], B.M. Zhang[2], S. Cross[3], R.J. Weeks[1], J.L. Ludgate[1], U. Khatoon[4], J.L. Zehnder[2]

[1]University of Otago, Dunedin, New Zealand, [2]Stanford University, Stanford, United States, [3]Canterbury District Health Board, Christchurch, New Zealand, [4]University of Auckland, Auckland, New Zealand

Familial ITP, which is extremely rare, has the potential to reveal causative mechanisms of disease. A four generation family with incompletely penetrant autosomal dominant ITP affecting six individuals was documented. Affected family members presented at the ages of 1 to 47 years (mean 19 years) with severe thrombocytopenia that was responsive to intravenous immunoglobulin or immunosuppressive therapy and usually had relapsing disease. The aim was to identify the causative genetic variant and to determine the mechanism by which the variant predisposes to immune destruction of platelets.

Whole exome sequencing and targeted sequencing were used to identify and confirm the variant and its effect on RNA splicing.

A pathogenic variant affecting the canonical splice donor site in intron 12 of *MYB* was identified in three distantly related affected individuals and in four unaffected individuals. Affected and unaffected carriers of the *MYB* variant had HbF levels of 2.0-7.2% (mean 4.2%; normal < 1%). The mean platelet count of unaffected carriers was 278 x 10(9)/L (normal 150-400). Amplification of peripheral blood cDNA showed alternative splicing: reference and alternatively spliced transcripts from exons 12 to 13 were present in equal amounts. The alternative transcript codes for a protein in which the final 111 amino acids are replaced with 52 alternative residues. Collation of genome-wide association study results shows a strong association of the *MYB* locus with HbF and other red cell, platelet and granulocyte parameters, but very few, low probability associations with immune- mediated diseases.

Familial ITP, with associated HPFH, was attributable to a pathogenic variant of *MYB*. The absence of association of the *MYB* locus with other autoimmune diseases implies that the predisposition to ITP may be driven by alteration of platelet antigens. These result challenge the definition of ITP (non- genetic) and also challenge the paradigm that ITP is driven by dysregulation of the immune system.

From Genotype to Phenotype: Understanding idiopathic disease

Megan J. Wilson

Developmental Biology and Genomics Lab, Department of Anatomy, School of Biomedical Sciences, University of Otago, Dunedin.

Adolescent Idiopathic Scoliosis (AIS), affects 2-10% of the population and is the most prevalent spinal deformity in children and adolescents, it also exhibits a dramatic sex bias with 90% of severe curves, requiring surgical intervention, arising in females. Genome Wide Association Studies have identified a number of variants associated with AIS, only one of these has been successfully identified across multiple ethnicities, rs11190870 found in a putative enhancer of the *LBX1* gene. The role of this enhancer with respect to *LBX1* and surrounding genes remains to be investigated functionally *in vivo*.

Using CRISPR-Cas9 mediated gene editing, we have deleted a 189 bp region in the homologous putative enhancer located near the *Lbx1* gene in mice. To characterise/phenotype this novel mouse line, which we have called *Lbx1EHΔ*$^{-/-}$, we have employed the use of a combination of RT-qPCR, and *In Situ* Hybridisation. We have used publicly available 4C data to determine another five genes potentially associated with this region. We have observed an increase in *Lbx1* expression across several critical developmental timepoints (E10.5, E12.5 and E15.5, $p<0.05$), while postnatally the differences in gene expression aren't statistically significant. Following this finding, we sought to determine whether these changes to gene expression lead to phenotypic changes in our mouse model.

The spines of adult mice underwent micro-Computed Tomography scanning to examine their vertebral columns in 3D. The *Lbx1EHΔ*$^{-/-}$ cohort significantly greater vertebral rotation and instability than their wild-type counterparts in the absence of morphological changes to the vertebrae, in line with clinical reports of adolescent idiopathic scoliosis.

We next sought to determine whether these changes might be due to difference in proprioceptive ability as is previously reported . Our model underwent a series of behavioural tests including a grid-walk test examining foot faults, grip strength and SNAP testing (simple neuroassessment for asymmetric impairment). We found that the *Lbx1EHΔ*$^{-/-}$ cohort performed significantly worse on the grid walk with 2.8-3.7-fold more foot faults than their wild-type counter parts post-puberty ($p<0.001$). SNAP testing suggested an early decline in proprioceptive ability with *Lbx1EHΔ*$^{-/-}$ mice performing significantly worse pre-puberty ($p<0.001$), that wild-type counterparts, a trend that continued in to adulthood ($p<0.01$).

The current results indicate an early deficit in proprioceptive ability of *Lbx1EH*$^{-/-}$ mice, resulting in vertebral instability and rotation with age. The next steps will seek to determine how altered gene expression and spatial expression patterns affect neuronal circuits in the spinal cord, which we believe drive the phenotypic changes observed in our *Lbx1EHΔ*$^{-/-}$ cohort.

Bus Stop Talks:

Investigating DNA methylation as a mechanism for paradoxical gene activation using epigenomic editing technology

Rakesh Banerjee, Jim Smith, Peter Stockwell, Mike Eccles, Robert Weeks, Aniruddha Chatterjee

Department of Pathology, University of Otago, Dunedin, New Zealand

Cutaneous melanoma is an aggressive malignancy accounting for 75% of skin cancer-related deaths. Globally, the incidence rate of cutaneous melanoma is increasing more rapidly than any other cancer type and widespread metastasis is the leading cause of mortality among melanoma patients. Advanced melanoma is well known for its propensity to metastasize, with 80% of patients presenting brain metastasis at the autopsy. As such, it is imperative to investigate the metastatic process and to develop new treatment options. Despite ongoing research of classic human genetics and its impact on disease phenotype, variations in DNA sequences alone are insufficient to explain the pathobiological changes involved in metastasis. Recently, the introduction of epigenetic drivers as a proposed mechanism of progression to metastasis in melanoma is a promising concept which warrants further exploration.

DNA methylation is a stable epigenetic regulator of gene expression. Hypermethylation at gene promoters has been widely established as a strong down-regulator of gene expression in many diseases, particularly in cancer. However, recent studies from our group and several others have demonstrated the opposite phenomenon, where high gene expression occurs in the presence of high promoter methylation, challenging the dogma of promoter methylation being only a silencing mechanism and raising the possibility that for a group of genes, promoter hypermethylation may activate gene expression.

Established methylation manipulation methods commonly involve the use of demethylating agents such, as 5-azacytidine, which act globally, altering methylation levels in a replication dependant manner. Thus, to precisely investigate the activating role of promoter hypermethylation, we employ a novel methylation-editing approach using CRISPR-based editing technology. Our modified system utilises a deactivated Cas9 protein, allowing for manipulation of methylation at a specific genomic locus without changes in DNA sequence.

Prior investigation of the gene *EBF3* identified this association of promoter hypermethylation and transcriptional activation. We now strive to correlate these locus-specific changes in methylation with gene expression, and subsequently, corresponding changes in the 3D architecture of the genome. Consequently, we aim to determine whether decreases in promoter DNA methylation result in the repression of gene expression in this context, and thus begin to elucidate the underlying mechanisms by which paradoxical gene activation could occur.

Whole body regeneration and wound healing comparison in *Botrylloides leachii*

Rebecca M Clarke[1], Michael Meier[1], Miles Lamare[2] and Megan J Wilson[1]
[1]Department of Anatomy, School of Biomedical Sciences, University of Otago, Dunedin, New Zealand, [2]Department of Marine Science, University of Otago, Dunedin, New Zealand

Whole body regeneration (WBR) is the ability to regrow an entire body or adult from a small collection of cells. Wound healing is the process that occurs after injury, and which utilises components of the immune system, blood coagulation cascade and the inflammatory pathways to repair the wound. We study these processes using the colonial ascidian, *Botrylloides leachii*. *B. leachii* are chordates, they live in colonies that consist of adults, referred to as zooids, that share a common gelatinous matrix or tunic which is embedded with a vascular system. *B. leachii* have the ability to regenerate from only a few hundred cells, to form a fully functioning zooid within 8 days. Regeneration arises from a small section of blood vessels but it must not contain any adult zooids for the process to occur. Wound healing occurs in *B. leachii* when injury results in at least one zooid being left on the colony. It is currently unknown, besides the presence or absence of zooids, what triggers regeneration verses wound healing.

We are using RNA-sequencing at 1, 3, 5, and 10 hour times points to determine the genes and pathways unique to wound healing and regeneration. Only 2 genes were differentially expressed at 1h, suggesting this period is largely important for healing of the injury site. At 3 hours, 159 genes were differentially expressed between healing and WBR fragments, and by 10 hours, this increased to 296 genes, suggesting that they were following different developmental trajectories. One pathway common across all the time points (for WBR) was calcium signalling. Calcium ion binding helps co-ordinate physiological responses to injury and the absence of calcium signalling prevents regeneration in planarians and drugs that alter calcium signalling result in regeneration polarity defects. Preliminary experiments, inhibiting calcium signalling in both regenerating and wound healing colonies, indicate that it is essential to trigger the regeneration process in tissue fragments, however, an injured colony is still able to heal and continue to grow and reproduce asexually.

Understanding what triggers regeneration and wound healing may give insight as to why certain species have lost the ability to regenerate, along with the triggers of *B. leachii* WBR.

Step Change for Ryegrass Breeding

Caitlin Harris[1], Colin Eady[2], Richard Macknight[1], Lynette Brownfield[1]
[1]University of Otago Department of Biochemistry , [2]Barenbrug NZ

Perennial ryegrass (*Lolium perenne*) is the primary pasture crop supporting New Zealand's agricultural industry. Limited genetic gains have been made with current breeding strategies compared to other grass crops such as rice and maize which have benefited from hybrid breeding. One of the largest constraints on hybrid breeding in *L. perenne* is self-incompatibility (SI), a genetic mechanism that stops pollen fertilizing flowers from the same plant. SI prevents the production of inbred lines and limits the ability to both purge deleterious alleles and fix agronomically advantageous traits in breeding populations.

There have been several *L. perenne* populations identified that have a mutant allele (*SF-locus*) which provides the ability to overcome the SI mechanism. Barenbrug NZ was granted access to a European self-fertile *L. perenne* population. This project aims to introgress the *SF-locus* into elite New Zealand *L. perenne* breeding populations and produce inbred lines with agronomically advantageous traits specific to New Zealand. This involves the development of molecular markers for the germplasm being used, crossing the SF locus into elite material and confirming the ability of the offspring to self-fertilise, as well as assessing the genomic impact of several rounds of inbreeding using GBS (genotyping by sequencing). Having elite self-fertile ryegrass lines would be the first step in a hybrid breeding programme which could lead to a step-change for ryegrass breeding in New Zealand.

"The more the merrier"? Making Better Use of GBS Data

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Recent sequencing methods, such as Genotyping-by-Sequencing (GBS), enable us to characterise the genomic variation of non-model but agriculturally important species at relatively low cost. Many thousands of GBS markers provide a valuable resource to accelerate genetic gains in animal and plant breeding via Genomic Selection (GS), but also raise the question of how to make better use of the genomic information?

Perennial ryegrass (*L. perenne*) is one of the most economically important forage species in the temperate regions. The highly polymorphic nature of ryegrass offers another type of markers: shortHaps ('short haplotypes'), which are defined as multiple variants located within small genomic segments (GBS fragments). We aim to enhance existing GS strategies of ryegrass by utilising shortHaps.

In this talk, I will describe how to identify and utilise shortHaps from GBS data.

Understanding pollen abortion in female kiwifruit

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Kiwifruit (the genus *Actinidia*) is unusual amongst crop plants in that it is dioecious, meaning there are separate male and female plants. The dioecious nature of kiwifruit reduces breeding efficiency and impacts upon commercial production as growers must dedicate orchard space to non-fruiting males.

Sexuality in kiwifruit is controlled by at least two genes in an approximately 1.3 MB male- specific sex-determining region (SDR). One of these genes, a fasciclin-like arabinogalactan protein *Friendly Boy* (*FrBy*), has recently been found to be crucial for male fertility, and its absence in female kiwifruit alters the development of the tapetal layer in the anther resulting in sterile pollen. While *FrBy* has now been identified, its biological role and why its absence leads to pollen abortion is not yet understood. In addition, there are a number of other genes on the SDR, some of which are likely to be required for pollen fitness.

We have performed low-input RNAseq on single male and female anthers and their isolated meiocytes/microspores at key developmental stages. Comparison of gene expression between male and females shows developmental differences arise prior to meiosis in anther tissue.

We will next identify candidate genes which may contribute to the female pollen phenotype, characterize the downstream effects of *FrBy* on pollen fertility, and identify additional genes that are required for male fitness.

Results from this project will help guide the development of hermaphroditic kiwifruit capable of rapid breeding through selfing. Further, understanding the gene expression dynamics of young sex chromosomes such as the kiwifruit SDR may show how plants evolve two distinct sexes from a hermaphroditic ancestor.

Restriction enzyme based next generation sequencing effectively recapitulates tumour mutation burden and cancer mutation signatures

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The use of next generation sequencing (NGS) technologies to profile tumours has resulted in the association of genomic biomarkers, such as tumour mutation burden (TMB), with response to therapies such as immunotherapy. Other cancer mutation signatures have been refined to detect clinically relevant associations such as *BRCA1/2* insufficiency in the absence of detectable genetic alterations. However, NGS applications to detect these genome wide parameters are not currently cost effective, presenting a barrier to this research and diagnostic capability for labs and clinicians. Reduced representation approaches such as panel sequencing target a small proportion of the genome and are less expensive than whole genome sequencing (WGS) approaches. We hypothesised that these approaches could recapitulate genome wide patterns detected by WGS. We focused on restriction enzyme based library preparation methods due to their availability and low costs.

An *"in silico* library" was prepared to simulate regions captured for restriction enzyme based NGS (REBaNGS). Restriction enzyme sequence recognition sites were mapped in the human genome. REBaNGs libraries were generated for 135 enzymes by filtering regions based on typical library fragment lengths. The coverage of the REBaNGS libraries varied between 1-10% of the genome.

We defined REBaNGS profiles for a cohort of 560 breast cancer samples that had undergone whole genome sequencing (WGS) by filtering mutations to those that overlapped the mapped REBaNGS libraries. For comparison to an approved assay, we generated a FoundationOne Cancer Diagnostics (F1 CDx) mutation profile for each sample by subsetting mutations to those present in genes in this panel. We compared genome wide parameters captured from REBaNGS profiles to the WGS profiles. We found a high correlation between WGS TMB and REBaNGS TMB values. We then classified each mutation into it's genomic context for signature analysis. Mutation context (MC) values had consistently high correlation between REBaNGS and WGS profiles. Notably, REBaNGS outperformed the F1 CDx profiles in terms of TMB and MC correlations. REBaNGS profiles allowed for elucidation of clinically relevant mutation signatures. We sequenced a mouse cell line using REBaNGS and the mutations captured recapitulated those seen in WGS data for this cell line.

Our *in silico* and sequencing analyses demonstrated that REBaNGS is an effective surrogate marker for mutation profiles and TMB captured by WGS. These methods may represent a sensitive, more cost effective approach to capture the information in these biomarkers, allowing patients to be matched to targeted therapies.

Circulating tumour cells as a model to identify tumour-specific epigenetic signatures of colorectal cancer metastasis

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New Zealand currently has the highest incidence and death rate for Colorectal Cancer (CRC) accounting for 4 deaths/day. Metastasis is responsible for 90% of cancer related deaths, but if detected early, the prognosis improves substantially. Circulating Tumour Cells (CTC) are tumour seeds that arise from both primary and secondary tumours, responsible for metastatic dissemination and can be detected in patient blood. Hence, CTCs possess great potential to improve CRC management. However, there is limited knowledge of the molecular profile and the role of CTCs in metastasis. Recent work in breast cancer has shown loss of DNA methylation in CTCs in stem cell associated genes (SOX2 and NANOG), which contributed to the metastatic potential of the CTCs in breast cancer. The epigenetic landscape of CTCs remains largely unknown, particularly in colorectal cancer patients. A major challenge for studying epigenetics of CTCs is their low number in patient blood and lack of user-friendly, yet robust methods to isolate or enrich for CTCs in a standard laboratory setting. The only FDA approved method CELLSEARCH has many limitations such as cost, set up and the use of only one type of marker (i.e., EpCAM expression). We are employing a well validated size-based isolation method (MetaCell) to enrich for CTCs in CRC patients. MetaCell based enrichment is fast, easy to use, relatively inexpensive and could be deployed in laboratory and clinical settings. The isolated CTCs will be cultured and characterised and used for generating the first DNA methylomes and transcriptomes of circulating tumour cells, first *in vitro* and then from CRC patients. This work is likely to identify the methylomic and transcriptomic signatures/markers for CTCs, facilitate exploring the role of these markers in CRC metastasis and also provide an opportunity to further explore the potential of these markers in predicting metastatic potential and response to therapy in patients in future.

Ancient DNA reveals strong phylogeographic structure within the extinct New Zealand bush wren (*Xenicus longipes subsp.*)

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New Zealand (NZ) contains a unique endemic avifauna. Regrettably, much of this avifauna has been driven to extinction following the arrival of humans and mammalian predators, including the extinctions of multiple species of Acanthisittid wren (the sister taxa to all other Passerines). The NZ bush wren (*Xenicus longipes subsp.*) was one such species, rendered extinct following the invasion of Big South Cape Island by ship rats. Three bush wren subspecies are currently recognised, each endemic to one of the three largest islands of NZ (North, South, and Stewart Island), and their near-shore islands. However, the genetic relationships between bush wren populations and subspecies are unknown. Here we sequence mitochondrial genomes from bush wren museum specimens to examine the validity of each subspecies, and the phylogeography of the South Island subspecies. We find that the North Island and South Island subspecies are genetically distinct and could potentially be considered separate species. Furthermore, the South Island bush wren complex is highly phylogeographically structured, forming four geographically discrete clades, including a clade specific to the Stewart Island subspecies. The divergence between the North and South Island subspecies is likely associated with the closing of the Manawatu strait, facilitating dispersal to the North Island. While the phylogeographic structure present within the South Island/Stewart Island subspecies complex is possibly driven by glaciation, with glaciers restricting populations to ice-free refugia during glacial periods. This research highlights the importance of museum specimens as genetic resources, and sheds light on the biogeographic processes which shaped New Zealand's avifauna.

Investigating the role of DNA methylation in paradoxical gene expression using a CRISPR-based system

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DNA methylation is a stable and somatically heritable epigenetic process. It is classically understood as a gene silencing mechanism, whereby hypermethylation of gene promoters is largely associated with transcriptional silencing. However, emerging research in the past decade has found a different association, particularly in the context of cancer and metastasis. These studies have demonstrated a seemingly paradoxical correlation between high levels of promoter methylation and subsequent transcriptional activation. As such, we are yet to fully unravel the complex mechanism underlying this relationship. This warrants further investigations to better understand this dynamic molecular mechanism and thus its implications in cancer pathobiology and metastasis.

Previously established methods of manipulating DNA methylation have largely involved the use of chemical drugs, such as decitabine. These drugs act globally on the epigenome and are thus non-specific. Importantly, as they are inhibitors of DNA methylation, they cannot definitively demonstrate that an increase in promoter methylation is directly responsible for an increase in gene expression. Therefore, by using a CRISPR-based system for targeted epigenetic editing, the precise mechanism of paradoxical gene activation by DNA methylation can now be investigated.

Our group has recently demonstrated this paradoxical correlation in a study characterising epigenetic changes in metastatic melanoma. The *Early B-cell Factor 3 (EBF3)* gene promoter region was found to be substantially hypermethylated in metastatic cell lines compared to paired, primary cell lines. This increase in methylation was also found to be associated with a subsequent increase in gene expression. These findings suggest that *EBF3* in human melanoma cell lines is a good candidate gene to investigate this complex relationship.

Here, I aim to establish a methylation editing system and apply it to the *EBF3* promoter region. This is a three-component CRISPR system incorporating the SUperNova Tag (SunTag), which allows for the recruitment of multiple effector proteins. In this project, I use the DNA methyltransferase 3A (DNMT3A) effector protein to induce active methylation at a target locus within the *EBF3* promoter region in melanoma cell lines.

Methylation changes have been evaluated using methylation-specific Illumina iSeq sequencing. Variable levels of targeted methylation have been observed, of up to 38.0% absolute methylation change between edited and unedited samples. This project represents important progress in investigating the promoter methylation of *EBF3* in more detail, as well as laying out an essential platform to further investigate methylation changes and causal mechanisms of gene expression alteration in future studies.

Activity of Bacillus sp. as a biocontrol agent in controlling green mould disease in oyster mushrooms (*Pleurotus sp.*)

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Pleurotus sp., which is commonly known as oyster mushroom is one of the most important commercial edible mushrooms cultivated worldwide. However, main producers of *Pleurotus sp*. including South Korea, Hungary, Romania and Thailand have reported severe yield loss resulted from the attack of green mold disease, caused by some *Trichoderma sp*. Other *Trichoderma sp*. are well – established biocontrol agent which is used in inhibiting plant pathogens and improving plant growth, certain Trichoderma sp. are reported to be the causal agents of green mould disease. The reason behind the pathogenicity of *Trichoderma sp*. towards *Pleurotus sp*. is still unclear but literature suggested that the abundance of carbohydrate active enzymes (CAZYmes), proteins involved in secondary metabolites production and peptaibols in *Trichoderma sp*. might be the determinants of its pathogenicity.

Tremendous efforts have been made to control green mould disease including the use of fungicides such as prochloraz, steam sterilization, steam pasteurization, hot water immersions and chemical treatment during substrate preparation and biopesticides such as *Bacillus sp*. Prochloraz has been selected as the most effective fungicide but high concentration of plochlolraz is harmful to the mycelia growth and fruiting body development of *Pleurotus sp*. Steam sterilization and pasteurization, hot water immersions and chemical treatments are not always successful too as growers reported recurring contamination during handling and spawning. Biopesticides, in contrary, have shown promising results in controlling green mould disease. For instance, native Bacillus strains have shown effective growth inhibition (> 60 %) against *T. pleuroti* and *T. pleuticola* in vitro. In addition, commercially available *Bacillus velezensis* strain QST713 is currently used in France to protect white button mushrooms from *Trichoderma aggressivum f. europaeum*.

Therefore, this study is carried out to investigate the effectiveness of *Bacillus sp*. found in New Zealand in controlling green mould disease in *Pleurotus sp*. and to compare the ITS regions of *Trichoderma sp*. which are pathogenic and non – pathogenic to *Pleurotus sp*.


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