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GO SYMPOSIUM 2022

1st & 2nd December

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A UNIVERSITY OF OTAGO RESEARCH THEME

LAB SUPPLY

MICROBIOLOGY & IMMUNOLOGY

Te Tari Moromoroiti me te Ārai Mate

Thursday, 1st December 2022

Posters will be on display for the duration of the Symposium in the foyer, please take time to view these during the breaks.

Session 1 - *Tangata ako ana i te whare*		**Chair: Alana Alexander**
12:50 pm – 1:00 pm	Welcome	A/Prof Louise Bicknell Peter Williamson
1:00pm – 2:00pm	Tikanga and Mātauranga Māori in Genetics Education and Research: What, Why and How.	A/Prof. Phillip Wilcox *Ngāti Rakaipaaka, Rongomaiwahine, Ngāti Kahungunu ki te Wairoa* *Department of Mathematics & Statistics*
2:00 pm – 2:20 pm	A long and winding road – learning to work with communities in genomic research	Prof. Lisa Matisoo-Smith *Department of Anatomy*
2:20 pm – 2:40 pm	The Māori Strategic Framework can greatly broaden the appeal of scientific research.	Peter Williamson *School of Biomedical Sciences*
2:40 pm – 3:00 pm	Māori Health Advancement: Aspirations, Considerations and Commitments	Howard Maxwell Te Whakatōhea, Te Whānau A Apanui, Ngāti Porou *University of Otago*
3:00pm – 3:30pm Tea/coffee break		

Session 2 - *Retirement celebration for Prof Clive Ronson*		**Chair: Greg Cook**
3:30 pm – 4:30 pm	Reflections on a symbiosis island	Prof. Clive Ronson *Department of Microbiology & Immunology*
4:30 pm – 4:50 pm	An epigenetic switch activates bacterial quorum sensing and horizontal transfer of an integrative and conjugative element	Dr. Josh Ramsay *Curtin Medical School*
4:50 pm – 5:10 pm	How a previously unrecognized variant of the helix-turn-helix domain can be a transcriptional activator or antiactivator	Dr. William Jowsey *Department of Microbiology & Immunology*
5:10 pm – 5:30 pm	Carbohydrate signalling in microbe-legume symbiosis	Dr Simon Kelly *Lincoln Agritech*
5:30 pm – 7:00 pm Drinks and Nibbles		

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Friday, 2nd December 2022

Posters will be on display for the duration of the Symposium in the foyer, please take time to view these during the breaks.

8:50 am – 9:00 am	Welcome	A/Prof Louise Bicknell
Session 3		**Chair:**
9:00 am – 9:15 am	The rise of the Lilliputians: palaeogenetic insights into our neglected minifauna	Dr Nic Rawlence *Department of Zoology*
9:15 am – 9:30 am	Piecing together the settlement of the Pacific with kiore genomics	Dr Catherine Collins Ngāi Tahu, Pakeha *Department of Anatomy*
9:30 am – 9:45am	Cloacal virome of an ancient host lineage – the tuatara (*Sphenodon punctatus*) – reveals abundant and diverse diet-related viruses	Stephanie Waller *Department of Microbiology & Immunology*
9:45 am – 10:00 am	Characterising the molecular interactions between jumbo phages and *Serratia*	Kate Harding *Department of Microbiology & Immunology*
10:00 am – 10:15 am	CRISPR-Cas targeting of a nucleus-forming jumbo phage induces abortive infection	Dr Leah Smith *Department of Microbiology & Immunology*
10:15 am – 10:30 am	A mobile restriction–modification phage defence system resolves an epigenetic conflict with an antagonistic endonuclease	Dr Nils Birkholz *Department of Microbiology & Immunology*
10:30am –11:00am Tea/coffee break		

Session 4		**Chair: Louise Bicknell**
11:00 am – 12:00 pm	Claire Aldrich: A Legacy	Sally Crouch *Founder, Claire Aldrich Legacy Fund* *Student, University of Otago*
12:00 pm – 12:15 pm	Working towards Pacific genomes - 4 Ws and 1 H.	Dr Allamanda Faatoese *Department of Medicine, University of Otago Christchurch*
12:15 pm – 12:30 pm	Why Genetics?	Dr Michelle Thunders *Department of Pathology and Molecular Medicine, University of Otago Wellington*
12:30pm – 1:30pm Lunch		

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Session 5		Chair: Mary Hawkes
1:30 pm – 1:45 pm	Where did that come from? Genomic detection of dispersal	A/Prof Ceridwen Fraser *Department of Marine Science*
1:45 pm – 2:00 pm	Pātai about parāoa: molecular ecology of sperm whales	Dr Alana Alexander Te Hikutu: Ngāpuhi, Pākehā *Department of Anatomy*
2:00 pm – 2:15 pm	Buffering brain development: Sex-bias miRNA and gene expression	Rachel Cannon *Department of Anatomy*
2:15 pm – 2:30 pm	"Evolution gives you wings": A Comparative study of Gene Regulatory Networks involved in Wing Development of *Galleria mellonella* & *Drosophila melanogaster*	Kate McPhail *Department of Biochemistry*
2:30 pm – 2:45 pm	Using a zebrafish stem cell model to understand the molecular basis of Cornelia de Lange Syndrome	Anastasia Labudina *Department of Pathology*
2:45 pm – 3:00 pm	Using *Xenopus laevis* to evaluate two novel epilepsy variants	Cabriana Earl *Department of Zoology*
3:00pm – 3:30pm Tea/coffee break		

Session 6		Chair: Amie Siemonek
3:30 pm – 4:00 pm	The Silent Genomes Project: Construction of and Indigenous governance over an Indigenous genetic variation reference database for rare disease diagnosis in Canada	Dr Wyeth Wasserman *Professor of Medical Genetics, University of British Columbia, Vancouver BC, Canada*
4:00 pm – 4:15 pm	Metal dyshomeostasis in CLN5 Batten disease	Josh Clegg *Department of Biochemistry*
4:15 pm – 4:30 pm	Cognitive abilities at behaviours, brain and genetic levels	Dr Narun Pat *Department of Psychology*
4:30 pm – 4:45 pm	Successful modelling of a single nucleotide polymorphism in cells and mice using CRISPR-Cas9 and CRISPR-Cas12 methodologies	Dr Nicholas Fleming *Department of Pathology*
4:45 pm – 5:00 pm	Investigating *ELAVL2* – a novel neurodevelopmental disorder disease gene	Meghan Mulligan *Department of Biochemistry*
Closing		

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Abstracts

Keynote Speakers:

Tikanga and Mātauranga Māori in Genetics Education and Research: What, Why and How.
Phillip Wilcox
Associate Professor | *Ahorangi Tuarua*
Department of Mathematics and Statistics | *Te Tari Pāngarau me te Tatauraka*
Also: Affiliate Faculty, Bioethics Centre | *Te Pokapū Matatika Koiora* and Kaikōkiri Māori, Genetics Teaching Programme | *Mātai Ira*
University of Otago | *Te Whare Wānanga o Otāgo*
(Iwi Affiliations: *Ngāti Rakaipaaka, Rongomaiwahine, Ngāti Kahungunu ki te Wairoa)*

Over the past two decades the inclusion of Te Ao Māori at the interface of modern genetics/genomics has been steadily increasing. Māori have their own concepts of hereditary inheritance which are deeply embedded in Māori culture, and broadly and extensively utilised, particularly prior to British colonisation. Following a lengthy period of suppression and denigration of Māori culture and knowledge as a result of colonisation, the current trend of increased Māori involvement began in the 1990s with requirements for consultation with Māori communities, and consideration of Māori perspectives in regard to genetics research. This era included the development of tikanga-based research frameworks, and in some cases, publication of guidelines specifically aimed at implementing best practices for working with Māori. More recently, Māori involvement as researchers and participants in applying modern gene technologies has increased across health primary sector and environmental sectors. Moreover, initiatives such as MBIE's Vision Mātauranga, HRC's Māori Health Advancement guidelines, and the University of Otago's (UoO) Māori Strategic Framework as well as the Ngāi Tahu Research consultation process, has meant genetics education and research now needs to include tikanga and Mātauranga Māori. In this talk, I will describe Māori content in the Genetics Teaching Programme at UoO – which contains knowledge that most practicing geneticists, researchers and academics don't have – as well as some examples of how tikanga and Mātauranga are informing study design and analyses in existing research projects.

Claire Aldrich: A Legacy
Sally Crouch

I am the mother of a little girl who passed away. We do not know why she died, but her doctors were sure it was a genetic condition or mutation that has simply not been discovered. I am honoured to be able to share Claire's story with you, and our hopes for an outreach program that will allow school students to learn about genetic mutations and the importance of genetic research in our community, and worldwide.

Invited Speakers:

The Māori Strategic Framework can greatly broaden the appeal of scientific research.
Peter Williamson

When you engage with the Māori Strategic Framework, look carefully for how it can add value to your research. Whakatauki, narratives, karakia and even individual words within te reo Māori can often align in novel and powerful ways with science. If as a researcher, you harness and profile these alignments well, you are potentially availing your work to a much wider audience and to wider opportunities for funding.

A long and winding road – learning to work with communities in genomic research
Lisa Matisoo-Smith

After nearly 30 years of working on taonga species and with communities on both ancient and modern DNA, many people have asked me for advice about consultation. My answer is generally that there is no one way to engage or consult and that each project will be different. I will discuss many of the lessons I have learned about engaging with communities and provide examples of things that have worked and things that sometimes haven't worked but have provided learning experiences.

How a previously unrecognized variant of the helix-turn-helix domain can be a transcriptional activator or antiactivator

William J. Jowsey[1], Calum R.P. Morris[1], Drew A. Hall[2,3], John T. Sullivan[1], Robert D. Fagerlund[1], Karina Y. Eto[3], Paul D. Solomon[4], Joel P. Mackay[4], Charles S. Bond[2,5], Joshua P. Ramsay[3], Clive W. Ronson[1*]*

[1]Department of Microbiology and Immunology, University of Otago, Dunedin, 9016, New Zealand

[2]School of Molecular Sciences, University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia

[3]Curtin Medical School and Curtin Health Innovation Research Institute, Curtin University, Perth, WA 6102, Australia

[4]School of Life and Environmental Sciences, University of Sydney, Sydney, NSW 2006, Australia

[5]Marshall Centre for Infectious Disease Research and Training, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia

The mobile genetic element ICE*Ml*SymR7A can transfer to non-symbiotic *Mesorhizobium* species, rendering them capable of forming a nitrogen-fixing symbiosis with *Lotus* legumes. Initiation of transfer of ICE*Ml*SymR7A is tightly controlled by the regulators FseA and QseM that both contain the domain of unknown function (DUF) 2285. The FseA protein, which activates transcription of transfer genes, contains DUF2285 in its C-terminus and the DUF6499 in its N-terminus. The antiactivator QseM that represses FseA is comprised entirely of DUF2285. Structure prediction of FseA and NMR structure determination of monomeric QseM revealed DUF2285 is a variant of the helix-turn-helix (HTH) domain that deviates from the canonical HTH by an extended turn. We renamed DUF2285 the helix-extended-turn-helix (HeTH) domain to reflect this deviation from the HTH domain. The HeTH of FseA exhibits a positively charged face and makes interdomain contacts with DUF6499 through a distinct face. DNA binding assays revealed homodimeric FseA binds DNA, with HeTH residues being critical for strong DNA binding. In contrast, monomeric QseM does not exhibit a positively charged face and does not bind DNA. Protein interaction studies of mutant FseA and QseM proteins revealed DUF6499 of FseA binds QseM, and that QseM binds to DUF6499 in a fashion analogous to the FseA HeTH domain. Therefore, QseM has lost the capacity to bind DNA but retained DUF6499 binding to repress FseA.

In summary, DUF2285 is a newly discovered variant of the HTH domain that controls horizontal gene transfer of ICE*Ml*SymR7A through transcriptional activation and antiactivation in distinct proteins.

Carbohydrate signalling in microbe-legume symbiosis
Simon Kelly

Plant roots are exposed to a diverse soil microbial population, yet only a restricted subset of these are permitted to colonise the root interior. Our research aims to understand the molecular communication between plants and microbes enabling root colonisation. The accommodation of symbiotic bacteria and fungi by legume plants provides an ideal system for studying microbial signalling molecules and their perception by plant receptors.
I will present our research into the function of legume carbohydrate receptors in facilitating both bacterial and fungal symbiotic infection. Rhizobia bacteria induce and colonise root nodules on legumes where the symbiotic process of nitrogen-fixing occurs. Rhizobia produce exopolysaccharide that we have identified as being perceived by the legume receptor EPR3 to monitor symbiont compatibility during colonisation. Symbiotic arbuscular mycorrhiza fungi assist plants with nutrient uptake. We have identified a second legume carbohydrate receptor that potentially perceives fungal-derived glucans during fungal colonisation of the root.

The rise of the Lilliputians: palaeogenetic insights into our neglected minifauna
Nicolas J. Rawlence, Lachie Scarsbrook, Alex Verry, Kerry Walton
Otago Palaeogenetics Laboratory, Department of Zoology, University of Otago, Dunedin

Since the inception of ancient DNA, size has mattered. Most research has focused on charismatic megafaunal species that have captured the public attention. The bones of these large animals can also sustain levels of sampling needed for palaeogenetic analysis, without destroying important osteological characters or morphometric landmarks. In contrast, those of small vertebrates or the shells of molluscs cannot be sampled without invariably destroying the specimens. New techniques developed in our lab, including non-destructive palaeogenomics and the ability to cost-effectively sequence palaeogenomes from mollusc shells and small vertebrates, are unlocking previously hidden chapters of our biological heritage. In turn, these techniques are opening up vast swathes of natural history and archaeological collections to genetic analysis. In this talk I will highlight some of our discoveries focusing on geckos, wrens and molluscs.

Piecing together the settlement of the Pacific with kiore genomics
Catherine Collins

The Lapita people, whose culture is first documented in the archaeological record of the Bismarck Achipelago in the Pacific around 3,500 years ago, quickly settled previously uninhabited islands east of the Bismarck Archipelago. As the Lapita people moved through the Pacific, settling new islands, they carried important plants and animals with them. The kiore (Pacific rat, *Rattus exulans*) is one of these species that was transported across the Pacific. Understanding the movements of kiore can therefore inform us about the movements of the people who carried them. Early genetic studies focused on a short region of the mitochondrial control region identified the generic Hawaiki or homeland region of the Cook and Society Islands for East Polynesian populations, but could not distinguish the specific source population for the founding kiore populations because most of the East Polynesian samples shared a single mtDNA lineage. Today we can generate complete mitogenomes, which can break up previously identified haplotypes into multiple haplotypes. By sequencing mitogenomes and building a higher resolution genomic dataset for kiore we can better understanding some of the finer-scale interactions in this region.

Where did that come from? Genomic detection of dispersal
Ceridwen Fraser,
Department of Marine Science

Many plants and animals (and microbes) disperse long distances, either frequently or occasionally, but until recently we have rarely been able to confirm the sources of dispersing individuals. High resolution phylogenomic approaches can link new arrivals to their point of origin – revealing, for example, that rimurapa bull kelp rafts from South Georgia sometimes wash up on New Zealand or Antarctic beaches, after travelling tens of thousands of kilometres at sea! These approaches give exciting insights into biogeographic and broader evolutionary processes and could also provide a toolkit for understanding invasion dynamics and vectors.

Pātai about parāoa: molecular ecology of sperm whales
Alana Alexander
Te Hikutu: Ngāpuhi, Pākehā

Parāoa are a taonga species that hold cultural significance for Māori and Pacific peoples across Te-Moana-nui-a-Kiwa. As large, apex predators, they also fill an important role in marine ecosystems worldwide. This talk will cover ongoing efforts to utilise genetics and mātauranga Māori to better understand pātai [questions] like "Nō hea rātou?" - where are the whales in Aotearoa coming from - and who else are their whanaunga [relatives].

The Silent Genomes Project: Construction of and Indigenous governance over an Indigenous genetic variation reference database for rare disease diagnosis in Canada
Dr. Wyeth W. Wasserman, Professor of Medical Genetics, University of British Columbia, Vancouver BC, Canada.

The Silent Genomes Project aims to establish Indigenous governance for and creation of a reference genetic variation database to support the equitable diagnosis of the causes of rare disorders for Indigenous patients in Canada. At present there are inequities in the capacity to diagnose Indigenous children in Canada with rare diseases. It can be unclear if observed genetic differences between an Indigenous patient and international reference databases of genetic variation reflect an absence of Indigenous participants in the reference data or a genetic cause of a patient's disease. To address this inequity, the Silent Genomes Project pursues three technical aims shaped by an encompassing aim of Indigenous data and research governance. The three technical aims include: (Aim 2) the use of whole genome sequencing to diagnose Indigenous patients with rare disorders; (Aim 3) the creation of a reference Indigenous Background Variant Library (IBVL) reporting the frequency of genetic variants across the human genome for Indigenous participants; and (Aim 4) a health economics assessment of the impact (under multiple measures) of reference data on diagnosis. Aim 1 focuses on the development of Indigenous governance over the research and the data, which wraps around and within all other aims. The presentation will provide an overview of the Indigenous peoples of Canada, and will focus on Aims 1 and 3, highlighting how bioinformatics can be enabling to the process and implementation of governance models. The similarities and differences between the Silent Genomes Project and the Genomics Aotearoa Variome Project will be explored.

“Evolution gives you wings”: A Comparative study of Gene Regulatory Networks involved in Wing Development of Galleria mellonella & Drosophila melanogaster

Kate McPhail

Decades of research has gone into our understanding of wing development in *Drosophila melanogaster*; however, this same area has been critically understudied in insects beyond this species. The process of wing development in *D. melanogaster* is a leading model for how gene regulatory networks pattern epidermal tissues. Our understanding of how the genes involved control the growth of the wing blade and pattern it into sections, is a crucial model for how we understand the functions and interplay between growth control, patterning and planar cell polarity in all organisms [1-3] Lepidoptera are known for their wide diversity of wing morphology and patterns, yet we are only just starting to investigate the genetic processes that allow for this diversity. Whilst some species of Lepidoptera are increasing in use as model organisms in other areas of research, like the Greater Wax Moth (*Galleria mellonella*) [4-7], this species has not yet been utilised to its full potential as a model for Lepidopteran wing development. This research aims to determine to what extent the gene regulatory network that patterns *D. melanogaster* wings is also involved in the patterning of the wings of *G. mellonella*. As *G. mellonella* has a completely different wing shape & pattern to *D. melanogaster*. So, by investigating how that difference in wing morphology is created, I aim to further understanding of how these patterning mechanisms evolve and their flexibility to produce the diversity & variation seen across species.

There are six genes of interest that are involved in D. melanogaster wing development that I hypothesise to carry out similar roles in G. mellonella, these genes are *decapentaplegic, engrailed, wingless, brinker, optomotor blind* and *spalt* [2, 8-10]. The past year of research has been centred around establishing an *In-situ* Hybridisation V.3 (Hybridisation chain reaction) methodology for observing the patterns of mRNA expression of these genes within the imaginal discs of *G. mellonella,* I have also begun the process of generating a transcriptome of the Imaginal discs and will continue this into next year. Finally, I aim to produce RNAi knockdown phenotypes of the six genes, over the course of next year, to allow for comparison of gene function in *G. mellonella* to that of *D. melanogaster*. At this time, I have successfully identified the Imaginal Wing Disc tissue in vivo and can dissect this from caterpillars, I have developed an HCR protocol that has been able to show the mRNA expression patterns of just over half of my genes of interest. I have also completed the RNA extractions for the transcriptome.

References:

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Metal dyshomeostasis in CLN5 Batten disease

Josh Clegg, Genetics (Hons)
NLD laboratory, Otago University

Batten disease is the most common cause of childhood dementia and is one of the leading inherited neurological disorders in children. A key characteristic of Batten disease is the dysfunction of the lysosome, an organelle with the role of recycling and removing waste from within the cell. A recent transcriptomic screening in a neuronal model of a sub-type of Batten disease (CLN5 Batten disease) revealed changes in metal ion transport expression. In particular, eight metal transporters were downregulated in the CLN5 deficient model compared to control cells. The irregular transport and accumulation of metals is seen in many lysosome-related neurological diseases such as Parkinson's and Alzheimer's. However, whether metal dyshomeostasis plays a role in Batten disease progression is largely unknown. Here we show that the neuronal model used in the NLD lab has a cytotoxic response to metal ion treatment and metal transporter dysregulation. Treatment with potassium and zinc metal ions showed a dose-dependent cytotoxic reaction compared to untreated controls. Following this, CRISPRi-mediated knockdown of a potassium channel (KCNH4) and glutamate transporter (SLC17A7) led to increased cell death compared to lentiviral-treated controls. Metal treatment or metal transporter knockdown studies have never been completed in the context of Batten disease; hence, these findings represent novel ideas in this area of research. As metal dyshomeostasis is broadly seen in neurodegenerative disorders, I anticipate this report to be the beginning of further research regarding the role metals play in Batten disease. Whether metal ion treatment in Batten disease models shows a differential response to control cells has not been tested and is a crucial focus point as we advance. Lastly, treatments of Batten disease are currently palliative, with gene therapy trials and enzyme replacement therapies underway. Whether supplementing metal transporter proteins that are downregulated in Batten disease could restore lysosomal function and cell viability is an exciting future avenue.

Selected Oral Presentations:

Cloacal virome of an ancient host lineage – the tuatara (*Sphenodon punctatus*) – reveals abundant and diverse diet-related viruses

Stephanie J. Waller[1], Sarah Lamar[2,3], Benjamin J. Perry[1], Rebecca M. Grimwood[1], Edward C. Holmes[4], Jemma L. Geoghegan[1,5]
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[3]Centre for Biodiversity and Restoration Ecology, School of Biological Sciences, Victoria University of Wellington, Wellington, New Zealand.
[4]Sydney Institute for Infectious Diseases, School of Life and Environmental Sciences and School of Medical Sciences, The University of Sydney, Sydney, NSW 2006, Australia.
[5]Institute of Environmental Science and Research, Wellington, New Zealand.

Tuatara (*Sphenodon punctatus*) are one of the most phylogenetically isolated species and provide a unique host system to study virus evolution. While the tuatara genome, sequenced in 2020, revealed many endogenous viral elements, we know little of the exogenous viruses that infect tuatara. We performed a metatranscriptomics study of tuatara cloaca samples from a wild population on Takapourewa (Stephens Island), Aotearoa New Zealand. From these data we identified 49 potentially novel viral species that spanned 19 RNA viral families and/or orders, the vast majority (48) of which were likely dietary related. Notably, using a protein structure homology search, we identified a highly divergent novel virus within the *Picornaviridae* which may directly infect tuatara. Additionally, two endogenous tuatara adintoviruses were characterised that exhibited long-term viral-host co-divergence. Overall, our results indicate that the tuatara cloacal virome is highly diverse likely due to a large number of dietary related viruses.

Characterising the molecular interactions between jumbo phages and *serratia*

Kate R. Harding[1,2], Lucia M. Malone[1], Natalie Kyte[1] & Peter C. Fineran[1,2,3]
[1]Department of Microbiology & Immunology, University of Otago, Dunedin, New Zealand.
[2]Genetics Otago, University of Otago, Dunedin, New Zealand.
[3]Bioprotection Aotearoa, University of Otago, Dunedin, New Zealand.

Bacteriophages are viruses that infect bacteria. 'Virulent' phages replicate within the host cell via the lytic cycle with subsequent cell lysis leading to bacterial cell death. The lytic cycle consists of attachment of the phage to a bacterial receptor(s), entry of the phage genome into the host cytoplasm, replication of new phage particles and the release of the resulting phage progeny. Opportunity arises at each stage for phages to hijack host cell machinery to ensure a successful infection. We recently discovered a *Serratia* sp. ATCC 39006 jumbo phage (>200 kb genome) that can form a protective nucleus-like structure upon host infection. The nucleus-like structure acts to protect the genome of the jumbo phage from DNA-targeting CRISPR-Cas systems, but the export of phage mRNA into the bacterial cytoplasm leaves it vulnerable to the type III-A RNA-targeting CRISPR-Cas system of *Serratia*. To identify bacterial genes required for jumbo phage infection, transposon sequencing (Tn-Seq) was used. We revealed over one hundred Serratia genes that were involved in jumbo phage infection. Validation of the Tn-Seq findings was performed by CRISPRi knockdown and assessing the involvement of the host genes during phage infection. We demonstrated that this jumbo phage utilizes the flagella as a primary receptor, but multiple other genes were involved in infection. Many of those genes may have a role in flagella synthesis or function, which may interfere with jumbo phage attachment. A conserved set of host genes may be used by flagellotropic phages, as many similar host genes employed by jumbo phages during infection were also used by flagellotropic non-jumbo phages. Understanding phage-host interactions should allow more effective utilisation of nucleus-forming jumbo phages as antimicrobials in the fight against antibiotic-resistant bacterial infections.

CRISPR-Cas targeting of a nucleus-forming jumbo phage induces abortive infection

Leah M. Smith[1,2], David Mayo-Muñoz[1], Carmela García-Doval[3], Lucia M. Malone[1,5], Kate Harding[1], Simon A. Jackson[1,2], Hannah G. Hampton[1,6], Laura F. Gumy[4] & Peter C. Fineran[1,2]*
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Bacteria harbor multiple lines of defense against bacteriophages, including the adaptive CRISPR-Cas immune response. Upon infection, some jumbo (>200 kb genome) phages shield their DNA behind nucleoid-like 'shell' structures. These shells render jumbo phages resistant to degradation by DNA-targeting CRISPR-Cas systems. However, mRNA exported from the shell – for translation in the cytoplasm – can trigger activation of RNA-targeting CRISPR-Cas systems. The type III-A system of *Serratia* sp. ATCC 39006 encodes an accessory DNase – NucC – which is activated upon targeting of jumbo phage PCH45. Using deep sequencing and confocal microscopy, we demonstrate that NucC activation leads to preferential degradation of host DNA, while phage DNA remains largely protected throughout infection. In agreement, we show that NucC is excluded from the shell structure. Interestingly, *in-vivo* and *in-vitro* degradation experiments suggest that NucC demonstrates a preference for certain nucleotides at binding and/or cleavage positions. Using flow cytometry, we demonstrate that cells with active type III-A targeting halt the spread of PCH45 infection through abortive infection, as cell membranes remain largely intact well-beyond expected burst times. This work has uncovered the mechanism of RNA-targeting CRISPR-Cas systems when arresting infection of nucleoid-forming jumbo phages.

A mobile restriction–modification phage defence system resolves an epigenetic conflict with an antagonistic endonuclease

Nils Birkholz, Simon A Jackson, Robert D Fagerlund, Peter C Fineran
Department of Microbiology and Immunology, University of Otago, Dunedin
Bioprotection Aotearoa, University of Otago, Dunedin
Genetics Otago, University of Otago, Dunedin

Epigenetic DNA methylation plays an important role in bacteria by influencing gene expression and allowing discrimination between self-DNA and intruders such as phages and plasmids. Restriction–modification (RM) systems use a methyltransferase (MTase) to modify a specific sequence motif, thus protecting host DNA from cleavage by a cognate restriction endonuclease (REase) while leaving invading DNA vulnerable. Other REases occur solitarily and cleave methylated DNA. REases and RM systems are frequently mobile, influencing horizontal gene transfer by altering the compatibility of the host for foreign DNA uptake. However, whether mobile defence systems affect pre-existing host defences remains obscure. Here, we reveal an epigenetic conflict between an RM system (PcaRCI) and a methylation-dependent REase (PcaRCII) in the plant pathogen *Pectobacterium carotovorum* RC5297. The PcaRCI RM system provides potent protection against unmethylated plasmids and phages, but its methylation motif is targeted by the methylation-dependent PcaRCII. This potentially lethal co-existence is enabled through epigenetic silencing of the PcaRCII-encoding gene via promoter methylation by the PcaRCI MTase. Comparative genome analyses suggest that the PcaRCII-encoding gene was already present and was silenced upon establishment of the PcaRCI system. These findings provide a striking example for selfishness of RM systems and intracellular competition between different defences.

Buffering brain development: Sex-bias miRNA and gene expression

Rachel Cannon, Susie Szakats, Megan J. Wilson
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Developing the embryonic brain requires specific regulations. Alternations to gene expression can result in neurological disorders such as attention-deficit/hyperactivity disorder, schizophrenia, and autism. These disorders demonstrate sex differences in presentation, and it is believed that oestrogen is a key player due to differences in levels between the sexes during brain development. The testis produces testosterone early in development, which, in the developing brain, is converted to oestrogen via aromatase activity, while the ovaries do not produce oestrogen till near birth. Small non-coding RNA, called microRNA (miRNA), has known roles in brain development. We have previously determined that 119 miRNAs in embryonic (15.5) mice brains are expressed in a sex-dimorphic manner. Many of these miRNAs have also been linked to neurological disorders, and we predict a link between miRNA expression and oestrogen in the phenotype of sex-biased neurological disorders. Cell culture was used to understand if these sexually dimorphic miRNA responds to hormones present and if ESR2 mediates it. Embryonic (12.5) mice neurons were cultured, and the cells were treated with dose-dependent addition of estrogen and hormones anti-Müller hormone and dihydrotestosterone, followed by qPCR analysis. Cell culture showed the addition of oestrogen caused a change in the expression of the miRNA, and we aim to expand further our understanding with the treatment of other hormones and ESR2 inhibitors.

Chromatin immunoprecipitation of embryonic (15.5) mice brain of estrogen revealed ESR2 is binding near the regulatory region of miRNAs previously identified as sexually dimorphic in the mouse brain. Males had high fold enrichment in ESR2 and low ESR2 enrichment in the females. This suggests ESR2 plays a role in regulating these miRNAs. Currently, we are working to determine if estrogen and its co-activators, FOXA2 and NCOA1, are found bound with ESR2 at the enhancer regions of the miRNA of interest. This will demonstrate if ESR2 is either repressing or enhancing gene expression. The combination of these experiments helps us expand our knowledge of oestrogen's role in regulating sexually dimorphic miRNA in the embryonic mouse brain. Understanding this link can help us to understand how sex differences in neurological disorders arise.

Using a zebrafish stem cell model to understand the molecular basis of Cornelia de Lange Syndrome.

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Cornelia de Lange Syndrome (CdLS) is a multisystem disorder caused by germline mutations in cohesin subunit genes. Cohesin genes have important non-overlapping roles in cell division and gene expression. CdLS is likely caused by interplay of both the cell division and gene expression roles; however, their individual contribution is not understood. Our aim was to investigate cell division and gene expression in cohesin deficiency to better understand the molecular pathology of CdLS.

Cohesin subunit Rad21 is essential for cell division, whereas cohesin subunit Stag2 controls gene expression without affecting cell division. We used zebrafish embryos with mutations in *rad21* and *stag2* to investigate the consequences for stem cell fate choice. Multipotent cells in the embryonic tailbud, called neuromesodermal progenitors (NMPs), form mesoderm or neurons by pausing cell division and switching on lineage genes. We performed RNA-sequencing of wild type, *stag2* and *rad21* mutant tailbuds (4 replicates of tailbud pools per condition).

Thousands of genes were dysregulated in tailbuds of both *stag2* and *rad21* mutants. Genes downregulated in *rad21nz171* were strongly cell cycle associated. Moreover, *rad21* mutants had increased expression of NMP cell markers but decreased expression of lineage genes. In *stag2bnz207* mutants, genes of the Wnt signalling pathway were upregulated. Increased Wnt signalling was confirmed by confocal fluorescence microscopy. Moreover, *stag2* mutants increased expression of genes involved in actin cytoskeleton organisation and muscle formation. Genes expressed in endothelial cells were reduced *stag2* mutants. We suggest that *rad21* mutation disrupts the cell cycle of NMPs which in turn leads to the cells failing to differentiate. Muscle forms when Wnt signalling is high, while endothelial cells form when Wnt signalling is low. Therefore, transcriptional dysregulation upon *stag2* mutation might alter cell fate choice through dysregulation of Wnt signalling.

Using *Xenopus laevis* to evaluate two novel epilepsy variants

Cabriana Earl – BSc (Hons) (Genetics) Project
Supervised by A/P Caroline Beck

Developmental and epileptic encephalopathies (DEEs) are a rare, heterogeneous collection of severe seizure disorders encompassing developmental deficiencies with a high prevalence for de novo mutation. Here, *X. laevis* was used as a model system for replicating DEE-associated variants. Separate genetic variants from two Aotearoa patients were investigated via the use of *X. laevis* tadpoles, utilising the literature-supported seizure associated swimming phenotype as an indicator for genetic contributions to the patients DEE. Microinjections to induce replications of these patient mutations were utilised at the 1-cell stage of embryos, allowing tadpoles to develop with these genetic changes. In terms of injections, two methods were explored; using mRNA to simulate a gain-of-function phenotype for a *de novo CACNA1D* variant, or CRISPR/Cas9 knockdown to create a loss-of-function phenotype to investigate the role of *AP3B2*. These showed differing results; giving insight into the function of the *AP3B2* gene in epileptic behaviour, while outlining the importance of expanding on behavioural phenotype in terms of the *CACNA1D* variant.

Cognitive abilities at behaviours, brain and genetic levels

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Based on the US National Institute of Mental Health's Research Domain Criteria (RDoC), cognitive abilities are one of the major transdiagnostic domains across psychiatric disorders. RDoC stipulates that cognitive abilities should not be studied as a unitary construct, but should rather be studied through different units of analysis, from behaviours to the brain and genes. To empirically model this, we leveraged the unique power of the large-scale, longitudinal data from the Adolescent Brain Cognitive Development (ABCD) study (n ~ 11 k in children 9- 10 years old). Following RDoC's integrative approach, we captured cognitive abilities at behaviours, brain and genetic levels, through cognitive tasks, neuroimaging (resting-state fMRI, structural MRI and DTI) and polygenetic scores, respectively. More specifically, we treated (1) the brain as a mediator, (2) the behaviour as the dependent variable, and (3) genetics along with sociodemographic and psychological factors as independent variables. We found that the brain accounted for variance in behavioural performance of cognitive abilities due to (1) genetic variation (proportion mediated = 15.6% [11%–20.7%]) and (2) key socio-demographic and psychological factors (proportion mediated = 18.65% [17.29%–20.12%]). Thus, our work supports the RDoC's Framework in integrating cognitive abilities at behaviours, brain and genetic levels and paves the way to use cognitive abilities at different units of analysis to understand psychopathology, trans-diagnostically.

Successful modelling of a single nucleotide polymorphism in cells and mice using CRISPR-Cas9 and CRISPR-Cas12 methodologies

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The single nucleotide polymorphism (SNP) rs1800795 resides within the interleukin-6 (IL-6) gene promoter and alters IL6 transcription1. The SNP has reported associations for multiple pathologies, including infections, inflammatory diseases, and the risk of various cancers. Importantly, the SNP roughly trisects the population and so has high value for clinical stratification of patients. Recently, we found that rs1800795 genotype also associates with disease recurrence in colorectal cancer (CRC) and so may have value as a prognostic biomarker in that malignancy as well as others. The advent of CRISPR-Cas9 based technologies allows SNPs to be rapidly modelled and directly studied. The IL6 gene promoter is highly conserved between humans and mice with only minor differences in and around the rs1800795 SNP. But unfortunately, a difference did alter PAM site availability. We first established protocols based on Cas9 for cell lines of both species, but then moved to a Cas12 strategy to improve success rates for mouse cells. At the same time, we first attempted to generate genetically modified mice using Cas9, and then shifted later to Cas12 for that application as well. Success rates and additional unwanted modifications were mirrored between our cell line and animal work. We now have multiple useful modified cell lines and two different versions of rs1800795 mice. The animals display clear alterations in IL-6 regulation, and phenotypes that match the reported human associations for the SNP. The mice are powerful pre-clinical models for studying the prognostic and treatment response biomarker value of rs1800795, and reinforce the value of whole animal CRISPR approaches to studying SNP effects.

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Investigating *ELAVL2* – a novel neurodevelopmental disorder disease gene.

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Neurodevelopment is a highly complex process which requires tight regulation. Disruption can result in neurodevelopmental disorders (NDDs) such as autism, developmental delay and epilepsy. Despite extensive investigation into NDDs, the underlying genetic causes can often be difficult to identify. Through international collaborations and databases, we have collated a cohort of nine patients that present with neurodevelopmental phenotypes and have de novo variants in *ELAVL2*. ELAVL2 is an RNA-binding protein implicated in regulation of RNA metabolism, particularly in the brain and is yet to be associated with NDDs. My PhD aims to determine whether the variants identified in our patient cohort are responsible for the observed NDD. It also aims to shed light on the exact function of ELAVL2 in the cell and how this may be disrupted in our patients.

Currently, four truncating variants cause a null allele, confirming that haploinsufficiency for *ELAVL2* is the likely disease mechanism, however the remaining missense variants require further molecular investigation. We hypothesise that these variants are acting in a haploinsufficient manner, resulting in the disruption of downstream processes, ultimately disrupting neurodevelopment. To determine the pathogenicity of these variants, protein stability assays and western immunoblotting is underway. RNA-seq and RIP-seq analyses will also be undertaken to gain insight into the consequences at the cellular level as a result of ELAVL2 haploinsufficiency. To date, we have identified *ELAVL2* as a novel NDD gene. Further investigation is ongoing to prove the pathogenicity of all identified variants, as well as elucidate the function of ELAVL2.

Posters:

1. Identification and Development of Novel Drug Combinations for Diffuse Gastric Cancer

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Gastric cancer is the third most lethal and fifth most common cancer worldwide. Diffuse gastric cancer (DGC), often has a strong genetic aspect, characterised by mutation of the tumour suppressor gene E-cadherin (*CDH1*). Inactivation of *CDH1* causes hereditary DGC (HDGC) with 70% penetrance. Dasatinib, an ATP-competitive tyrosine kinase inhibitor has recently been identified as a novel therapeutic candidate for the chemoprevention of HDGC. Dasatinib targets pathways that are upregulated in *CDH1*-null cells, providing specificity for diseased cells. To improve this targeted therapy, we aimed to identify synergistic partners of dasatinib. These are other compounds that when combined with xdasatinib, increase its negative effects on *CDH1*-null cells.

Using large, publicly available gastric cancer gene expression and drug sensitivity datasets, we compared gene expression patterns between cell lines that were susceptible and resistant to dasatinib, to identify differentially expressed genes. Overrepresentation analysis was carried out on this gene list to identify those pathways targeted by dasatinib. These were clustered based on their gene composition, and combined with drug sensitivity data to identify FDA-approved compounds that have similar targets to dasatinib, providing a list of candidate synergistic drugs. These drugs were tested on a cell line model, singularly and in combination with dasatinib, and analysed to identify synergistic pairings.

Ten compounds were selected for validation from the in silico results: these targeted a variety of the dasatinib-associated pathways, primarily those involved in extracellular matrix interactions and maintenance, along with PI3K-AKT signalling and ErbB family activity. EGFR inhibitors gefitinib and AZD3759, as well as *PI3K/mTOR* inhibitor AZD8055 and acetalax, which may act via AMPK/mTOR signalling, provided evidence of a synergistic relationship with dasatinib. These will be explored further in the development of dasatinib-based therapies for *CDH1*-null DGC. The methods developed here will be applied to other mutations that cause hereditary forms of gastric cancer.

2. ***Development of a gene silencing platform using the type I-D CRISPR-Cas system***

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CRISPR-Cas systems provide prokaryotes with adaptive immunity against bacteriophages and plasmids. These systems feature two genetic components – a Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR) array, and CRISPR-associated (cas) genes. The CRISPR array encodes CRISPR RNAs (crRNAs) that associate with Cas proteins to form ribonucleoprotein interference complexes. The crRNA portion guides the complex to bind complementary target sites in invading genomes, which are subsequently degraded by Cas nucleases. In recent years, diverse CRISPR-Cas systems have been repurposed into sequence-specific DNA and RNA-targeting biotechnologies that have revolutionized molecular biology and precision medicine. Several CRISPR-Cas effectors have been developed as CRISPR interference (CRISPRi) tools for targeted gene silencing. CRISPRi complexes consist of a crRNA guide and nuclease-deficient Cas proteins. CRISPRi complexes inhibit gene expression by sterically blocking RNA polymerase from transcribing the target gene. The type I-D system is a genetic and structural hybrid of two different CRISPR-Cas systems and possesses the unique ability to bind DNA and RNA. We expressed the type I-D crRNA and cas genes from the cyanobacteria *Synechocystis* sp. PCC6803 in a heterologous *Escherichia coli* host. The type I-D complex was repurposed into a CRISPRi platform that elicited targeted silencing of a plasmid-borne fluorescent reporter gene. Further in vivo studies of the chimeric type I-D complex will elucidate further nucleic acid-targeting capabilities that can be used to improve CRISPR interference activity.

3. Phage-derived antimicrobials against kiwifruit pathogen *Pseudomonas syringae* pv. *actinidiae*

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Horticultural diseases caused by bacterial pathogens provide a consistent obstacle to crop production globally. The 2010 outbreak of the gram-negative kiwifruit phytopathogen *Pseudomonas syringae* pv. *actinidiae* (*Psa*) in New Zealand caused an estimated >$1 billion impact on the NZ economy[1]. Agrichemical sprays including copper and antibiotics have been used to manage *Psa* to date[2]. The rising levels of bacterial resistance requires the development of new antimicrobials, particularly specific, environmentally friendly, sustainable solutions. Bacteriophages – viruses that specifically infect and kill bacteria – are one option as biocontrol agents. An alternative phage-derived biocontrol strategy is the use of endolysins, lytic enzymes produced by phages at the end of their replication cycle. These enzymes lyse bacterial cells from the 'inside out' by degrading the peptidoglycan (PG) of the cell wall[3]. Exogenous application of lysins has shown antimicrobial potential against gram-positive pathogens[4]. However, the additional outer membrane (OM) of gram-negative bacteria provides an impermeable barrier and prevents endolysins from accessing the PG. To overcome the OM, we propose the fusion of other phage proteins to endolysins to generate phage-inspired antimicrobial enzymes that we term 'Phagezymes'. Libraries of variant proteins were created using the DNA shuffling method, VersaTile5, to 'mix-and-match' phage 'parts' for screening of antimicrobial potential against *Psa*. We will present our current work into the use of phage proteins as alternative antimicrobials.

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4. Unraveling the mechanisms involved in the co-regulation of breast cancer associated genes at the 6q25.1 locus

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Oestrogen receptor positive breast cancer is the most common diagnosed cancer in women worldwide. Oestrogens are steroid hormones which promote growth, proliferation and differentiation in both healthy and malignant breast tissue (Saha Roy & Vadlamudi, 2012). In breast cancer, normal ER signalling is lost (Chi *et al.*, 2019). Reprogramming of the oestrogen regulated network appears to play an important role in neoplastic transformation, tumour progression and the development of endocrine resistance (Achinger-Kawecka *et al.,* 2020). The oestrogen receptor (ER) is encoded by the *ESR1* gene on chromosome 6q. A noncoding variant associated with genetic breast cancer susceptibility, upstream of *ESR1*, was identified by Stacey *et al.* in 2010 (Stacey *et al.,* 2010). The rs77275268 variant results in a C to T base-shift within a binding site of the CCCTC binding factor (CTCF). CTCF is a methylation-sensitive transcription factor and is thought to play an important role in genome architecture. An aberration in a CTCF binding site or a change in the methylation status of that site may led to alternate chromatin loops and dysregulated gene expression.

The expression of *ESR1* in ER positive tumours is highly correlated with the expression of three upstream genes which encode, *ARMT1*, *CCDC170* and *RMND1*, and this appears to be regulated at a transcriptional level (Dunbier *et al.,* 2011). We hypothesis that the rs77275268 variant could alter the three-dimensional organization of these correlated genes, which may contribute to genetic breast cancer susceptibility.

To investigate if the C to T variant alters the binding of CTCF we have performed bisulfite sequencing to assess the methylation status of the SNP site and CpG islands within the ROI. Results indicate that there is a complete loss of methylation at the SNP site in CRISPR-edited MCF7 cells. We have subsequently performed Cut&Run sequencing to establish if the loss of methylation at this site results in a change in CTCF binding. Preliminary results support this hypothesis suggesting that the loss of CTCF binding within the ROI could affect the expression of the aforementioned co-regulated genes.

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5. Discovering the Genetic Aetiology of a Rare Gastrointestinal Disorder Affecting a New Zealand Family
Kam Salt

Chronic intestinal pseudo-obstruction (CIPO) is a rare disease defined by recurrent intestinal dysmotility without a mechanical cause. Phenotypes are often severe and variable, with the disorder having heterogeneous associations.

A New Zealand family with four brothers congruent for severe intestinal dysmotility have been diagnosed with a presentation of CIPO with a suspected but unknown genetic cause. Previous attempts to formally diagnose the disorder using whole exome sequencing have failed to establish this suspected genetic aetiology. This project attempted to establish the genetic aetiology using whole genome sequence data of each individual family member through modification and optimisation of the bioinformatic pipelines used for variant curation. Whilst a candidate variant was not identified and classified to pathogenic mechanisms, the results increased our confidence of what genetic mechanism are likely to be involved.

This project initially used a modified but standard variant curation pipeline, and after it yielded limited findings, a non-parametric linkage analysis was designed. Whilst this also failed to curate a pathogenic variant in the timeline of this project, with further modification and refinement this approach could potentially overcome some of the limitations facing rare disease diagnosis. This identified a future approach that with improvements could be repeated and potentially capture the pathogenic variant responsible for this familial presentation of CIPO.

6. Sometimes it is okay not to open up, if you are a fungus
Finn Dobbie

Many fungi disperse their spores via the wind, however there are a few that use a more interesting strategy for spore dispersal. Truffle-like fungi have evolved, convergently, from many wind-dispersed fungi in many different countries. They have a notable ball-like fruiting body that requires their spores to be spread by non-wind related means, and have unique secondary metabolites, and a longer lasting fruiting body to assist in this task.

New Zealand is host to many unrelated truffle-like fungi, which are unique in that they are more brightly colored than those found in other countries. There are likely many unique genetic features that contribute to the brightness, longevity and ball-like appearance of these fungi. But this has not been investigated, until now.

By comparing the genome of the New Zealand truffle-like fungus, Leratiomyces erythrocephalus, with a closely related wind dispersed fungus, Leratiomyces ceres, and other wind-dispersed/truffle-like fungi. Novel candidate genes have been identified that, when removed, may reveal some answers as to how this unique fungus came to be.

7. Class II HDACs, an antiviral toolbox in Influenza A virus infection

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Introduction

Influenza A virus (IAV) is a primordial acute respiratory illness[1]. IAV interacts with a plethora of host factors in each stage of its life cycle[2]. Host acetylation plays a crucial role during IAV infection and different classes of the host histone deacetylases (HDACs) are central in negatively regulation. Recent findings exemplified that HDAC4 and 6 of class II have an antiviral role in IAV infection[3,4]. The class II contains four other, HDAC 5, 7, 9 and 10. We hypothesized that the rest of Class II [a (5,7,9) and b (10)] HDAC (RoCIIH) will have an antiviral role in IAV infection. We also delineate the mechanism involved in HDAC6 anti-IAV property.

Methods

RNAi and plasmid transfection were used to deplete and ectopically expression in mammalian system. The mRNA and polypeptide levels were determined through quantitative real-time PCR and western blotting. RNA-Seq performed on HDAC6 depleted cells using novaseq6000.

Results

We observed that IAV replication was elevated in depleted cells and plummeting in supplemented cells. HDAC10 ectopically expressed cells showed increased expression of pSTAT, IFITM3, ISG15, MX1 and viperin. In turn, IAV strongly antagonized RoCIIH at the mRNA and polypeptide level (HDAC7 & HDAC10) via IAV vRdRp (PA-X, PA, PB1 and PB2) in a time, dose, strain independent and cell line dependent manner by inducing host caspase (Cas3, 6 and 7) in infected cells. Global transcriptome analysis on HDAC6 depleted cells revealed the anti-IAV mechanism the host uses to delimit IAV growth.

Conclusion

These results demonstrate the potent anti-viral role of RoCIIH and could be promising factors in the anti-influenza arsenal. RoCIIH uses the host-innate response as defense and restricts virus growth. IAVinduced global host transcriptome reveals the mechanism involved in HDAC6 directed anti-IAV mechanism. This knowledge will allow HDACs to be evaluated as potential anti-influenza virus targets.

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8. Effects of sex on vertebral development and the function of a nearby Pax1 regulatory region

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Adolescent idiopathic scoliosis (AIS), a 3-dimensional curvature of the spine with unknown aetiology exhibits a severe sex bias, with 9 out of 10 severe cases occurring in females. GWAS studies have identified a SNP (rs6137473) near the *PAX1* gene as significantly associated with AIS risk in females. Using gene editing, a mouse model was generated carrying a deletion of this *Pax1*-associated enhancer.

Micro-CT analysis showed a significant increase in vertebral rotational degree at T4 in the Pax1EHΔ**-/-** mice; however, when stratified by sex, it can be seen that this increase in vertebral rotation in the mid-thoracic region is due to the female Pax1EHΔ**-/-** mice which saw a significant increase in rotation. IVD grading results supported our findings with female Pax1EHΔ**-/-** mice having higher degenerative scores in the mid-thoracic region while RNA-sequencing data showed significant downregulation of genes critical in cartilage development, maintenance and survival in both Pax1EHΔ**-/-** mice including *Col2a1, Acan, IL-10, Prg4* and *XYLT1*.

The current results indicate knockout of a *Pax1*-associated enhancer, linked to AIS in humans, results in poor cartilage development and maintenance, disc degeneration and rotational curvature in females.

9. Genetic/genomic resources for biogeographic research in terrestrial Antarctica

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Genetic and genomic data can provide powerful insights into the evolutionary processes that have shaped – and will shape in the future – spatial patterns of biodiversity. Biogeographic research requires data from many different locations, yet the challenges of working in the Antarctic mean that most studies only include a limited number of sites and samples, and often only one or a few genetic markers are sequenced. As a result, much of Antarctica's biodiversity has yet to be discovered.

Biogeographic studies that bring together disparate data sets can maximise our capacity to infer key processes. Data from all genetic and genomic studies are normally required, on publication, to be made freely and publicly available, although in some cases data are not made available with essential metadata. Here, we present a meta-analysis of Antarctic terrestrial species using pre-existing genetic and genomic data from hundreds of studies over recent decades. We highlight the taxonomic groups and regional locations that are well- versus poorly represented in the data, as well as the most common genetic markers used across studies. We examined diversity patterns and phylogenetic relationships for key markers and groups, enabling us to carry out spatial environmental analyses to reveal the likely drivers of biodiversity patterns in Antarctica

11. Single-cell RNA sequencing reveals potential synthetic lethal targets for chemoprevention of hereditary diffuse gastric cancer

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CDH1 is a tumour suppressor gene encoding Epithelial cadherin, a protein responsible for cell-cell adhesion, polarity and differentiation in epithelial tissues. Heterozygous germline mutations in CDH1 are an established cause of hereditary diffuse gastric cancer (HDGC). HDGC confers up to a 70% lifetime risk of diffuse gastric cancer as well as a 40% lifetime risk of lobular breast cancer; for which there are limited, invasive, preventative measures. Tumorigenesis is initiated upon somatic loss of the second *CDH1* allele.

This project aims to identify genes which have a synthetic lethal (SL) relationship with CDH1; revealing cellular vulnerabilities specific to CDH1 deficient cells. These could be promising drug targets for chemoprevention and treatment of HDGC, as well as treatment of tumours lacking CDH1. Our lab has established a murine organoid model of HDGC, derived from mice with a cre-lox inducible CDH1 knockout to study these SL interactions.

Using single cell RNA sequencing (scRNA-seq) of non-cancerous organoids, with wild-type CDH1 (n=2) and an induced knock-out of CDH1 (n=2) we have characterized their cellular composition and performed differential expression analyses on normalized count data to compare cell-type specific gene expression between conditions. This identified 19 genes which appear to have a SL relationship with CDH1 and could be involved in tumorigenesis of HDGC. Of these, 9 were shown to be upregulated in diffuse gastric cancer samples from a recently published scRNA-seq dataset (GSE150290).

Further investigations aim to identify transcriptomic changes in stem cells and cancer-associated fibroblasts within the GSE150290 dataset and assess their role in development of HDGC as potential chemopreventative targets. Pathways involving promising genes from these analyses will be targeted with existing drugs in cell lines and our organoid model to assess their viability as a chemopreventative agent.

12. What's your poison? Differential transcriptomic responses to diverse stressors in the Lake Baikal sponge *Lubomirskia baikalensis*

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Lake Baikal is the oldest and deepest freshwater environment on earth. Despite its status as a World Heritage site, its unique ecosystem is now under a variety of threats, both from directly anthropogenic pressures (climate change and pollution)[1], and downstream consequences of these (disease)[2]. One of the keystone species for this ecosystem is *Lubomirskia baikalensis*, but to date there is little data regarding how this species can adapt to the threats facing it. To address this issue, we have sampled independent *L.baikalensis* from a variety of conditions (control "normal" samples direct from wild habitat, specimens suffering from Brown Rot Syndrome, those under thermal stress, some exposed to nitrate pollution, and treatment controls).

A reference transcriptome was assembled *de novo*. Assays of completeness (BUSCO) indicate a well-assembled dataset, with in excess of 95% of the expected metazoan and eukaryotic gene complement. Differential expression analysis (edgeR) of individual conditions revealed highly variable gene expression between *L. baikalensis* samples. Comparison of transcriptional profiles showed that while thermal stress had less impact, disease and pollution dramatically changed the transcriptional profile of sponges compared to natural conditions. These results suggest that *L. baikalensis* may be resilient to thermal changes, which reflects their broad tolerance in the wild. Our results will inform conservation of these key species, which are foundational for the Baikal ecosystem, and provide a raft of information on the means by which sponges can adjust to a range of external stresses, and the extent to which these adjustments share a common transcriptional cassette.

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2. Khanaev IV , Kravtsova LS , Maikova OO , Bukshuk NA , Sakirko MV , Kulakova NV , Butina TV , Nebesnykh IA , Belikov SI. 2018. *Current state of the sponge fauna (Porifera: Lubomirskiidae) of Lake Baikal: sponge disease and the problem of conservation of diversity*. J Great Lakes Res . 44 1 :77 –85.

13. Improving read accuracy for ctDNA diagnostics

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The flexibility of next-generation sequencing (NGS) makes it an attractive option for early-stage diagnosis of diseases such as cancer. The Oxford Nanopore Technologies (ONT) minION device is a small, portable real-time sequencer that has the potential to be set up in community healthcare clinics, making it ideal for point-of-care testing. Recently, ONT released a short fragment mode feature, making it potentially tractable for circulating tumour DNA (ctDNA) diagnostics. However, to detect low abundance ctDNA based on somatic variants, extremely sensitive and accurate sequencing methods are needed. Currently, the ONT platform still has a high sequencing error-rate relative to other NGS platforms and thus requires an increase in basecalling accuracy for applications such as ctDNA mutation detection.
One potential mechanism for improved basecalling accuracy is to generate consensus calls through the use of Unqiue Molecular Identifiers (UMIs) ligated to distinct ctDNA fragments, to evaluate this approach, we used the ONT platform to sequence an Illumina ctDNA library from a 21-gene targeted cancer panel applied to a gastric cancer sample. ONT adapters were ligated to the Illumina library, and the sample was sequenced using short fragment mode on a minION sequencer. We tested a dilution range of ctDNA amount (5-40ng) to establish the optimal amount of input sample. UMIs were also included for each DNA molecule, thereby allowing us to perform consensus basecalling, and assess the potential for applying this to somatic variant detection in ctDNA samples.

14. Exploiting genetic interactions to design improved therapeutic regimens for *Mycobacterium tuberculosis*

Cassandra Chapman

Mycobacterium tuberculosis is an obligate human pathogen that remains a leading global cause of infectious disease morbidity and mortality. In 2020, *M. tuberculosis* was responsible for 1.5 million deaths and 10 million new infections. Whilst drug susceptible strains of *M. tuberculosis* can be treated with a combination of four antibiotics for six months, toxicity issues and treatment noncompliance have driven to the emergence of drug resistant (DR) *M. tuberculosis* strains that have rendered many antibiotics ineffective and account for one in four deaths associated with antimicrobial resistance. Treatment options for drug-resistant M. tuberculosis are woefully inadequate with long treatment times (>18 months) and low cure rates. Combination antibiotic regimens are favoured for their ability to increase efficacy, delay the development of resistance and reduce toxic side effects. Favourable interactions are typically, the result of either (I) therapeutic synergy, when the effect of drug combinations on bacterial growth is greater than the sum of the individual drugs, or (II) synthetically lethal interactions, when the individual drugs alone are non-lethal, whilst the combination results in killing. A better understanding of the interactions between antibiotics, will allow for the design of drug combinations that rapidly sterilize both drug-susceptible and drug-resistant *M. tuberculosis* to reduce treatment times and improve therapeutic outcomes.

15. Investigating the role of immunotherapy in the treatment of metastatic oestrogen receptor positive breast cancer

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Approximately 80% of breast cancer diagnoses present as oestrogen receptor-positive (ER+), with ~10% of these patients developing metastatic ER+ breast cancer (mBC). The estimatedfive-year survival rate of mBC is between 20 – 30% and is incurable with current treatments. Within the past decade, a rapid increase in the number of promising new therapies, including immunotherapies, has fuelled hope that effective treatment regimens may be identified. However, the almost impossibly large number of potential therapy combinations means that relevant pre-clinical models are required to assess which will be effective in mBC. This research aims to develop a syngeneic mouse model of mBC and investigate the use of antioestrogen therapies in combination with immunotherapies in mBC.

Trackable, ER+ breast cancer-like SSM3 cells were developed, making it possible to visualise tumour growth with bioluminescence in the syngeneic 129S6/SvEv background. Fluorescently labelled, antibiotic selectable, sleeping beauty expression vectors encoding Antares2 or Firefly luciferase were developed and introduced to SSM3 cells via electroporation mediated transfection. Luciferase expression was confirmed through fluorescent microscopy and *in vitro* protein lysate and whole-cell bioluminescence assays. Tagged SSM3 cells were then introduced to the mice by mammary fat pad or tail vein (IV) injection. Mammary fat pad injections of SSM3-Luc2, developed into primary tumours with clear luciferase activity. Following primary tumour removal via mastectomy, the mice were imaged to monitor metastasis. Seven days following IV delivery of SSM3-Luc2 cells, luciferase signal can be detected in the chest of mice. Further analysis, including immunohistochemistry and RNAseq will be utilised to determine immunological and genomic landscapes of the metastatic tumours. This will be the first biologically relevant preclinical model of ER+ mBC in mice with fully intact immune systems. Continued development of this model will allow immunotherapies in combination with anti-hormone therapies to be evaluated as a new therapeutic options for metastatic ER+ breast cancer.

16. Novel *CASC5* intronic splicing variant in three siblings with primary microcephaly

Bridget Fellows, Karen Knapp, Louise Bicknell
Department of Biochemistry

Microcephaly primary hereditary (MCPH) is an autosomal recessive neurodevelopmental disorder characterised by head circumference of at least two standard deviations below the mean for age and sex. MCPH is caused by disruption of essential genes in the mitotic pathway, including those encoding centrosome and spindle pole localising proteins. Homozygous splicing variants in the essential kinetochore gene *CASC5* have recently been identified as a cause of MCPH. CASC5 is the central component of the KNL1-MIS12-NSL1 (KMN) network which acts as the signalling hub of the kinetochore and is required for correct chromosomal segregation during mitosis.

We have identified biallelic *CASC5* variants in three siblings from a non-consanguineous family with microcephaly and moderate-to-severe intellectual disability. The three siblings share a frameshift variant predicted to prematurely truncate the transcript and prevent protein expression, and an intronic single nucleotide variant (SNV) predicted to disrupt splicing. An in vitro splicing assay revealed the canonical transcript is not produced by the variant allele and instead an exonic splicing enhancer (ESE) is activated producing a transcript which skips *CASC5* exon 23. As *CASC5* exon 23 is an inframe exon, this alternative transcript is predicted to produce CASC5 protein lacking the RWD domain, which mediates a key interaction within the KMN network and would likely destabilise the kinetochore signalling hub and disrupt mitosis.

20. Fast and accurate prediction of protein thermal stability from gene sequences

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We wish to select genes that encode proteins of value for biotechnology from metagenomes. There are often many candidates with a particular enzymatic function, so we would like to choose genes that are easy to express, soluble and stable. Surprisingly, we were able to develop quite an accurate method (PPV=0.95) to predict proteins that are stable over 50 degrees Celcius from gene sequences. We derived a set of 20 thermal stability indices (TSI), one for each amino acid. Then we applied a simple metric based on mean-TSI of proteins. Other sequence-only and scalable methods are being developed and compared to the current one. We are now applying this mean-TSI metric to proteins with specific value in biotechnology.

21. Type III CRISPR–Cas provides resistance against nucleus-forming jumbo phages via abortive infection

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Bacteria harbor multiple lines of defense against bacteriophages, including the adaptive CRISPR-Cas immune response. In response, some jumbo (>200 kb genome) phages protect their DNA behind nucleus-like structures. We previously demonstrated that RNA-targeting type III CRISPR–Cas systems provide jumbo phage immunity by recognising viral mRNA exported from the nucleus for translation. Here, we demonstrate that jumbo phage PCH45 targeting by the type III-A system of *Serratia* sp. ATCC 39006 results in cA3 production that activates an accessory endonuclease, NucC. Although phage DNA remains largely protected throughout infection, NucC activation leads to preferential degradation of host DNA, what triggers cell death and disrupts phage replication and maturation. Hence, type III-mediated jumbo phage immunity occurs via abortive infection with suppression of the viral epidemic protecting the population. We further show that type III systems targeting jumbo phages have diverse accessory nucleases, including RNases that provide immunity. Our study demonstrates how type III CRISPR–Cas systems overcome the inaccessible nature of jumbo phage DNA to provide robust immunity. Nucleus-forming jumbo phages are excellent candidates for phage-based therapies against bacterial infections in clinical settings for their ability to protect their genomes. However, their vulnerability to type III immunity means we must find natural – or develop engineered – phages capable of evading RNA-targeting systems to increase the therapeutic potential of jumbo phages.

22. Pathogen hunters: Using ancient DNA to authenticate the presence of *Mycobacterium tuberculosis* and other microbes in early Polynesian settlers of Aotearoa New Zealand

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Historically, it was believed that tuberculosis was brought to Aotearoa New Zealand in the 18-19th Centuries by European voyagers, but pre-European skeletal evidence consistent with tuberculosis challenges this theory. A new hypothesis suggests that tuberculosis may have been introduced by seals and sealions, and subsequently jumped host.

This poster outlines the methods and results of our study, that uses advanced ancient DNA technologies to detect *Mycobacterium tuberculosis complex species* and other microbes in the people from Wairau Bar (~750 BP). While we could not confirm the presence of *M. tuberculosis spp.*, we did detect several ancient oral microbiome species.

23. CasRx as a suitable tool for plant biology research

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Supervisor: Lynette Brownfield

To determine the roles of specific genes involved in plants, specific knockdown and mRNA localisation could potentially be performed using the CRISPR/Cas13 system. Recently the CRISPR/Cas13 system has been identified to be able to target single-stranded RNA for cleavage leading to gene silencing, and for mRNA localisation in animals (using an inactivated version of Cas13 fused with a fluorescent protein) (Cao *et al.,* 2021; Burmistrz *et al.,* 2020). Cas13 has shown vast potential within different animal models, but its usefulness for mRNA knockdown and mRNA localisation has not been well explored in plant systems (Mahas *et al.,* 2019; Cao *et al.,* 2021).

Due to pollen having thick cell walls, it is difficult to identify key components involved in asymmetric division and cell-fate specification involved in plants, making it difficult understanding how pollen development works in flowering plants. The CRISPR/Cas13 system could be used to understand the importance of specific genes involved in pollen development and the role they have in regulating it. It can also be used to characterise and validate potential genes thought to be involved in pollen development.
Preliminary results will be shown focusing on optimising the CRISPR/Cas13 system for stable transformation in *Arabidopsis thaliana* and transient transformation in *Nicotiana benthiana*.

24. Characterizing a Non-Heading Phenotype Found in the Ryegrass Cultivar 'Array'

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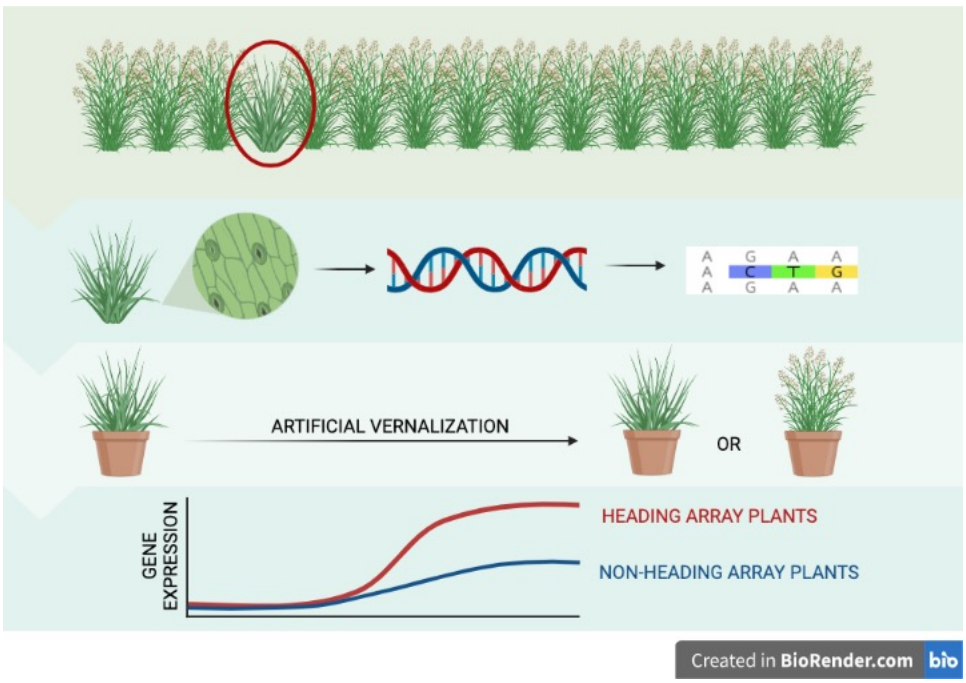
Ryegrass is New Zealand's primary forage crop, but animal production decreases when ryegrass heads (flowers) in spring. Heading is regulated by vernalization, which occurs during the cold winter months. Ryegrass genes in the vernalization pathway respond to environmental cues to control if and when ryegrass heads. Genetic variation in these genes can produce stricter vernalization requirements. To maintain agricultural productivity year-round, there is ongoing research to develop a cultivar of ryegrass that does not head under field conditions but can be induced to head for seed production by taking advantage of genetic variation in the vernalization genes.

The seed producing company Barenbrug is currently developing a cultivar called Array. In the summer of 2020/21, Barenbrug breeders noticed that approximately 1 in 500 Array plants did not head after a normal vernalization. It was hypothesized that a recessive homozygous mutation was responsible for the non-heading phenotype. This project aimed to characterize:

- genetic variation in heading and non-heading Array plants
- gene expression during vernalization in heading and non-heading Array plants
- the heading phenotypes of heading and non-heading Array plants after artificial and natural vernalizations

Targeted and genome-wide sequencing did not reveal any regions of homozygosity just in the non-heading plants, and there was no difference in gene expression levels of key vernalization genes. After a second natural vernalization, genetically identical Array plants had different heading phenotypes. It was concluded that weak vernalization signals in combination with complex genetic backgrounds preventing heading in some Array plants.



25.
Catie Wylie

Unlike most flowering plant species, kiwifruit (*Actinidia* species) are dioicous having separate male and female plants. Female kiwifruit flowers contain ovaries which develop into fruit (the commercial product) and produce non-viable pollen. On the other hand, male kiwifruit flowers produce viable pollen to fertilize the eggs of female kiwifruit but have no ovary and therefore unknown fruit genetics.

Recently the primary kiwifruit pollen fertility gene was identified as *Friendly Boy* (*FrBy*) which is only inherited by male kiwifruit plants. A functional homolog of *FrBy,* has also been identified in *A. thaliana* which also has a significant effect on male fertility and pollen development. Both kiwifruit and *A. thaliana* FrBy homologs have been previously predicted to be part of the Fasciclin-like arabinogalactan protein (FLA) family of extracellular glycoproteins predicted to be important for signalling and structural functions.

To test the biological function of FrBy, *A. thaliana* overexpression models were developed. When *A. thaliana* plants are transformed with the *A. thaliana FrBy* homologue under the high expression constitutive *UBQ14* promoter, the plants lacked developed seed pods indicating they failed to self-fertilise. These plants also lack typical fluffy pollen grains indicating male sterility. Using light microscopy, it has been found that these plants make pollen that gets aborted during the later stages of development. This is a very similar phenotype to the pollen made by female kiwifruit making these overexpression lines a valuable tool for studying female kiwifruit pollen development.

27. Fast and scalable prediction of protein-protein interactions

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Almost all cellular processes require protein-protein interactions. Common interaction types include binding (e.g., haemoglobin assembly), post-translational modifications (e.g., phosphorylation), and catalysis (e.g., ubiquitylation). However, existing prediction tools do not take these interaction types into account and do not scale well on proteome-wide prediction. In this study, we show that a random forest classifier trained on per-residue physicochemical and biochemical properties is useful for predicting protein-protein interactions. Interestingly, we find that training random forests by individual interaction types improves accuracy. Furthermore, a combination of these specialised classifiers improves generalisability. Our tool LazyPair outperforms the state-of-the-art in accuracy, generalisability and scalability. LazyPair is freely available at https://tisigner.com/lazypair/

28. Investigating transposable element derived genes in pre-eclampsia

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Pre-eclampsia (PE) is a dangerous pregnancy condition that presents around 20 weeks gestation and is associated with abnormal placental development. It is characterised by shallow invasion of placental trophoblast cells and poor remodelling of maternal spiral arteries. PE occurs in approximately 5% of pregnancies worldwide, however the underlying molecular mechanisms are not well understood. Interestingly, transposable elements (TEs) make up ~50% of the human genome and have created new genes that play important roles in placental development. To date, the role of TE-derived genes has not been explored in PE, and our preliminary data suggests TEs may be involved in PE.

RepExpress, our bioinformatic pipeline, was used to identify differentially expressed TE-derived genes in PE. The expression of these candidate genes has been validated in two independent PE cohorts by RT-qPCR. Functional assays will be carried out in a placental stem cell line to evaluate the role of TE-derived genes.

Five publicly available RNA-seq datasets enabled discovery of differentially expressed TE-derived genes in PE. Some TE-derived genes have shown expression differences between two PE cohorts. Genetic knockdown will be carried out in a human placental cell line to investigate the role on trophoblast differentiation.

Examining the expression profiles of TE-derived genes and their potential role(s) in placental development may improve our understanding of the abnormal placentation that occurs in preeclampsia. These genes may also be useful in the development of novel therapies or diagnostics. This research aims to help improve mothers' and perinatal health during pregnancy.

29. Transcriptome analysis of Cellaria immersa (Phylum: Bryozoa) provides insights into marine invertebrate biomineralization and consequences of climatic changes

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One of the most salient features of marine bryozoans is their well-calcified skeleton, and many species in this phylum are important reef-builders. To date, the molecular machinery responsible for skeletal formation in these key animals, and how it will be affected by environmental stressors, remains unknown. In this study we performed de novo transcriptome assembly from erect articulated *Cellaria immersa* colonies collected in New Zealand. *Cellaria* species form erect, heavily calcified arborescent colonies which when abundant can create micro forests or meadows on the ocean floor.

RNA was extracted separately from younger distal and older proximal parts of 10 colonies, aiming to identify the key genes involved in biomineralization. Differential expression analysis was carried out to identify genes that are expressed at a higher rate in distal and proximal parts of the colonies. The assembly resulted in a set of 21,219 transcripts. Of the proteins translated from these, 11,148 had predicted function when tested for protein similarity against a range of databases using Interproscan. Over 50 proteins were identified as candidates involved in the biomineralization process and skeletal resorption in *C. immersa*. Many represent structural proteins, transmembrane transport channels and enzymes known to be involved in skeletal processes in other metazoans. This is the first such study on a heavily calcified species from the phylum Bryozoa, significantly increasing the amount of 'omic data available for *C. immersa* and the phylum. Identifying and cataloguing this 'biomineralization toolkit' is critical for understanding the potential effects of environmental stressors and climatic changes on these vital ecosystem engineers.