QMB Epigenetics Abstracts

Q1: Gene-environment interactions and epigenetic modulators of cognitive and affective function in mouse models

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Huntington's disease (HD) is one of over 40 tandem repeat disorders and involves a triad of psychiatric, cognitive and motor symptoms. In a transgenic mouse model of HD we have shown that expansion of the CAG tandem repeat encoding a polyglutamine tract of the mutant huntingtin protein leads to a spatiotemporally specific cascade of molecular, cellular and behavioural abnormalities. We have also demonstrated that environmental enrichment can delay onset of the affective (depression-like), cognitive and motor endophenotypes. These findings have been extended to included stress and stress hormone (glucocorticoid) manipulation in HD mice, and environmental manipulations in other mouse models of cognitive disorders, including schizophrenia. Most recently, we have discovered that gut microbiota are altered at an early stage of HD pathogenesis. We are pursuing this first evidence of gut dysbiosis in HD using a variety of approaches including metagenomics and metabolomics. This will inform both pathogenic mechanisms and novel therapeutic targets. These approaches may also facilitate the development of 'enviromimetics' for a variety of brain disorders known to be modulated by environmental stimuli. We have also explored the transgenerational effects of paternal environmental exposures. Our findings reveal significant experience-dependent effects on cognitive and affective function of offspring via intergenerational and transgenerational epigenetic inheritance. We are exploring the impact of specific environmental and pharmacological factors, including exercise and stress hormone elevation, and the relevance of these discoveries in mice to human transgenerational epigenetics and associated 'epigenopathy'.

Q2: DNA Methylation Changes in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome

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Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is a severely disabling and lifelong disease that currently affects around 20,000 people in New Zealand. There is currently no molecular diagnosis or clear understanding of the etiology or disease pathophysiology. Research has shown multisystemic dysruptions affecting patients indicating a 'hypometabolic syndrome'. DNA methyation is a common epigenetic modification that can affect the expression of genes such as those involved in metabolism. This research investigates whether changes in DNA methylation can explain the uncertain onset and sustained progression of ME/CFS. DNA was extracted from the PBMCs of 10 patients and matched age/gender controls from a Dunedin ME/CFS cohort that we have characterised comprehensively for changes in different molecules in plasma and immune cells. The methylation status of the subjects were interrogated by Reduced Representation Bisulfite Sequencing (RRBS). Two pipelines (DMAP and Methyl kit) were used to analyse the methylation data generated across the genome by RRBS. DMAP analysis identified 775 differentially methylated fragments ($P < 0.05$, Difference 5+) in the ME/CFS patients compared to controls; 390 within gene regions and 540 within promoter regions. MethylKit analysis found 282 differently methylated individual cytosines ($Q < 0.05$, Difference 5+) and 253 fell within gene regions and 94 within promoters. Pathway analysis identified affected functional pathways for metabolic functions and immune related functions, suggesting an explanation for the 'hypometabolic state' in ME/CFS, and as well the documented immune dysfunction. This first study investigating the methylation state of ME/CFS patients with RRBS enables a precision medicine approach to better understand the disease pathophysiology and the impact of ME/CFS at a personal level.

Q3: Alterations in the Cancer Epigenome

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A three-dimensional chromatin state underpins the structural and functional basis of the genome by bringing regulatory elements and genes into close spatial proximity to ensure proper, cell-type specific gene expression profiles. However it is still unclear if the epigenome is involved in shaping the three-dimensional (3D) chromatin architecture and how this is altered in cancer. We performed Whole Genome Bisulphite Sequencing (WGBS), ChIP-seq, Hi-C chromosome conformation capture and replication timing sequencing to investigate how 3D chromatin organization in relation to the epigenome is disrupted in cancer. To our surprise we find a direct relationship between chromatin modifiying enzymes and the pattern of DNA methylation at CpG island promoters. In addition we reveal a conserved class of CTCF sites that are important in the maintenance of chromatin structure and gene expression. Moreover our study provides new insights into the relationship between replication timing, long-range epigenetic deregulation and changes in higher-order chromatin interactions in cancer.

Q4: Long non-coding RNAs as regulators of gene expression in cancer

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Recent genome-wide studies revealed that 1-2% of the human genome encodes for proteins, while as much as 50% of the genome can be transcribed. Of these "non-coding" transcripts, long non-coding RNAs (lncRNAs) represent the largest and most diverse class. LncRNAs can be spliced and polyadenylated, lack a significant open reading frame, and are expressed in a tissue-specific manner. They have been implicated as regulatory molecules in a variety of cellular functions, including epigenetic gene regulation, splicing, mRNA stability and translation. However, a detailed molecular mechanism is lacking for most lncRNAs.

We previously identified 30 potentially oncogenic lncRNAs in breast cancer, termed *Mammary Tumour Associated RNAs* (*MaTAR*s). To functionally validate the role of *MaTAR*s, we performed knockdown experiments and observed reduced cell proliferation, invasion and/or organoid branching in a cancer-specific context. One of the identified lncRNAs, *hMaTAR17*, is over-expressed in several different types of cancer compared to normal tissue. Notably, injection of antisense oligonucleotides targeting *MaTAR17* into a transgenic mouse model of breast cancer resulted in a significant decrease of tumour size, and increased tumour differentiation. We generated loss-of-function cell lines using CRISPR/Cas9 genome editing, and were able to reproduce the reduced proliferative potential both *in vitro* and *in vivo*.

Ongoing studies to investigate the molecular mechanism by which *hMaTAR17* acts include RNA-seq, single molecule RNA-FISH and Chromatin Isolation by RNA Purification in breast and colorectal cancer cells. Our results suggest that this lncRNA is likely an important driver of mammary tumour progression, and represents a promising new therapeutic target in cancer.

Q5: Creodes and cancer

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C. H. Waddington is best remembered for his contributions to epigenetics, but he also wrote on the relationship between art (especially painting) and science in the 20th century[1]. He advocated for an intensified dialogue between the two, convinced that it would increase our understanding of the natural world. Waddington's articulation of the epigenetic landscape is a prime example of this[2]. The concepts depicted and explained therein often required the appropriation of existing words for new purposes or the creation of entirely new words. For example, he coined the word "creode" to describe a property of the embryo whereby it returns to its normal developmental trajectory when disturbed by external forces. His thinking encouraged those working on genetics, evolution and development to reach across disciplinary boundaries. Importantly, he helped to reframe the important questions for biology, providing fertile ground for the development of epigenetics. Nearly 80 years later, there is now extensive molecular evidence supporting these concepts. This is all the more impressive given that the structure of genes was not known at the time.

Of interest to us today is not just the content of his science but how he integrated information into the bigger picture, and how this integration helps to frame better questions. In our lab, we are investigating the role that *TET2* mutations play in myeloid leukaemia, as well as the impact of neutrophil oxidants on methylation in lymphocytes and cancer cells. Drawing examples from these projects, this paper will put some of the language and ideas presented in *The Nature of Life*[3] in dialogue with our work.

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3. Waddington, C. H. (1961). *The Nature of Life*. London : George, Allen, & Unwin.

Q6: Expression of TETs in epithelial cells across the menstrual cycle

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Ten Eleven Translocation Proteins (TET) mediate DNA hydroxymethylation, an epigenetic mechanism that is known to activate gene expression and potentially critical for endometrial health. Previously, our data demonstrated significant and dynamic changes in the mRNA expression of TETs across the menstrual cycle. The aim of this study is to localise TET proteins and determine the hormonal regulation of TETs in the endometrium.

Endometrial tissues were obtained after informed written consent from women with normal cycles. TETs were localised using colorimetric immunohistochemistry. To determine the hormonal regulation of TETs, HES cells (endometrial epithelial cell-line) were first treated with estrogen for 24hours and subsequently treated with a combination of estrogen and progesterone for 24, 48 and 72hours, in vitro. RNA was extracted and TET gene expression was determined using real-time PCR.

Data suggest, nuclear localisation of TET proteins in epithelial and stromal cells, throughout the cycle. Strong immunostaining of TET1 and 2 were observed in the glandular epithelium, during proliferative, early and mid-secretory phases which reduced during the late-secretory phase. Conversely, TET3 immunostaining was the highest during proliferative phase, then reduced during the mid to late-secretory phases. Strong universal staining was seen throughout the glandular and luminal epithelium. In contrast, staining in the stroma was not universal with immunostaining present in some cells and absent in others throughout the stroma. TET 1 and TET 3 transcriptions in HES cells were up-regulated in response to combined treatment of estrogen and progesterone for 72hours compared to control. While, TET2 transcription was the highest with the combined treatment for 48hours.

Our data imply that TETs are expressed in a cell-specific and dynamic manner in the endometrium and they are responsive to varying levels of estrogen and progesterone. Further investigations are underway to elucidate their role and interaction with other epigenetic machineries in the endometrium.

Q7: Pseudouridine in small RNAs

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RNA is made up of four key nucleotides, cytosine, guanine, uridine and adenosine. Pseudouridine (psi) is an isomer of uridine and is the most abundant modified nucleotide in RNA, garnering it the nickname "the 5th nucleoside". Historically, psi was thought to be only present in structural RNAs such as tRNA, rRNA and snRNA; however, new techniques have allowed sensitive identification of psi in a genome-wide manner.

We hypothesised that psi would be present in small RNAs from *Arabidopsis thaliana*, given their similarities to tRNAs in terms of processing and nuclear export, as well as interactions between AGO proteins and pseudouridylation machinery. To test this hypothesis we developed methods to detect psi in small RNAs in high- and low-throughput manners using chemical treatment with CMC. Using these techniques on inflorescence and pollen tissue, we were able to detect psi in small RNAs, particularly a unique class of pollen small RNA called epigenetically-activated siRNA or "easiRNA". The function of psi in easiRNA is currently unknown, although a number of hypotheses are being investigated.

Q8: RNA interference in replication and quiescence

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Heterochromatin comprises tightly compacted repetitive regions of eukaryotic chromosomes and has widespread roles in chromosome integrity and epigenetic inheritance. The inheritance of heterochromatin requires RNA interference (RNAi), which guides modification of histones deposited on daughter strands upon DNA replication, and is required for chromosome segregation. In *S. pombe* pericentromeric heterochromatin the alternating arrangement of origins of replication and non-coding RNA transcribed during S phase provokes the collision of RNA polymerase with the replication machinery. We propose that collisions are resolved by co-transcriptional RNAi, which releases Pol II, allows replication to complete, and couples the spreading of heterochromatin with fork progression. In the absence of RNAi, stalled forks accumulate damage and are repaired by homologous recombination without histone modification, and Pol II accumulates at the 3' end of highly transcribed genes, and, surprisingly, at rRNA and tRNA genes as well as in heterochromatin. Recently, we have found that RNAi mutants (*Δdcr1*, *Δrdp1*, *Δago1*) are impaired in the ability to enter quiescence and lose viability rapidly in G0. Suppressor screens have uncovered genes involved in transcription, heterochromatin and chromosome segregation. We propose that Dicer releases RNA polymerase I from rDNA in quiescent cells, and failure to release results in heterochromatinization and DNA damage. Dicer knockouts in mouse embryonic stem cells (ESC) also have extensive DNA damage and chromosome segregation defects. CRISPR-based suppressor and enhancer screens have revealed some of the same chromatin modifiers discovered in *S. pombe* suppressor screens, and indicate that dicer mutant ESC have characteristics of both dividing and quiescent cells.

Q9: Pre-trained for climate change

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Climate change is an accepted issue that needs to be addressed. Climate modelling shows that over the next 20-50 years, considerable changes to crop growing practises and locations will need to change, with breeding programmes needing to keep pace.

The potato is considered one of our major food crops that will be impacted by climate change. Commercial potato crops are clonal, being grown from seed tubers. Seed tubers are susceptible to external environmental influences during growth which can have a major impact on yield. As such, changes in plant environment through water availability pose a considerable threat to the potato industry.

Given the above, we asked the question: Can we pre-train potato cultivars to handle more extreme drought situations, and is this change retained in the emerging seed potato, pre-arming tubers for drought conditions?

We will present our initial findings of drought impact on three potato genotypes, including preliminary single-nucleotide resolution methylation findings through whole-genome bisulfite sequencing.

Q10: Epigenetic regulation of transposon bursts in plant genomes

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Mobile genetic elements or transposons (TEs) are important components of all genomes. In higher eukaryotes, they occupy from 20% and over 80% of genome space depending on species. Their biology is characterised by their ability to contribute to new genetic diversity (through formation of new insertion loci, contribution of cis-regulatory units to existing promoter regions, and exon shuffling) and alteration of the epigenetic landscape of the genome in response to their transcriptional activation.

There has, therefore, been a continuing debate around the role of TEs in genomes; are they solely genomic parasites? Or have they been exapted to provide genetic and epigenetic plasticity in response to environmental queues? Aside from the obvious evolutionary question as to why genomes retain such mutagenic potential, we have begun to explore the potential to utilise the biology of TEs in plant species to both generate new genetic diversity and to explore the role of TEs in modulating epigenetic responses within the genome that contribute to economically important phenotypes

Grapevine (*Vitis vinifera*) is an ideal model system in which to study transposon activation biology due to its small diploid genome (~512 MBp) and the extensive clonal propagation of the crop in which we have observed evidence of considerable TE activity – some of which has been shown to contribute to the formation of new varietals. Using a combination of whole genome bisulphite sequencing, small RNA sequencing, Illumina short read RNA-seq and ONT long read RNA-seq, and with the development of new bioinformatics pipelines we have sought to determine which TE containing loci represent transcriptionally active autonomous TEs and the epigenetic conditions at these loci that contribute to their potential to contribute to a burst of transposition.

Our model system has focused on somatic embryogenic cell cultures of grapevine in which we have determined that there is a global shift in methylation where on one hand there is a decrease in CHG methylation while at the same time there is an increase both in transposon transcription and the associated increase in small RNAs and small RNA derived CHH methylation. Application of a range of biotic and abiotic stressors to these cultures combined with extensive deep RNA-sequencing of the transcriptome has allowed us to determine that only a very small portion (<0.15%) of TE loci may be able to transpose. In addition it appears that these loci are found almost exclusively in the introns of expressed genes, indicating a link between the epigenetic state of the genome and the ability of TEs to initiate and complete their life cycle despite clear targeted silencing of these same loci.

We will present data on the epigenetic state of grapevine cell cultures, and the results of our deep mining of the TE transcriptome using both Illumina and ONT long read cDNA sequencing, which has allowed, for the first time, identification of individual TE containing loci contribute to TE transcription.

Q11: The histone deacetylation inhibitor 4-phenylbutyric acid elevates transcriptional activation of transposable elements in grapevine (*Vitis vinifera*) embryogenic callus

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Mobilization of transposable elements (TEs) is an endogenous source of mutagenesis with major impacts on host genome integrity and evolution. Although TEs are implicated in diseases such as cancer, TE mobilization might sometimes benefit host genomes by enhancing genetic diversity. To proliferate throughout the host genome, TEs harness host's transcriptional and translational machinery to generate proteins that catalyse transposition processes. Consequently host genome has evolved epigenetic surveillance systems targeting multiple stages of TE's life cycle to prevent or minimise TE mobility and therefore mutagenic load. However, successful transposition can be achieved with biotic or abiotic stimuli[1,2]. Furthermore, compromising various components of the epigenetic signalling pathway can lead to an increase in the efficiency of TE mobilization. Arabidopsis epigenetic recombinant inbred lines deficient in METHYLTRANSFERASE 1 (*met1*) or DECREASED DNA METHYLATION 1 (*ddm1*) demonstrated a wide range of TE activation[3,4], whereas pharmacological inhibition of DNA methylation and RNA Pol II activity respectively by zebularine and α-amanitin, also results in exaggerated stress-dependent TE mobilization[5]. It is likely that along with direct inhibition of transcription of TEs and transcript degradation, these interventions prevent the formation of methylation marks that signal modification of the heterochromatin at these sites further repressing transcription of the underlying DNA. We therefore hypothesised that pharmacological inhibition of the formation of heterochromatin should also lead to a transcriptionally permissive state for transposon activation. Histone deacetylation inhibitors (HDACi) can suppress chromatin deacetylation thus lead to the transition of chromatin state from compact to relaxed structure[6,7]. Here we applied two HDACi Trichostatin A (TSA) and 4-phenylbutyric acid (4PBA) on wound-treated *Pinot noir* embryogenic callus with series of time-points up to 72 hours. Within this time frame, a TE transcription storm indicated by an increased number of significantly up-regulated TE loci, including an increase of potential origins of autonomous LTR-TE transcriptions, was observed in 4PBA but not TSA treated cells. The increase in TE transcriptional activity was not only shown in the growth of active individual loci in TE families (e.g. Copia-3 and Copia-23) previously found to be responsive to wound and biotic stressors, but also demonstrated in the broadened range of active TE families. These findings suggest that suppression of the maintenance of compact chromatin structure, commonly associated with TEs, can give rise to TE de-repression. Extending from this, a combination of HDACi (e.g. 4PBA) and biotic or abiotic stimuli may efficiently increase the frequency of TE mobilization and thus enrich endogenous mutations in response to specific environmental queues.

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Q12: An overview of the temporary and persistent epigenetic changes that accompany somatic embryogenesis in grapevine

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Transposable elements (TEs) are an essential source of genetic and epigenetic diversity on an evolutionary scale. In eukaryotic genomes they play key roles in genome architecture and establishing gene regulatory networks. Plants, which typically carry high TE loads in their genomes, have a germline that segregates multiple times from somatic tissue. The reversible nature of TE-silencing enables plants to inhibit their mutagenicity by default, while potentially exploiting it for occasional stress adaptation. This offers the potential that artificially intervening in the silencing of endogenous TEs could allow access to the future evolutionary potential of a plant genome.

Grapevine, with its small genome and history of clonal propagation offers a useful model in which to push the boundaries of genome plasticity. We have used somatic embryogenesis to reduce the likelihood of chimeric somaclones, but have found that this tissue also has reduced of cytosine methylation across TEs that are co-located with genes. Furthermore, unlike vegetative tissue, TE transcripts are greatly increased in these cells when exposed to biotic stressors. This burst of TE activity is accompanied by the accumulation of TE-specific siRNAs and RNA-dependent DNA methylation of TEs across the genome.

Interestingly, chemical demethylation of the genome using 5-Azacytidine prior to embryogenesis was found to have a persistent effect on the epigenome of regenerated vines. In contrast to what has been reported in other species, the genomes of these vines demonstrate a moderate but widespread hypermethylation compared with plants regenerated from untreated embryogenic callus three years post-treatment. We are now exploring the dynamics of the epigenetic changes associated with stress response in callus and tissue differentiation following embryogenesis.

Q13: Characterization and hormonal regulation of histone deacetylases in human endometrium

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Epigenetic regulation plays a crucial role in normal and aberrant endometrial development. Histone acetylation is generally associated with gene activation. Histone acetylation is regulated by histone acetyl transferases (HATs) and histone deacetylases (HDACs, which include sirtuins (SIRTs). Previous study from our lab has demonstrated that global histone acetylation changes in the endometrium, correlates with the expected transcriptional activity during the menstrual cycle. However, the expression and regulation of HDACs in the endometrium have not been fully elucidated. The aim of our study is to characterize the gene and protein expressions, and hormonal regulation of HDACs in human endometrium during the menstrual cycle. Normal endometrial tissues were obtained from pre-menopausal women and gene expression patterns were determined using real-time PCR. Study of protein expression patterns using western blot analysis is currently underway. Gene expression profile of HDACs shows significant temporal changes in many of the HDACs across the menstrual cycle. Transcription of the majority of HDACs were significantly up-regulated during the early secretory phase compared to other phases in the endometrium. These include HDAC1, HDAC4, HDAC7, HDAC9, HDAC11, SIRT1, SIRT5 and SIRT7. Only SIRT6 transcription was up-regulated during the mid-secretory phase, while the gene expressions of the remaining HDACs did not show any significant changes in the endometrium during the menstrual cycle. Observed gene expression changes correlate well with the global histone acetylation changes in the endometrium during the menstrual cycle. The results imply that HDACs can be involved in the regulation of endometrial gene expression during the menstrual cycle and that abnormal HDAC expressions may be associated with endometrial pathologies. Studies are underway to determine the exact functional significance of these expression patterns in the endometrium.

Q14: Epigenomic remodelling in trained immunity

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The adaptive immune system develops memory following specific antigen exposure, but is the same true for the innate immune system? An emerging field of research, called 'trained immunity (TRIM)', has described how innate immune cells, such as monocytes and macrophages, form non-specific memory in response to a variety of exogenous signals. Exposure-induced epigenetic remodelling governs their future response to a range of pathogens. This process can be modelled in vitro, using both yeast and bacterial antigens and metabolites (Novakovic et al. Cell 2016), metabolites (Bekkering et al. Cell 2018), vaccines (Arts at el. Cell Host Microbe 2018) and a range of other stimuli. In vivo, the hyper-responsive TRIM phenotype is observed in allergy (Neeland et al. JACI 2018) and obesity (Bekkering et al. Cell Metabolism 2019). By applying a multi-omics approach on monocytes exposed to a range of TRIM-inducing stimuli, we identify a pan-TRIM molecular signature and the transcriptional regulators that play a role in the establishment of this signature.

Q15: Imputing environmental exposures from DNA methylation

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The epigenome provides a mechanism for an organism to adapt to its environment. Changes to the epigenome – in particular, DNA methylation – have been observed in response to a range of environmental exposures. Through accurate studies of the effects of environmental exposure on DNA methylation, we are able to in turn use an individual's DNA methylation to predict historic environmental exposures. These predictors have potential uses across a range of fields including medicine, foresenics, and epidemiology.

Methylome-wide association studies have been preformed using cohort studies for a range of course environmental exposures. These studies have identified regions of epigenetic change caused by the environment and found some of these changes can remain stable for a long period post exposure. Using these results, highly accurate DNA methylation based predictors have been contructed for smoking and age. Building predictors for other medically relevant environmental expsoures is limited due to the extensive confounding across exposures and smaller effects on DNA methylation.

Q16: Understanding the relationship between maternal tobacco smoking and offspring conduct disorder: are metastable epialleles present?

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Metastable epialleles (MEs) are described as loci at which epigenetic regulation is established during development and maintained throughout life. Consequently, individuals can have the same genetic sequence, yet their epigenetic regulation of the underlying sequence can vary. This variation can be induced by environmental exposures. For instance, we know that maternal tobacco smoking during pregnancy can alter offspring DNA methylation. Thus, there is potential for MEs to be induced in developing human offspring in response to maternal tobacco smoking during pregnancy. Furthermore, associations between maternal smoking and offspring conduct disorder has been observed. However, currently, we do not know what links these associations. We wish to provide a molecular link between maternal smoking and later life outcomes of the offspring.

A cohort will be sub-selected from the Christchurch Health and Development Study, a longitudinal cohort of children born in Christchurch in 1977. These will consist of: those exposed *in utero* who are now non-smoking adults, those exposed *in utero* who reported as being a smoker as an adult and individuals who were not exposed to smoking *in utero* who are non-smoking adults. Bisulfite-based amplicon sequencing (BSAS) was used to investigate DNA methylation differences and potential MEs between the different groups. We identified permanent DNA methylation differences in the genomes of offspring exposed to tobacco smoke *in utero*, that associated with their conduct disorder phenotype.

This research implicates epigenetic mechanisms, specifically DNA methylation, in the aetiology of the observed link between maternal smoking and childhood/adolescent conduct disorder, which could provide new insights into the mechanisms involved in the detrimental outcomes associated with *in utero* tobacco smoke exposure.

Q17: Inferring cancer population history from a single-cell sequencing DNA methylation data

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DNA methylation is an epigenetic change in which cytosine is modified to methylcytosine. If this change occurs within a promoter region, this causes silencing of a particular gene. This process has been found to play an important role in carcinogenesis[1]. As DNA methylation changes are usually more frequent than genetic changes, they have also been a target of considerable interest in attempts to reconstruct the development and progression of cancer. However, most methods based on DNA methylation data do not use phylodynamic models. Instead, clustering approaches are used, which can reconstruct general patterns, but not a detailed model-based history of cancer spread.

Kingman's coalescent theory[2] allows quick and efficient reconstruction of population history but assumes a constant well-mixed population with no internal structure or migration. To account for this, coalescent theory can be extended to the structured coalescent[3] to allow population structure and migration. Recent progress in the structured coalescent approach enables efficient simulation of migration between multiple populations of interest[4]. However, current implementations allow only for constant population, which is unsuitable for modelling the spread of rapidly-growing populations of cancer cells.

This can be alleviated by extending the structured coalescent to allow nonparametric models of population size change through time, thus allowing sudden changes in the population dynamics to be uncovered, such as a metastatic development or the start of cancer treatment.

In this work, we extend the structured coalescent approach to estimate population changes during various stages of cancer development and introduce a novel substitution model for DNA methylation data that explicitly account for changes on a DNA level. We then demonstrate these methods by reconstructing a demographic history of cancer from a single-cell sequencing data and compare them to a standard approach that does not account for an internal structure of cancer cell populations within the body.

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Q18: Shared regulatory pathways reveal novel genetic correlations between grip strength and neuromuscular disorders

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Pathological muscle weakness can develop during the course of aging or due to a range of neuromuscular diseases. Genome-wide association studies have identified single nucleotide polymorphisms (SNPs) associated with grip strength (GS; an inverse measure of age-related decline of muscle strength) and neuromuscular disorders (multiple sclerosis (MS), myasthenia gravis (MG) and amyotrophic lateral sclerosis (ALS)). However, little is known about the functional roles of these SNPs and how or if they contribute to the comorbidity between muscle weakness caused by aging and that caused by neuromuscular disorders.

We combined chromatin interaction data (Hi- C)[1] and functional expression quantitative trait loci (eQTL)[2] data using a bioinformatics algorithm (CoDeS3D)[3] to identify genes that are spatially regulated by SNPs (*i.e*. eQTL- eGene pairs) associated with GS, MS, MG and ALS. Biological pathways enriched with these spatial eGenes were identified using pathway enrichment analyses and the drug-gene interaction database were used to identify those eGenes that have the property of druggability.

None of the eQTLs associated with GS, MG, MS and ALS were shared but we identified eGenes that are commonly regulated by these eQTLs. Particularly, GS eQTLs share eGenes with all three disease-associated eQTLs indicating shared gene regulatory mechanisms between GS and neuromuscular diseases causing muscle weakness. Furthermore, pathway analysis revealed 24 pathways shared between GS and MG, 70 pathways between GS and MS and 18 pathways between GS and ALS. Strikingly, three pathways: mTOR signaling pathway, axon guidance pathway, and alcoholism were identified to be shared by all four phenotypes. A few eGenes implicated in these shared pathways were identified as druggable. Collectively, these findings identify significant biological overlap between age- (GS) and disease-related (MG, MS and ALS) muscle weakness, demonstrating the utility of spatial genetic analysis for the identification of potential therapeutic targets and mechanisms underlying multimorbidity.

Q19: The Humble Guinea Pig: Cute, Furry and Deserving of a Better Genome Assembly?

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For centuries the domestic Guinea pig has been used as an experimental animal, thus the term 'Guinea pig' was coined. Their utility as a platform to study whole system integrated physiology remains integral to advances in our understanding of health and disease. Unlike other laboratory species they share many key physiological similarities with humans, especially with respect to pregnancy and newborn development, and the regulation of fundamental cardio-metabolic, immune and inflammatory pathways.

The new wave of ultra-long-read next generation sequencing is making it a reality to resolve repeats and close gaps in genome assembly. While improvements in base-calling software for this data continue, higher error rates are still an issue. To this end the accuracy of more 'traditional' shorter read technology provides a useful means with which to error correct and infer accurate genomic variation.

We will provide a high-quality reference genome utilising hybrid-assembly of both Nanopore (PromethION) and Illumina (X10) data. We performed long-read sequencing on 3 outbred females to generate longer scaffolds, complementing this with Illumina sequencing on the same 3 females as well as 3 additional males. This short-read data will be used to polish the long-read scaffolds and generate a comprehensive map of genomic variation. An additional benefit to long-read sequencing is the detection of base modifications, i.e. cytosine methylation. Establishing the Guinea pig methylome will improved the framework for epigenetic research in this animal model.

We present our work towards a more complete reference genome for the laboratory Guinea pig and demonstrate the value of lower coverage long-read sequencing in improving both genome assembly contiguity and completeness. In addition we will profile our initial methylome findings, highlighting these alongside more traditional RRBS data. We believe these findings and datasets will be an invaluable molecular resource and further support the translational strength of this animal model.

Q20: Hi-C detects novel structural variants in HL-60 and HL-60/S4 cell lines

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Cancer cell lines often have large structural variants (SVs) that evolve over time. There are many reported differences in large scale SVs between HL-60 and HL-60/S4, two cell lines derived from the same acute myeloid leukemia sample. However, the stability and variability of inter- and intra-chromosomal structural variants between different sources of the same cell line is unknown. Hi-C and RNA-seq are often used to study gene regulation, but can also be exploited to study the linear genome architecture as well. The high contact frequency of linearly proximal loci can identify genomic rearrangements in Hi-C heatmaps, and RNA-seq read pairs spanning breakpoints can identify putative gene fusions. Using the two methods in combination can improve sensitivity and specificity of SV detection. hic_breakfinder and STAR-fusion were used to identify breakpoints in Hi-C and RNA-seq data respectively, and characterisation of complex and multi-chromosome SVs was performed by visual inspection of Hi-C heatmaps. Here, we used Hi-C and RNA-seq to identify and compare large SVs in HL-60 and HL-60/S4 cell lines. Comparisons with previously published karyotypes identified novel SVs in both cell lines. Hi-C was used to characterise the known expansion centered on the MYC locus. The MYC expansion was integrated into known locations in HL-60/S4, and a novel location (chr4) in HL-60. The HL-60 cell line has more within-line structural variation than the HL-60/S4 derivative cell line. Collectively we demonstrate the usefulness of Hi-C and with RNA-seq data for the identification and characterisation of SVs.

Q21: Epigenetic impact of cannabis use in the Christchurch Health and Development Study

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The New Zealand Parliament recently passed a Medicinal Cannabis bill, effectively legalising the use of cannabis for terminally ill people, and is considering a referendum on law changes concerning recreational cannabis use in light of the recommendation to decriminalise personal use of cannabis. The Christchurch Health and Development study (CHDS), a birth cohort of 1265 Christchurch children born in 1977, provides evidence of harms caused by the prohibition of cannabis. However the CHDS also shows that cannabis use is associated with negative psychosocial outcomes, most strongly in youth (<18 years).

There is clear evidence that environment factors, including the use of cannabis and tobacco alters epigenetic marks across the genome. Hence we explored the association of cannabis use with methylation in the DNA from purified peripheral blood of heavy long term cannabis smokers, long term cannabis and tobacco smokers and matched controls who smoked neither cannabis nor tobacco.

We confirmed the most differentially sites in tobacco and cannabis users were hypomethylation in the AHRR and F2RL3 genes (adjusted P $3x10^{-6}$, 0.002 respectively) replicating previous studies into the epigenetic effects of tobacco. Cannabis users who did not use tobacco had no evidence of differential methylation at any sites in these genes (all adjusted P>0.97). There was no differential methylation in cannabis only users at the epi-genome wide significance level ($P<10^{-8}$). However there were multiple sites differentially methylated at more than 1% beta value at a nominal significance of P<0.001 in 218 genes. Pathway analysis of these genes involved neuronal development and neural signalling.

We conclude that the effects of cannabis use on the mature human methylome differ from the effects of tobacco use. We did not identify any methylation loci associated with exclusive cannabis use at the epi-genome wide level of significance. However loci differentially methylated at nominal significance levels were enriched in pathways expressed in the brain, consistent with psychosocial phenotypes associated with cannabis use in the CHDS cohort. Further studies are required to elucidate the long term effects of cannabis use on the developing and mature human brain.

Q22: Integrating the genome organisation into mechanisms of complex diseases

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Non-communicable diseases (NCDs) commonly occur together (multimorbid), affecting 1 in 4 New Zealand adults. How does this affect the way we approach the use of genetic information to interpret a person's risk of developing a NCD? The epigenome – and in particular the 3-dimensional structure of the genome – can be used to better understand how genetic variants that are located outside of genes can contribute to NCD risk. Although the genetic variants that contribute to one's risk of developing different disorders are unique, our team has found that the genes and pathways they impact are highly overlapping. Thus the NCD multimorbidities reflect the contribution of shared biological pathways to phenotype development. Given that disease-associated genetic variants are enriched in gene control regions, we developed a computational method that identifies how genetic variants contribute to the diseases with which they are associated by genome wide association study ($p<10^{-6}$). I will discuss the genetic inter-relations between disparate phenotypes (muscle wasting, mood disorders, cognition) to provide evidence of how these findings further current understandings of multimorbid disorders. We have also begun applying risk prediction algorithms to our multimorbid models to examine the different pathways that contribute to disease, demonstrating that the onset of Type 1 diabetes emerges from the accumulation of many tissue-specific contributions to an individual's disease risk. In conclusion, the 3-dimensional structure of the genome provides a powerful framework for considering how disorders and diseases develop, and why certain disorders and diseases co-occur. Identifying common pathways involved in the development of complex diseases opens the door to novel and shared strategies to prevention and treatment.

Q23: The placental potential: harnessing insights from placental epigenetics to predict disease

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Epigenetic modifications that regulate early human placental development may inform early-life diseases such as pre-eclampsia and later-life diseases such as cancer. Pre-eclampsia is a dangerous pregnancy condition caused by abnormal placental development, requiring early-induced delivery of the placenta and baby despite fetal immaturity. Infants surviving pre-eclamptic pregnancies can have early-life complications associated with low birthweight, as well as increased risk of developing later-life hypertension and endocrine and metabolic diseases. We have identified abnormal methylation in pre-eclamptic placentas by performing reduced-representation bisulfite sequencing, targeted deep bisulfite sequencing and a cross-cohort analysis of publicly available 450K methylation data. Our goal in this project is to identify a DNA methylation signature of pre-eclampsia that can be used clinically (by circulating cell-free fetal DNA analysis) to improve health outcomes for mothers and infants.

Strikingly, the epigenetic landscape of the healthy placenta is more similar to malignant tissue than to healthy somatic tissue. Placental cells share many phenotypic similarities with cancer cells: they invade surrounding tissues to initiate angiogenesis, they exhibit control over the immune system to prevent rejection, and most intriguingly, they share an unexplained epigenetic phenomenon – they lack DNA methylation at retrotransposon sequences. Retrotransposons are usually silenced by methylation in somatic tissues to maintain genome function. Yet in the placenta, retrotransposon hypomethylation has given rise to new genes, some of which are critical for placental development and function. Surprisingly, these unique placental genes are overexpressed in many cancers, but their role in cancer is unknown. We are investigating the functional role of placental retrotransposon-derived genes in cancer. We have developed a pipeline to identify new retrotransposon-derived genes in the placenta, and will determine whether these genes drive invasion in cancer. Our research ultimately aims to harness the insights gained from placental epigenetic research to advance our evolving paradigms of human health and disease.

Q24: The jejunal methylome differs in individuals with and without type-two diabetes.

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Type-two diabetes (T2D) is a global epidemic. To date, the most effective treatment for T2D is bariatric surgery. Bariatric surgery results in improved glucose homeostasis, and changes in energy expenditure, gut hormone and metabolite secretion. Epigenetic mechanisms are involved in the interaction between the genome and the environment; they respond to changes in environmental stimuli such as those found in the gut (e.g. diet, microbes and metabolites) and include DNA methylation, histone modifications and small, non-coding RNA. Interactions between the host genome and environmental stimuli play a role in T2D, and can be mediated by epigenetic mechanisms.

Therefore, we hypothesised that there would be significant differences in the small intestine of individuals with and without T2D, including the epigenome. We performed a pilot study of 22 individuals with severe obesity and with (n=11) and without (n=11) T2D comparing the jejunal DNA methylome

Jejunal biopsies were taken at Roux en Y gastric bypass surgery, immediately snap frozen in liquid nitrogen and stored at -80°C. DNA and RNA were extracted (20mg tissue) using the Qiagen AllPrep kit. DNA methylation was assayed using RRBS. Analyses were performed using custom bash scripts, BS_seeker2, and the R statistical environment.

We observed 157 differentially methylated regions between case vs control (mean methylation difference 11%). A KEGG pathways analysis of genes in these regions identified enrichment for Insulin Signalling (FDR 3.2x10-2) and Glycolysis/Gluconeogenesis (FDR 4.4x10-2). We will present the results of this analysis.

Jejunal DNA is differentially methylated in individuals with obesity but with and without type-two diabetes. These differences are observed in regions encoding genes with roles in insulin signalling and gluconeogenesis. Such genes may present novel targets for treatment of T2D within the context of the gut.

Q25: Hypomethylation of *TXNIP* in type-two diabetes female case with Māori ancestry: a sex difference reported

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Type-two diabetes (T2D) is a non-communicable disease which can cause cardiovascular diseases, including heart attack and stroke, induce sight and kidney failure, and even lead to lower limb amputation. It affects over 420 million people worldwide, and more than 240,000 New Zealanders have been diagnosed with this condition, and a further 100,000 are not yet aware they have diabetes[1,2]. Moreover, according to the 2013/14 Tatau Kahukura: Māori health statistics report[3], female Māori (15+ years) have an estimated T2D prevalence of 4.3, which is around 2.5 times that of female New Zealanders of non-Māori ancestry, while Māori men were approximately 1.7 times more likely to have the disease. In this study, we investigated DNA methylation profiles in whole blood of 80 individuals (40 female, 40 male), with Māori ancestry. A case:control design was used whereby 20 individuals with and 20 without T2D were age- and BMI-matched for each sex. These individuals compose a subset from the New Zealand Gout Case-Control cohort. A methylation site mapped to *TXNIP*, cg19693031, was identified as the most significantly differentially methylated site overall (nominal $p = 4.35 \times 10^{-6}$). Nonetheless, when each sex was separately tested for differentially methylated sites, we observed an increased difference in methylation levels for cg19693031 within female cases (nominal $p = 2.64 \times 10^{-7}$), which was not present for males (nominal p = 0.11). *TXNIP* is involved in glucose concentrations sensitivity, results in beta cell apoptosis when overexpressed, and has been previously linked to differential methylation/gene expression in the context of type-two diabetes. Although hypomethylation of cg19693031 in individuals with T2D was previously reported in a number of studies, this is the first time a sex difference is observed for this site, and also the first analysis of this association in individuals with Māori ancestry.

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Q26: Good genes gone bad: are placental genes hijacked by cancer cells to facilitate invasion?

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The human placenta may offer novel insights into the complex molecular mechanisms that drive cancer invasion and metastasis. During early pregnancy the placenta invades into the uterus and overrides the local immune response, showing striking similarities to cancer. Many cancers reacquire an epigenetic landscape reminiscent of early developmental stages and express early developmental genes. To this end, dedifferentiation is recognised as a hallmark of cancer and there is compelling evidence to support that the path to dedifferentiation can contribute to invasion and metastasis. The placenta and cancer lack DNA methylation at some retrotransposon sequences. Demethylation of retrotransposons in the placenta has given rise to new genes and regulatory elements, some of which are critical for placental development. Surprisingly, these placental genes are also overexpressed in a number of cancers, but their function is not yet known. This project seeks to document the abundance and diversity of functional retrotransposons in the placenta and reveal the functional role of these elements in cancer. We have developed a bioinformatic pipeline to identify functional retrotransposons in the placenta. Expression of these elements has also been investigated in melanoma revealing that they are upregulated in comparison to corresponding somatic tissue. Currently we are investigating the mechanism which permits activation of these functional retrotransposons in cancer along with the functional significance. We expect that reactivation of these genes and regulatory elements occurs as a consequence of dedifferentiation associated epigenetic changes. These elements are likely to provide novel diagnostic and therapeutic targets due to their specificity to early developmental stages and cancers.

Q27: Erasing epigenetic memory: Understanding the kinetics and specificity of active DNA demethylation

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Epigenetic memory in the form of cytosine methylation is essential for vertebrate development and the formation of cellular identity. Active removal of DNA methylation by the action of the TET hydroxylase family helps enable developmental potency, both *in vivo* and during creation of induced pluripotent stem cells. Despite this, little is known about how TET proteins are targeted to DNA.

We report that mammalian TET enzymes show strong preference (>500 fold) for oxidising certain CG containing hexamers *in vitro*, and during global methylation reprogramming in cultured cells and during embryogenesis. These preferred sequences constitute recognition sites for developmental transcription factors whose binding activity is sensitive to DNA methylation. X-ray structural analysis and molecular dynamics simulations suggest that TET use indirect readout to sense the sequence context flanking CG sites.

These results are significant for understanding epigenetic reprogramming during development and have implications for synthetic biology and epigenetic editing.