Human Genome and Disease 2015: Abstracts

GD1: Targetted resequencing in gout

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Gout is very prevalent (>6%) in the Maori and Pacific (Polynesian) populations of New Zealand compared to people of European ancestry (3%). Genome-wide association studies have identified 28 loci associated with serum urate levels and risk of gout. However only a relatively small minority of loci have had likely causal genes identified. In order to identify candidate causal genes the loci were resequenced in European and Polynesian people with the highest and lowest serum urate levels, testing for a burden of rare functional variants in candidate causal genes. We also searched for Polynesian-specific variants associated with urate and gout. Candidate genes were identified by rare burden analysis, however they tended not to be replicated between the European and Polynesian sample sets. Common and rare Polynesian-specific variants were found in the ABCC4/MRP4 and ABCG2 uric acid transporters with association with gout being replicated in additional samples. While the rare burden analysis was restricted by power, the resequence data have and will continue to identify Polynesian-specific genetic variants that contribute to the increased prevalence of gout in the Maori and Pacific populations of New Zealand.

GD2: Exome sequencing identifies rare variants in a family with alcohol-induced sudden cardiac death

Hannah Kennedy[1], Kit Doudney[1], Kym Mina[4], Howard Potter[1], Chris Florkowski[1], Richard King[1], Richard McKay[1], Martin Sage[2], Wandy Chan[3], Ian Crozier[3,] Peter George[1]

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Massively parallel sequencing is rapidly being implemented as a diagnostic tool for unidentified genetic disorders segregating within families. Previously these cohorts have proved difficult to diagnose using traditional approaches including biochemical tests, Sanger sequencing, MLPA and microarray studies.

To test whether we can incorporate these sequencing technologies into our laboratory, we have performed exome sequencing in a family initially identified over 15 years ago and investigated extensively. The Caucasian family of two parents and 4 siblings (3 male, 1 female), were referred to us following the sudden death of two of the young males at 15 and 20 years of age, from cardiac arrhythmia following the consumption of very small volumes of alcohol. Post-mortem examination revealed mid-myocardial fibrosis in both boys. Following examination, the two other children also displayed cardiac fibrosis.

In this study we performed exome analysis in four living family members using an Illumina HiSeq platform. We identified two compounding non-synonymous variants in a nuclear encoded mitochondrial enzyme gene not previously associated with this phenotype. Both variants were predicted to be pathogenic based on SIFT and POLYPHEN scores.

To confirm the pathogenicity of these variants and their association with a new genetic basis of cardiomyopathy, we are undertaking functional analyses in a zebrafish model using morpholino expression knockdown and CRISPR-Cas9, and yeast functional studies. Preliminary data indicates that knockdown of the candidate gene in zebrafish leads to similar features seen in fish cardiomyopathy models, including cardiac adema and abnormal heart function.

GD3: Genomes, earthquakes and broken hearts

Martin Kennedy[1], Kit Doudney[2], Vivienne Bickley[2], Paul Bridgman[2], Vicky Cameron[1], Murray Cadzow[3], Mik Black[3], Tony Merriman[3], Klaus Lehnert[4], Allison Miller[1], Julie Zarifeh[2], Bridget Kimber[2], Peter George[2], Cameron Lacey[1]

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Amongst the devastation and tragedy of the 2010-2011 Christchurch series of major earthquakes, a remarkable opportunity arose to better understand a perplexing and serious acute cardiac illness called stress cardiomyopathy (SCM), also known as Takotsubo cardiomyopathy, or more colloquially, broken heart syndrome. The two largest earthquakes each precipitated a cluster of SCM cases (EQ-SCM) presenting to the emergency department of Christchurch hospital. Many of these patients received critical care and some required ventilatory support, but all survived. The resulting well characterised, homogenous cohort of 28 patients is unprecedented, and provides a unique opportunity to study the causes and presentation of this disorder.

The precise aetiology of stress cardiomyopathy remains unknown. Amongst the models that may be proposed for SCM aetiology, it is worth considering the possible contribution of genetic factors. We have carried out an extensive range of genetic analyses on the Christchurch SCM cohort, including exome analyses and array comparative genomic hybridisation analysis of structural variants. Although these data did not support a consistent Mendelian basis for predisposition to this condition, we discovered a surprising rate of large copy number variations of unclear clinical significance in this cohort. These findings and their possible significance to aetiology of EQ-SCM will be described.

GD4: An expression quantitative trait locus (eQTL) map for human heart

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Some genetic variants may influence susceptibility to disease by modulating gene expression. To identify functional variants for heart disease, we investigated ~50,000 genetic variants previously associated with cardiovascular traits, for association with global gene expression in heart tissue from 102 heart donors with no diagnosed heart disease and 100 heart transplant patients (explanted failed hearts). We genotyped variants associated with cardiovascular and metabolic traits with Illumina CardioMetabo Chips and generated individual gene expression profiles for each tissue sample with Affymetrix Human Gene ST 1.0 microarrays. Next, we tested associations between genotype and gene expression (expression quantitative trait loci, eQTLs) using an additive genetic model. For each gene we tested proximal (*cis*) and distal (*trans*) eQTL effects by analyzing variants <1Mb and >1Mb from the transcription start site, respectively. Finally, we adjusted our results to correct for multiple comparisons.

After quality control filtering, 54,908 variants in donors and 56,976 variants in heart transplant patients were tested for association with expression of 30,412 genes. We found evidence for *cis*-eQTL effects in ~1% of genes and evidence for *trans*-eQTL effects in 0.3% of genes, in both donors and transplant patients (adjusted $p<0.05$, effect size: 0.1-1.8). Fewer than half the genes with *cis*- or *trans*-eQTL effects were shared by donors and transplant patients. For most genes with eQTL effects, the disease-associated variant was located outside the coding region. In summary, we have identified a shortlist of candidate functional variants, mostly non-coding, that contribute to susceptibility to heart disease, with some eQTLs only apparent either in the presence or absence of disease. This study provides the first eQTL map of disease-associated variants in established heart disease.

GD5: Dysregulated splicing of a receptor tyrosine kinase disrupts osteogenesis in humans

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In contrast to the detailed understanding of the regulation of bone development that occurs within the growth plate, the growing ends of long bones, factors that regulate the thickness of the bone cortex are poorly characterised. Osteofibrous dysplasia (OFD) is a self-resolving congenital disorder of osteogenesis characterised by radiolucent lesions centered on the periosteal surface of the shafts of the tibia and fibula. The periosteum is the sheath-like lining of all bones and is responsible for the maintenance of bone cortex strength by appositional growth of bone. Although the lucencies in OFD resolve with time as cells populate the lesions and promoste ossification, the basis of the condition is unknown. We identified mutations in a gene encoding a receptor tyrosine kinase, in three families segregating an autosomal dominant form of OFD and in a fourth sporadic case. All mutations abolished the splice inclusion of an exon in transcripts resulting in receptors lacking a juxtamembrane cytoplasmic domain. Exclusion of this domain is a physiologically regulated event in the mouse periosteum and is spatially partitioned during development. Forced induction of this exon skipping event in vitro retarded osteoblastic differentiation in vitro and inhibited matrix mineralisation. Together these data indicate that the production of this receptor isoform is a developmentally regulated event during mammalian embryonic development and that mutations that render this alternative splice event constitutional subvert core functions of this receptor that regulate osteogenic functions within the periosteum.

GD6: Epigenetic regulation of genes associated with breast cancer risk and progression

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No abstract

GD7: Using splicing assays to interpret variants of unknown clinical significance in BRCA1 and BRCA2

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Routine diagnostic *BRCA1* and *BRCA2* gene screening for deleterious mutations is typically performed for individuals from suspected high-risk breast-ovarian cancer families to identify the genetic cause for their disease. However, for most women with breast and ovarian cancer, the genetic changes contributing to their disease remain poorly understood. Furthermore, many people within our population at high-risk of inherited breast/ovarian cancer remain unidentified. High-throughput sequencing technologies are being adopted by diagnostic laboratories worldwide, enabling mutation screening of *BRCA1* and *BRCA2*, and other cancer related genes in a greater number of people. Determining the clinical meaning of newly discovered genetic changes will be a central challenge facing the future of genomic medicine worldwide.

A statistical multifactorial likelihood model is the most established tool for determining the clinical significance of previously unclassified BRCA variants. However, this tool is limited by the amount of information available from the variant carriers and family members. Laboratory assays assessing the regulatory effect of an unclassified variant on mRNA splicing may contribute to classification by offering molecular evidence for the multifactorial likelihood model. We have used new targeted RNAseq and RNA *in situ* hybridisation (RNSscope) technologies to measure the expression behaviour of *BRCA1* and *BRCA2* activity in lymphoblastoid cells from unclassified variant carriers and non-carriers. Our data provide a measure of 'normal' isoform expression in LCLs, and will provide an important resource for both research and diagnostic laboratories in the interpretation of splicing assays. Moreover, we have identified isoforms that are differentially expressed in relation to genetic variants in the patient DNA. Our data demonstrate the utility of targeted RNAseq and RNSscope to evaluate the effect of genetic variants on mRNA splicing.

GD8: Patterns of placental methylation and implications for placental disease and cancer.

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The human placenta shows lower levels of DNA methylation than somatic tissues. Following fertilisation there is active demethylation of the gametic methylation, followed by remethylation in the inner cell mass and the trophoectoderm. Historical studies have implied almost complete demethylation of the early embryo and very low levels of methylation in the trophoectoderm compared to somatic tissues, but recent studies have challenged the accuracy of these assumptions.

Using reduced representation bisulphite sequencing we have compared the methylation of placentas to that of purified neutrophils (a readily available homogeneous somatic tissue). Methylation in the placenta was 22% lower than neutrophils. Since we and others have previously observed lower methylation in retrotransposon-derived genes in the placenta, and since activity of some of these genes is essential for normal placental function we hypothesised that the lower levels of DNA methylation predominantly reflect hypomethylation of retrotransposons. Unexpectedly, DNA fragments not containing retrotransposons showed similar, and even lower, levels of methylation. This observation is consistent with the evolution of hypomethylation in the placenta. The hypomethylated state of the placenta, which is observed over many clades within eutheria, and in the endosperm of plants, has repeatedly facilitated recruitment of invading retrotransposons to enhance its function.

Since methylation of the placenta is less complete than that of somatic tissues, it is plausible that its unusual control mechanisms are dysregulated in placental diseases, especially preeclampsia which reflects early failure of trophoblast function. We have preliminary evidence in support of this hypothesis.

A hallmark of cancer is genome-wide hypomethylation which is associated with genomic instability and reactivation of retrotransposons, features remarkably similar to the placenta. Because of the epigenetic parallels with cancer we propose that a thorough understanding of placenta will elucidate mechanisms of cancer onset and progession.

GD9: Analysis of paired primary and metastatic cell lines identifies common DNA methylation changes in melanoma metastasis.

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Melanoma accounts for approximately 75% of skin cancer related deaths primarily due to its highly invasive and metastatic properties. The molecular mechanisms underlying metastasis remain poorly understood, however increasing evidence suggests epigenetic events are crucial to melanoma metastasis. In our study, genome-wide methylation analysis using reduced representation bisulphite sequencing (RRBS) showed the *Early B-cell Factor 3* (*EBF3*) gene promoter was significantly hypermethylated in three metastatic melanoma cell lines compared to their matched primary melanoma cell lines derived from the same patients (Chatterjee *et al*., manuscript in preparation 2015). In contrast to the notion that hypermethylation reduces transcript expression levels, real-time qPCR analysis showed that *EBF3* expression was 27 and 680 fold increased in the two *EBF3* hypermethylated metastatic cell lines that were investigated compared to their matched primary melanoma cell lines. Functional studies using small interfering RNA transfections to knock down *EBF3* expression in the primary and metastatic melanoma cell lines demonstrated that depletion of *EBF3* reduced the melanoma cell growth capacity. In addition, depletion of *EBF3* significantly reduced the migratory potential of one melanoma cell line pair in two-dimensional wound-repair assays and Boyden chamber migration assays ($P \leq 0.001$). This is in contrast to studies that report *EBF3* as a tumor suppressor in pediatric acute myeloid leukemia and gastric carcinoma [1,2]. Altogether, our studies provide preliminary evidence that *EBF3* expression is epigenetically upregulated via the acquisition of promoter methylation in metastatic melanomas and that this change plays a contributory role to melanoma metastasis.

1 Kim, J., Min, S. Y., Lee, H. E. & Kim, W. H. *Aberrant DNA methylation and tumor suppressive activity of the EBF3 gene in gastric carcinoma*. Int J Cancer **130**, 817-826, doi:10.1002/ijc.26038 (2012).

2 Tao, Y. F. *et al. Early B-cell factor 3 (EBF3) is a novel tumor suppressor gene with promoter hypermethylation in pediatric acute myeloid leukemia.* J Exp Clin Cancer Res **34**, 4, doi:10.1186/s13046-014-0118-1 (2015).

GD10: Genetic predisposition to neuroblastoma mediated by a LMO1 super-enhancer polymorphism

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No abstract

GD11: Biobanking with Māori communities: He tangata kei tua

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The recognition of Maori rights in New Zealand has led to greater consultation and involvement of those communities in the ethical review processes for health research. Recent publications, *Guidelines for Researchers on Health Research involving Maori (2010)* and *Te Ara Tika Guidelines on Maori Research Ethics (2010),* provide a framework for understanding Maori views on health research and ethics. These documents note the heightened sensitivities that exist for Maori around the use of human tissue and genetic information in research but do not provide practical advice for genetic researchers or biobanks. Maori views on biobanking and genomic research have been explored through a Health Research Council (New Zealand) funded project 'Te Mata Ira'. Methods included, (a) meetings and interviews with five iwi (tribal groups), (b) interviews of Māori individuals that participated in genomic studies, (c) experts in Māori values and knowledge, (d) interviews with non-NZ indigenous experts in genetic research and/or biobanking, and (e) workshops with medical genomics researchers and health researchers. Key themes that have emerged include:

- A need for protection of Māori interests;
- Focus on Māori health priorities;
- Expectations of consultation & engagement;
- Accessibility to public education resources;
- Māori control over samples and data;
- Ongoing communication with, and feedback from, researchers;
- Expectations of consent; and
- Fair and equitable benefit sharing that recognises community contributions.

To optimise benefits for Māori from involvement in medical genomics technologies, we have developed guidelines for researchers grounded in the expectations and aspirations of communities. This paper will present the results of the project and the cultural foundation which informs a Maori framework for biobanking as it relates to the context of genomic research.

GD12: Genetic and Environmental Control of DNA Methylation in Humans

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The epigenome sits at the interface of an individual's genetics and environment. The most widely studied epigenetic mark is DNA methylation, primarily due to the relative ease of assaying its levels. The latest DNA methylation microarrays can accurately measure DNA methylation at 450,000 sites throughout the human genome. We have identified significant genetic control of DNA methylation, including mapping more than 50,000 QTL associated with DNA methylation levels. Using methylation measures across a number of time points, both genetic and environmental factors were demonstrated to constrain epigenetic drift across the human life-course. In addition, we can identify associations between DNA methylation levels and some phenotypes and use these >associates to improve phenotypic prediction beyond that obtained using genotype alone.

GD13: Telling the story of single nucleotide polymorphisms in human growth and disease

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Understanding how single nucleotide polymorphisms (SNPs) contribute to a phenotype is central to the evolution of personalized medicine. Yet current frameworks for understanding the possible mechanisms that mediate this genotype-phenotype linkage are limited by our current concept(s) of genes and gene regulation. The simplest illustration of this limitation is the categorisation of SNPs according to whether they occur within a gene (intragenic) or between genes (intergenic). In both theory and practice, intragenic SNPs are more easily linked to phenotypes as they are often directly associated with changes in the functionality of genes within which they are found. Yet re-sequencing efforts have confirmed causative linkages between many intergenic SNPs and phenotypes. Here we show that the spatial organization of the genome connects intergenic SNPs to both linked and unlinked genes as part of a regulatory network. Current data indicates that subsets of these SNP-gene connections are functional with significant effects on transcription levels. Our findings include networks that we propose are central to human growth and disease. Despite the limitations that are inherent in the current data, the subset of networks we identify serve as an example of how intergenic SNPs with no direct biological connection to disease can spatially associate with genes to contribute to disease phenotypes and growth. Thus, spatial information is a critical component of an expanded view of human genetics that enables us to understand how the genotype contributes to cellular function.

GD14: Obesity and type-two diabetes: the role of epigenetics in disease, and the potential of these mechanisms as biomarkers of disease.

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Epigenetics provides a mechanism whereby environmental factors can influence complex diseases such as obesity and type-two diabetes. Gastric bypass provides a model to investigate obesity and weight-loss in humans. Furthermore, type-two diabetes is substantially reduced in patients after gastric bypass.

Our work focusses on DNA methylation and miRNA. Recently, we published a comparison of global DNA methylation of two adipose tissues, subcutaneous abdominal and omentum before and after gastric bypass. Identifying significant changes in DNA methylation before compared to after weight-loss within genes involved in obesity, type-two diabetes and epigenetic regulation and development. We have extended this work, applying machine learning approaches to identify DNA methylation sites which distinguish between groups of tissues (DNA methylation biomarkers). The potential power of this approach is demonstrated by our identification of a single DNA methylation site which robustly differentiates subcutaneous and omental tissue from both normal weight and obese individuals. On-going analyses are also looking at other metabolically relevant tissues such as liver.

Our miRNA analyses include microarray profiling of miRNA in adipose tissue before and after gastric bypass as well as a comparison of miRNA in plasma from different phenotypes of obese individuals compared to lean controls.

I will present an overview of our current research in this area.

GD15: DNA Methylation in Patients with a Strong Family History of Early Onset Coronary Heart Disease

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Coronary heart disease (CHD) is a leading cause of morbidity and mortality in Aoteoroa/New Zealand[1]. Having a first-degree relative with early-onset CHD doubles the risk of CHD[2]. The mechanism by which our environment influences the expression of our genes is through epigenetics, such as methylation of the DNA[3-5]. DNA methylation has been associated with CHD at specific loci[6,7], but not in a cohort with a very strong family history of early onset CHD. The Family Heart Study (FHS) patients are unrelated individuals who have had a documented premature CHD event (<50 years in men, <60 years in women) and who have at least one first-degree relative with an early CHD event (total 80 to date). Controls from the Canterbury Healthy Volunteers (HVOLs) with no prior diagnosis of any cardiovascular disease were age and gender-matched to our patient groups.

To assess the association of DNA methylation on risk for those with a very strong family history of early onset CHD we have performed a screening study examining the methylation status of peripheral blood samples from 71 people, 48 from the FHS and 23 HVOLs, using illumina's HumanMethylation450 beadchips (485,000 methylation sites, covering 99% of RefSeq genes). The platform is sensitive to design and quality issues and we have applied well described methods to mitigate these issues, in consultation with ESR Wellington and University of Otago Vascular Disease Research. The results of our analysis pipeline confirm associations of methylation status with age and gender previously reported for population studies. In addition, using genotypes derived from the illumina iSelect Cardio-MetaboChip (220,000 SNPs associated with cardiovascular and metabolic traits), we can show clear evidence of sites where methylation is associated with genetic variations both local and at a distance, known as meQTL.

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2. Murabito J, Pencina M, Nam B-H, D'Agostino R, Wang T, Lloyd-Jones D, Wilson P, O'Donnell C. Sibling *Cardiovascular Disease as a Risk Factor for Cardiovascular Disease in Middle-Aged Adults.* JAMA. 2005;294(24):3117-23.
3. Barouki R, Gluckman P, Grandjean P, Hanson M, JJH. *Developmental Origins of Non-Communicable Disease: Implications for Research and Public Health. Environmental Health.* 2012;11:42.
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5. Heijmans B, Tobi E, Stein A, Putter H, Blauw G, Susser E, Slagboom P, Lumey L. *Persistent Epigenetic Differences Associated with Prenatal Exposure to Famine in Humans.* Proc Natl Acad Sci. 2008;105:17046-9.
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7. Sharma P, Garg G, Kumar A, Mohammed F, Kumar S, Tanwar V, Sati S, Sharma A, Karthikeyan G, Brahmachari V, Sengupta S. *Genome Wide DNA Methylation Profiling for Epigenetic Alteration in Coronary Artery Disease Patients.* Gene. 2014;541:31-40.

GD16: Circular RNA: A new enigma in genome function

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Circular RNAs (circRNAs) are known to be a large class of transcripts. Despite their discovery about thirty years ago, demonstrations of their widespread and substantial presence within transcriptomes have only recently come to the fore. However evidence as to their mechanism(s) of function is still lacking. The sequence conservation, developmental-stage and cell type-specific expression of circRNAs suggests a biological function for these transcripts.

It has been proposed that circRNAs function as stable sponges for other RNA molecules, although more recent studies have revealed that they also function in the assembly of complexes, in transport and in transcriptional control [1]. However, extensive studies in the last two years have suggested additional functional roles for circRNA.

We are investigating the roles of different species of long ncRNAs using breast cancer, melanoma and leukemia cell lines. In melanoma cell lines, we have identified several isoforms of the long ncRNA *ANRIL* transcribed as antisense to the *CDKN2A/B* locus. Circular forms of *ANRIL* (*circANRIL*) had been previously reported [2]. We have found the expression of circular *ANRIL* in melanoma cell lines. Examination of the subcellular localization of linear and circular forms of *ANRIL* has revealed exclusive expression of linear forms in the nuclear fraction of cells while *circANRIL* co-localises with light polysomes in the cytoplasm. The polysome association suggested a novel function for *circANRIL* either at the translational level or in ribosome biogenesis. Currently we are studying the presence of different isoforms of *circANRIL* in melanoma as well as in leukemia cell lines. We are also in the process of identifying novel *circRNAs* in cancer cell lines and investigating their functions in neoplasia.

1. Jeck WR, Sharpless NE (2014) *Detecting and characterizing circular RNAs*. Nature biotechnology 32: 453-461.

2. Burd CE, Jeck WR, Liu Y, Sanoff HK, Wang Z, et al. (2010) *Expression of Linear and Novel Circular Forms of an INK4/ARF Associated Non-Coding RNA Correlates with Atherosclerosis Risk.* PLoS Genet 6: e1001233.

GD17: Treatment of genetically-defined forms of lung cancer

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No abstract

GD18: Genomics related to drug response in melanoma

Peter Shepherd[1]

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No abstract

GD19: How the genome folds: now inside the loop

Erez Lieberman Aiden

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The human genome is over 2 meters long, but must fold up to fit inside the nucleus of a cell. How does it fold? We use in situ Hi-C to probe the 3D architecture of genomes, constructing haploid and diploid maps of nine cell types. The densest, in human lymphoblastoid cells, contains 4.9 billion contacts, achieving 1 kb resolution. We find that genomes are partitioned into contact domains (median length, 185 kb), which are associated with distinct patterns of histone marks and segregate into six subcompartments. We identify ~10,000 loops. These loops frequently link promoters and enhancers, correlate with gene activation, and show conservation across cell types and species. Loop anchors typically occur at domain boundaries and bind CTCF. CTCF sites at loop anchors occur predominantly (>90%) in a convergent orientation, with the asymmetric motifs 'facing' one another. The inactive X chromosome splits into two massive domains and contains large loops anchored at CTCF-binding repeats.

GD20: Optimisation of sequencing effort for relatedness estimation using genotyping-by-sequencing

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Genotype-based relatedness estimation underlies pedigree reconstruction, genomic selection and some association mapping methods. Genotyping-by-sequencing provides genotypes from sequencing data. Molecular biology techniques allow the use of genome subsets and for multiple individuals to be combined in a sequencing run, allowing flexibility in the number of single nucleotide polymorphisms (SNPs) assayed and read depth at each SNP position. SNP depth affects the ability to correctly call genotypes, as only one of the alleles might be observed, with low depth.

We use simulation to find the optimal depth for estimating relatedness at a fixed total sequencing effort (number of SNPs times mean depth), assuming random sampling of SNPs and alleles. Relationship estimation used a method we have recently developed which accounts for read depth for each genotype, including zero reads (missing). We also investigate the effect of estimating allele frequencies. The optimal depth was defined to be the depth that gave the lowest standard deviations of relatedness estimates, across sets of individuals of the same relationship type. We found that the optimal depth was around 3 for estimating relatedness between individuals, and around 8 for estimating self-relatedness. The optima were flat, so that depths of 1-5 and 5-10 were close to optimal for between-individual and self-relatedness, respectively. There were small biases in the relatedness estimates at very low (≤0.5) mean depths. These were corrected using the true allele frequencies, indicating that allele frequency estimates based on low total numbers of reads can influence relatedness estimation.

GD21: Tumour Transcriptomes: Going beyond expression profiling

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Genomic instability is a hallmark of cancer with structural rearrangements, chromosomal fusions and mutations all common occurrences. Sequencing the transcriptome using RNA-seq contains all the information about sequences and expression levels of the transcripts in the samples. However, the majority of analysis methods focus on summarising data into expression levels at either the gene or transcript level and often ignore the sequence information contained in the data. Here I will present our work on looking at sequences and mutations contained in the cancer transcriptome. I will begin by describing JAFFA, a tool we developed for detecting fusion genes in RNA-seq data. I will then move on to talking about our analysis methods for detecting of other transcriptional mutations commonly missed in RNA-seq data.

GD22: Translational Genomics Medicine Workshop

Chaired by Prof. Cris Print
Molecular Medicine & Pathology, University of Auckland.

Speakers:
Ben Lawrence[1], Mark McKeage[1], Jackie Evans[2], Bryan Betty[2]

[1]University of Auckland, [2]Pharmac, Wellington.

The focus of this session will be the changing nature of cancer medicine, such as expensive targeted therapies including immune checkpoint inhibitors. If clinical trials over the next few years show their value beyond doubt, can the use of these medicines, which may potentially transform the care of cancer patients, be sustainable within New Zealand's unique healthcare system? How are overseas countries similar to New Zealand using expensive targeted therapies for cancer patients? Can the new technologies for personalised medicine that scientists like my own group are trying to develop (including blood genomic diagnostics) really allow these therapies to be stratified to the right patients? If so, will this increase or decrease the overall cost?

The goal of this session is to allow the audience (and ourselves) to be informed by a range of viewpoints including: oncology, cancer science, government and health economics.

Prof. Print will briefly introduce the session, then each of the four speakers will have 10 minutes to talk, followed by an open discussion. While the session will be moderated, the intention is to have a relatively informal workshop with extensive participation from the audience.

GD23: Screening for predisposition for gastric cancer in Māori

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The incidence of gastric cancer is three times higher in Māori than New Zealand Europeans. While both environmental and genetic factors have been attributed to this difference, no clear cause has yet been identified. Using next generation sequencing and MLPA, we have searched for mutations in the tumour suppressor E cadherin (*CDH1*) in blood DNA collected from an unselected series of Māori gastric cancer patients diagnosed between 2009 and 2013. Germline *CDH1* mutations predispose to the cancer syndrome Hereditary Diffuse Gastric Cancer (HDGC). Overall, approximately 18% of all patients and 35% of patients with the diffuse subtype carried germline mutations in *CDH1*. This incidence increased to 58% for diffuse gastric cancer patients aged 50 years or less. We conclude that genetic predisposition to gastric cancer is especially common in Māori and that this is a major cause of the high incidence relative to New Zealand Europeans. These results suggest that in order to identify all whanau affected by HDGC and to optimise the clinical management of this aggressive cancer, germline *CDH1* testing should be considered for all Māori diffuse gastric cancer patients.

GD24: Can formalin-fixed paraffin-embedded (FFPE) tissue be sequenced consistently? Our experience of targeted hybridisation next generation sequencing of neuroendocrine tumours.

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Molecular Medicine and Pathology, University of Auckland.

Recently, with technical advances in Next Generation Sequencing (NGS) and rapidly declining costs, the field of cancer genomic analysis has exploded. International cancer genomics initiatives (e.g. TCGA, ICGC) as well as individual research programs are changing our understanding of tumour development and progression. Alongside this, techniques have developed to analyse genomes from formalin-fixed paraffin-embedded (FFPE) tissue. Since this type of tissue forms the majority of tumour tissue available for both research and clinical genomics, repeatable high quality sequencing from FFPE material is important. Our experience generating sequence information from a range of FFPE samples, many of which are several years old, is summarised in this talk.

We initially analysed FFPE neuroendocrine tumours and corresponding normal tissue a small number of patients using whole genome approaches. We are now expanding this analysis aiming for 1,000 x coverage of 580 cancer genes using targeted sequencing with Nimblegen's Sequence Capture Cancer Panel. Library preparation has been successful for 85% of these FFPE tumour samples and high quality genomic information was produced from all these libraries. However, this has required a long period of technical workup and protocol development. Methods are now being optimised for RNA Sequence Capture Hybridization to allow us to routinely examine mutation, copy number and expression in the same FFPE tumours, potentially replacing fresh tissue in our cancer genomics program. Based on our experience, we believe FFPE tissues can really replace fresh material for genomic analysis in both research and the clinic.

GD25: Adventures in next-generation sequencing

Taylor. J

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In this presentation I shall discuss some of the experiences of my laboratory with the analysis of next-generation sequencing (NGS) data, in particular, whole genome sequence and RNA-seq data. My laboratory has generated and traded whole genome sequences for 396 animals including 120 dogs, 3 bison, 19 Mediterranean Buffalo and 254 cattle. We also have generated RNA-seq data on 4-5 tissues on each of 154 cattle. We have comparatively analyzed these data to identify the mutation responsible for the spotted phenotype in Hereford which appears to be a structural variant, not present in the UMD3.1 and only partially present in the Btau4.6.1 reference assemblies. We have been fascinated by the fact that only 90% of NGS map to the reference assembly and have explored these unmapped reads to determine the reasons for their failure to align. As expected, the short-comings of the reference assembly are partially responsible for this result, but more interestingly, the results suggest that all NGS experiments are metagenomic analyses. Finally, we have been interested in utilizing these data to identify the causal variants underling the small numbers of large-effect QTLs underlying quantitative traits in cattle. To accomplish this we have begun to impute 50K and 800K data to (near) whole genome sequence to perform association analyses in the hope that multi-breed analyses will narrow the focus to relatively few candidate mutations. To improve the accuracy of imputation, we have designed a 250K bead assay containing 34K SNPs common to existing Illumina assays and 199K variants likely to be functional based upon annotation of the variants discovered in our sequence data. This assay will be publicly available late in 2015.

GD26: Mutation in MRPS34 Compromises Protein Synthesis and Causes Mitochondrial Dysfunction.

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The evolutionary divergence of mitochondrial ribosomes from their bacterial and cyto- plasmic ancestors has resulted in reduced RNA content and the acquisition of mitochon- dria-specific proteins. The mitochondrial ribosomal protein of the small subunit 34 (MRPS34) is a mitochondria-specific ribosomal protein found only in chordates, whose function we investigated in mice carrying a homozygous mutation in the nuclear gene en- coding this protein. The Mrps34 mutation causes a significant decrease of this protein, which we show is required for the stability of the 12S rRNA, the small ribosomal subunit and actively translating ribosomes. The synthesis of all 13 mitochondrially-encoded polypep- tides is compromised in the mutant mice, resulting in reduced levels of mitochondrial pro- teins and complexes, which leads to decreased oxygen consumption and respiratory complex activity. The Mrps34 mutation causes tissue-specific molecular changes that result in heterogeneous pathology involving alterations in fractional shortening of the heart and pronounced liver dysfunction that is exacerbated with age. The defects in mitochondrial pro- tein synthesis in the mutant mice are caused by destabilization of the small ribosomal sub- unit that affects the stability of the mitochondrial ribosome with age.

GD27: Investigation of the influence of DGAT1 K232A on gene expression in the bovine lactating mammary gland

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In *Bos taurus,* variants in diacylglyercol acyltransferase 1 (*DGAT1*) are associated with a myriad of milk production traits, the most significant of which is increased milk fat percentage. A non-conservative amino acid substitution lysine to alanine at position 232 (K232A) as a result of a dinucleotide substitution constitutes the most widely studied and validated variant in analyses of bovine milk composition. The mechanism of this variant is widely assumed to derive from enzymatic differences between the two protein isoforms, with recombinant DGAT1 bearing the 'K' allele shown to have enhanced activity over the 'A' allele in *in vitro* [1].

We have generated a large, bovine RNA-Seq dataset using lactating mammary gland biopsies, designed to detect expression quantitative trait loci (eQTL) associated with milk composition QTLs [2]. We report strong differences in the expression of DGAT1 transcripts, an effect which appears to derive from the K232A genotype. We propose that this effect is due to the status of K232A as a predicted exon splice enhancer, and that, in addition to increased enzymatic activity, the 'K' allele is associated with increased conversion of precursor mRNA to mature mRNA, and also modulates the production of an alternatively spliced mRNA isoform. Consequently, we propose that the major impacts on milk composition elicited by the K232A polymorphism may, at least in part, derive from these expression-based mechanisms.

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GD28: From Ancient History to Modern Medicine: Selection, Disease, and Genome Function

Elinor K. Karlsson

University of Massachusetts Medical School & Broad Institute of Harvard and MIT

Past natural and artificial selection leaves distinctive patterns of variation in the genome, highlighting functionally important genomic variation. By combining selection with trait association and functional data on a genome wide level, we can identify genes and pathways underlying polygenic traits, and pinpoint candidate functional variants. Applying this approach to cholera susceptibility in Bangladeshis, we identify genes acting in an innate immune response pathway that is activated in vitro by Vibrio cholerae - potentially yielding new insight into immune responses to cholera and other enteric pathogens, as well as mechanisms of intestinal homeostasis. In domesticated dogs, an excellent model for human disease, we find pathways, genes, and regulatory variants underlying osteosarcoma and obsessive compulsive disorder. The success of this approach in both humans and dogs suggests that finding and functionally dissecting naturally occurring variation underlying selected traits is powerful approach for translating genomics into improved medicine.

GD29: Using Epigenetics to Identify the Fetal Cell of Origin for Childhood B – Cell Acute Lymphoblastic Leukaemia

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The worldwide incidence of childhood B-cell acute lymphoblastic leukaemia (B-ALL) has increased since 1950s[1]. The disease originates before birth from the proliferation of neoplastic B lymphoid precursor cells[2]. Martin-Subero *et al* performed a microarray-based methylation analysis of 367 haematological malignancy cases including B-ALL to identify epigenetically modified genes[3]. Analysis of their data confirmed a common epigenetic signature consisting of more than 200 aberrantly methylated genes in B-ALL. For example, *TES* DNA hypermethylation and *CTGF* hypomethylation were highly associated with B-ALL[3].

The large number and consistency of the epigenetically modified genes identified by Martin-Subero *et al* raises the possibility that methylation-induced gene silencing in B-ALL may not be an acquired cancer-related phenomenon. We propose that B-ALL arises from fetal lymphocytes, comparable to murine B-1 lymphocytes, that do not regress after birth and that carry a specific epigenetic signature. Two distinct populations of B-lymphocytes have been identified in mice: fetal B-1 cells and postnatal B-2 cells[4]. Human B-lymphocytes with B-1 like features have been detected in fetal liver, spleen, bone marrow, and blood[5]. Putative B-1 cells were recently identified in human fetal peripheral and cord blood by their $CD20^{+}CD27^{+}CD43^{+}CD70^{-}$ expression[6].

We will identify neonatal B cells with an ALL-like epigenetic profile using sensitive assays that have been developed to detect methylated *TES* alleles (MiSeq; Illumina), and to detect *CTGF* expression (qRT-PCR). These assays will be applied to B-1 cells enriched from neonatal and cord blood on the basis of their immunophenotype ($CD19^{+}CD27^{+}CD27^{+}CD43^{+}$). In addition, these assays will permit monitoring of the expected changes in methylation level when babies develop and mature to term by utilising blood from premature neonates.

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GD30: Is Methylation a potential second hit mechanism in Polycystic Kidney Disease?

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Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited form of renal disease, and is characterised by the formation of fluid-filled cysts within organs, particularly in the kidneys. The two genes that are mutated in ADPKD, *PKD-1* and *PKD-2,* encode proteins in a plasma membrane complex that facilitates Ca^{+} ion release in the cell. Patients typically have an inherited mutation in one of these genes from birth and it is postulated that a second hit in the same gene is required for pathogenesis. Changes in downstream signalling pathways then give rise to the cysts, physically limiting the function of the kidney, and without intervention can lead to renal failure. Mutations in the second allele have been found to be responsible for the second hit in some but not all cysts. We postulate that this "second hit" could also be caused by epigenetic mechanisms[1], particularly involving changes in DNA methylation.

We have investigated two regions in the mouse *PKD-1* gene via bisulfite sequencing of kidney DNA samples from a del34 *PKD-1* mouse model[2] heterozygous for a *PKD-1* mutation. We found that the promotor CpG island was unmethylated whereas the gene body region examined was hypermethylated in both ADPKD and wildtype mouse kidneys. Investigating DNA methylation in mouse models is important to determine their suitability for pre-clinical studies involving epigenetic therapies for ADPKD.

Previous work by Woo *et al*., 2014[3] investigated genome wide methylation in human ADPKD. They examined methylation of the *PKD-1* gene in human kidneys and identified an increase in gene body methylation in ADPKD samples, when compared to wildtype kidneys. This increase in methylation correlated with a decrease in gene expression. Differences between our data may be due to the relative lack of cyst formation in the animal model; differences in species-specific methylation patterns may also contribute.

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GD31: The discovery of novel synthetic lethal compounds for the treatment of E-cadherin deficient tumours.

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The cell-cell adhesion protein E-cadherin (*CDH1)* is a putative tumour supressor and is frequently mutated in a range of both sporadic and hereditary cancers. Loss of *CDH1* expression is particularly common in both lobular breast cancer (LBC) and diffuse gastric cancer (DGC).

Current chemotherapeutic strategies for E-cadherin deficient tumours are limited; consequently, there is an urgent need to develop novel compounds for the treatment and/or chemoprevention of these diseases. We hypothesise that *CDH1* loss creates vulnerabilities within tumour cells that can be specifically targeted with drugs using a synthetic lethal approach (Telford et al., 2015).

To identify novel synthetic lethal compounds for the treatment of cancer arising from E-cadherin loss, we performed a three-staged 114,000 hit-like compound screening campaign on an isogenic pair of mammary epithelial cell lines with and without *CDH1* expression (Chen et al., 2014). The metabolism-based alamar blue assay and a high content imaging approach was employed to determine the impact of the compounds on cell viability and cell cycle phase distribution. This approach identified 84 lead-like compounds which were found to belong to 16 unique pharmacophore groups. Validation of these groups identified 13 novel compounds as being highly selectively lethal to E-cadherin deficient cells, demonstarting that E-cadherin loss presents druggable vulnerablities within tumour cells.

These novel synthetic lethal compounds have the potential to uncover new drug targets in the fight to improve the outcome of both sporadic and familial LBC and DGC.

Chen, A., Beetham, H., Black, M. A., Priya, R., Telford, B. J., Guest, J., et al. (2014). *E-cadherin loss alters cytoskeletal organization and adhesion in non-malignant breast cells but is insufficient to induce an epithelial-mesenchymal transition*. *BMC Cancer*, *14*, 552.

Telford, B. J., Chen, A., Beetham, H., Frick, J., Brew, T. P., Gould, C. M., et al. (2015). *Synthetic lethal screens identify vulnerabilities in GPCR signalling and cytoskeletal organization in E-cadherin-deficient cells*. *Molecular Cancer Therapeutics*, *14*, 1213)

GD32: Flexible analysis of TCGA DNA methylation and gene expression data using UNIX tools

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The Cancer Genome Atlas (TCGA) contains multiple levels of genomic data (mutation, gene expression, DNA methylation, copy number variation) for almost 13000 patients spanning 40 different type of cancer. It is an excellent resource for cancer biologists to explore molecular features of cancer. However, a dearth of appropriate software tools makes it difficult for bench scientists to use these data resourcefully. In recent years, several web-based tools have been developed for this purpose. However, these tools do not allow the flexibility for users to analyse data from different subgroups within a cancer, and impute the data according to the research question. We have developed a suite of tools using the awk programming language that allows flexible analysis of DNA methylation, mRNA and miRNA expression in any subgroup of patients. Using these tools it is possible to analyse TCGA data using command lines on simple desktop computers and obtain raw or processed values for further downstream analysis. We demonstrate the utility of these tools in retrieving meaningful biological data by analysing DNA methylation and gene expression signatures of primary and metastatic melanoma patients from the TCGA database.

GD33: Association analysis of the Beta-3 Adrenergic Receptor Trp64Arg (rs4994) polymorphism with urate and gout

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The Arg64 allele of *rs4994* (Trp64Arg) single nucleotide polymorphism in the β3-adrenergic receptor (ADRB3) gene has previously been associated with increased serum urate and risk of gout[1- 3]. The same variant is also associated with insulin resistance[4]. This research aims to investigate the association of *rs4994* with urate and gout in New Zealand (NZ) European, Māori and Pacific people.

A total of 1730 clinically-ascertained gout cases and 2145 controls were genotyped for *rs4994* by Taqman®. Māori and Pacific participants were subdivided into Eastern and Western Polynesian sample sets. Publicly available genotype data from the Atherosclerosis Risk in Communities (ARIC) and the Framingham Heart Study (FHS) was utilised for serum urate association analysis. Multivariate logistic and linear regression adjusted for potential confounders was carried out using R version 2.15.2.

No significant association of the minor Arg64 (G) allele of *rs4994* with gout was found in the combined Māori and Pacific Island cohorts (OR = 0.98, *P* = 0.88) although there was a strong trend towards association with opposing direction of effect in Western Polynesian (OR=0.61, *P*=0.08) and Eastern Polynesian (subjects with low Eastern Polynesian ancestry) (OR=1.49, *P*=0.07). There was also no evidence for association in the European participants (OR=1.11, *P*=0.57). However, the Arg64 allele was positively associated with urate in the Western Polynesian participants (β=0.036 $mmolL^{-1}$, *P*=0.004, $P_{Corrected}$=0.03).

Association of the Arg64 variant with increased urate in Western Polynesian participants was consistent with the previous literature. This association provides an etiological link between metabolic syndrome components and urate homeostasis. However, confirmation of a possible etiological contribution of this variant to gout requires further testing in larger sample sets.

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GD34: Trialling the MinION™ nanopore DNA sequencer for direct measurement of human telomere length

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Telomeres maintain the integrity of the genome but progressively shorten as cells age, with potential impact on morbidity and mortality later in life. Therefore, there is great interest in the use of telomere length as a biomarker of general health. However, current methods for measuring telomere length are technically challenging and imprecise. Oxford Nanopore Technologies (ONT) has developed a nanopore sequencing device (MinION™) capable of long-read sequencing at the single molecule level. We are exploring the use of the MinION™ as a novel means of accurately measuring telomere length.

For MinION™ sequencing, telomere-specific, biotinylated oligonucleotides were incubated with fragmented human genomic DNA, and captured with streptavidin coated magnetic beads. Enrichment was monitored on dot-blots. Two plasmids containing telomeric repeat inserts of 103 and 136bp respectively served as positive controls for all experiments. MinION™ sequencing data on the genomic "pull-down" fraction showed little evidence of telomeric or subtelomeric DNA sequence. Although generally quite repetitive, few perfect telomere repeats were observed with the longest repeat sequence only containing two tandem repeats. However, very long reads were obtained with the largest being 282,705 bases. MinION™ reads of the telomeric plasmid inserts similarly did not show characteristic hexanucleotide (T_2AG_3) repeats, although the plasmid sequences were of reasonable quality.

In conclusion, the MinION™ clearly struggled to generate readable sequence on telomeric repeat DNA. Telomeric repeats are well known to form G-quadruplex structures, and we are currently testing the hypothesis that G-quadruplexes are forming and blocking or impeding passage of the DNA strand through the nanopore, and thus disrupting accurate base calling Our results suggest that the MinION™ device cannot effectively read telomeric repeat sequences, but we propose that preventing G4 formation may lead to improved outcomes. The long-read capabilities of the device may prove useful for analysing individual telomeres, if the read accuracy issues can be resolved.

GD35: Genome-wide methylation analysis identifies a core set of hypermethylated CpG islands in CIMP-H colorectal cancer

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Aberrant DNA methylation profiles are a characteristic of all known cancer types, epitomised by the CpG island methylator phenotype in colorectal cancer (CRC). Hypermethylation has been observed at CpG islands throughout the genome, but it is still unclear which factors determine whether an individual island becomes methylated in cancer or not. Using the Illumina HumanMethylation450K array we characterised the DNA methylation profiles of 94 CRC tissues and their matched normal counterparts. Consistent with previous studies, unsupervised hierarchical clustering of genome-wide methylation data identified three subtypes within the tumour samples, designated CIMP-H, CIMP-L and CIMP-N, that showed high, low and very low methylation levels, respectively. Differential methylation between normal and tumour samples was analysed at the individual CpG level, as well as at the CpG island level. The distribution of hypermethylation in CIMP-N tumours showed high inter-tumour variability and appeared to be highly stochastic in nature, whereas CIMP-H tumours exhibited consistent hypermethylation at a subset of CpG islands, in addition to a highly variable background of hypermethylated islands. Three CpG islands were hypermethylated in more than 90% of all tumours examined, and 132 islands were hypermethylated in 100% of CIMP-H tumours studied. The set of islands hypermethylated in all CIMP-H tumours were highly enriched for functions relating to DNA-binding (Bonferroni adj. p = 5.00E-36), development (adj. p = 4.65E-34), neuron differentiation (adj. p = 1.57E-26), and cell fate commitment (adj. p = 4.02E-14). The identification of this core set of hypermethylated genes provides insights into the origin of epigenetic dysregulation in cancer.

GD36: Predicting altered methylation patterns in early pre-eclampsia

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Pre-eclampsia is a pregnancy condition that complicates about 5-10% of pregnancies worldwide, and has some of the highest rates of maternal and fetal morbidity and mortality around the world (1). Approximately 5% of live births from pre-eclamptic pregnancies also have low birth weight (less than 2,500 grams), which is shown to have poor long-term health outcomes for the infant (2). Pre-eclampsia is associated with a deficiency in the migration and differentiation of placental trophoblast cells at the maternal-fetal interface (3), yet the particular mechanisms for this condition are still unknown. Unfortunately, the only known cure is an early-induced delivery of the infant and placenta. Up to date, little progress has been made on treatment and management of pre-eclampsia because the condition commonly remains undiagnosed until after the 24th week of gestation, by which time the condition has manifested and treatment options are limited.

There is a growing interest in studying the epigenetics of the placenta as it provides a mechanism by which development can be altered in response to maternal-fetal signals and environmental effects, such as maternal nutrition, BMI, smoking and stress. Our lab has used a genome-wide DNA methylation analysis technique called reduced-representation bisulfite sequencing (RRBS) to determine methylation differences between pre-eclamptic placentas and matched controls (4-6). We have generated several DNA methylation maps and differential methylation datasets for pre-eclamptic and control placentas. A large number of differentially methylated CpG sites have been identified in preeclamptic placentas; however, these sites need further validation. We aim to increase our sample numbers (and power of the project) to detect reliable and robust methylation differences between pre-eclamptic and control placentas. We also aim to investigate downstream effects of these differences with expression analysis.

My PhD study is currently analysing global methylation data (produced by RRBS) to explore the biological effect of altered methylation in pre-eclampsia. The proposed work will enhance my investigation of DNA methylation as a marker for pre-eclampsia. Once the differences in methylation between pre-eclamptic and normal placentas have been validated, we aim to detect these differences in the circulating cell-free DNA (ccf-DNA) isolated from maternal blood plasma, since ccf-DNA is derived from apoptotic placental trophoblast cells. The use of epigenetic markers of ccf-DNA is becoming recognised as useful tool to screen for pregnancy conditions such as pre-eclampsia, intra-uterine growth restriction (IUGR) and preterm labor (7-9). In this project, we will differentiate between maternal DNA and cff-DNA using epigenetic markers that have shown opposite and extreme differences in methylation. We will then perform deep sequencing of PCR products using the MiSeq platform to target our candidate regions for pre-eclampsia. We ultimately hope to identify a DNA methylation signature of pre-eclampsia in maternal blood plasma that can be used clinically to predict women who are at risk of developing this threatening condition of pregnancy.

GD37: Multimodal assessment of oestrogen receptor status, expression and signalling activity in breast tumours

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Assessment of oestrogen receptor (ER)-α protein expression using immunohistochemistry (IHC) is used to stratify breast cancer patients for endocrine-based therapy. However, ER-α IHC provides no information regarding the functional status of the ER signalling pathway and it is estimated that up to 20% of ER status assessments may be inconsistent. Genomic indicators of ER activity have been proposed, however, these genomic indicators proposed to reflect ER signalling (usually represented by genes sets) are highly divergent, and the genomic consequences of ER pathway signalling remain incompletely understood.

We identified ER-α associated gene expression patterns in two complimentary ways. Firstly we used a simple well-controlled cell culture model of siRNA-mediated down-regulation of ESR1 (the gene encoding the ER-α protein) in ER positive MCF7 breast cancer cells, to capture the transcriptomic changes in the cells using microarrays. ESR1-dependent genes were significantly enriched for molecular pathways known to be associated with ESR1, CCND1, MYC and NFKB. Secondly, the expression patterns of ESR1 and ER-α associated genes were mapped, along with ER IHC status and 11 previously published ER-associated gene sets, across 1034 primary breast tumours of multiple subtypes. This analysis identified a continuous range of ESR1 mRNA expression across breast tumour subtypes, including low ESR1 expression in some luminal tumours and high ESR1 expression in some basal-like tumours. Using this data, we were also able to generate an inferred ER pathway activity using principal component analysis, which provided a putative measure of ER signalling activity.

We show that there is a continuous range of both ER expression and transcriptional targets of ER signalling in breast tumours. We propose that a combination of ER-α IHC, ESR1 mRNA expression, and bioinformatic inference of ER pathway activation can provide a multi-modal assessment of breast tumour ER pathway activity, useful for both research and treatment stratification.

GD38: Replication of association of the apolipoprotein A1-C3-A4 cluster with gout risk: evidence for a causal role in gout

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Problem Statement: Gout typically presents as an acute autoinflammatory response to monosodium urate (MSU) crystals. Gout is associated with dyslipidemia including increased very low-density lipoprotein (VLDL) triglyceride levels [1]. Consistent with this, association of the apolipoprotein A1-C3-A4 gene cluster with gout has previously been reported in a small study [2]. In order to investigate a possible causal role for this locus in gout we tested the association of genetic variants from APOA1 (rs670) and APOC3 (rs5128) with gout.

Subjects and Methods: A total of 2452 controls and 2690 clinically-ascertained gout cases of New Zealand Polynesian (Māori and Pacific) and of European ancestry were genotyped by Taqman for rs670 and rs5128. Data were also used from the publically available Atherosclerosis Risk in Communities (n=5367) study and the Framingham Heart Study (n=2984). Multivariate adjusted logistic and linear regression was used to test association of the SNPs with gout risk, serum urate, triglyceride and high-density lipoprotein cholesterol (HDL-C).

Results: In Polynesians the T-allele of rs670 (APOA1) increased (OR=1.53, P=4.88×10-6) and the G-allele of rs5128 (APOC3) decreased the risk of gout (OR=0.86, P=0.026). The effect size for both SNPs strengthened in comparison to asymptomatic hyperuricaemic controls (OR=1.74, P=1.6x10-4 and OR=0.74, P=0.002 for rs670 and rs5128, respectively). There was no evidence for association of rs670 or rs5128 with the risk of gout in Europeans (OR=1.11, P=0.059; OR=1.01, P=0.91 respectively). Association in Polynesians was independent of any effect of rs670 and rs5128 on triglyceride and high-density lipoprotein cholesterol (HDL-C) and there was no evidence for association with serum urate.

Conclusions: Collectively, these findings support the role of the apolipoprotein A1-C3-A4 gene cluster in gout etiology and support the hypothesis that apoliproprotein metabolism plays a causal role in MSU crystal accumulation and/or the inflammatory response in gout.

1 Rasheed, H. *et al. The relationship of apolipoprotein B and very low density lipoprotein triglyceride with hyperuricemia and gout.* Arthritis research & therapy **16**, 495 (2014).

2 Cardona, F. *et al. Contribution of polymorphisms in the apolipoprotein AI-CIII-AIV cluster to hyperlipidaemia in patients with gout.* Annals of the rheumatic diseases **64**, 85-88 (2005).

GD39: RNA-Seq analysis of gene expression changes caused by mood stabilizer drugs in a serotonergic cell line

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Sodium valproate and lithium are drugs of very different chemical classes that are widely prescribed in the treatment of bipolar disorder and other conditions. Molecular and pharmacological studies have revealed some relevant properties and targets of these drugs but their precise modes of action are not yet understood[1-2].

Our primary hypotheses are that genes displaying specific regulation by either or both of these drugs will be relevant to the mechanism of action, and that genes which are co-regulated by both drugs will highlight common pathways of action which may be of the greatest interest. To test these hypotheses we are using a serotonergic cell line called RN46A[3], which is an immortalized serotonergic precursor cell line derived from rat raphe nucleus. This cell line represents a relevant model for the neurological effects of valproate and lithium, as most mood disorders appear to involve the serotonergic system of the central nervous system. The cell line is exposed to therapeutically relevant levels of each drug for 72 hour, then RNA is extracted for RNA-Seq analysis. A preliminary RNA-Seq analysis yielded evidence for significant gene expression changes, particularly after exposure to valproate, and co-regulation of some genes by both drugs. In particular, a gene called *Dynlrb2* (Dynein light chain roadblock-type 2) known to be involved in the intracellular movement of vesicles along microtubules, was significantly upregulated by both drugs. We are carrying out more extensively replicated experiments to validate and extend these initial findings.

Understanding the broader gene regulatory effects of both drugs in a serotonergic context should help us to better understand how these drugs work, and perhaps will also provide insights into the molecular processes underlying mood control.

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2. Atmaca, M. (2009). *Valproate and neuroprotective effects for bipolar disorder*. International review of psychiatry. 21: 410-3.
3. White, L., et al. (1994). *Distinct regulatory pathways control neurofilament expression and neurotransmitter synthesis in immortalized serotonergic neurons*. The Journal of Neuroscience 14, 6744–53.

GD40: A Next Generation Sequencing PGx Panel For Predicting Adverse Drug Reaction And Unusual Reponse

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Recent advances technology advances have led to the development and launch of a wide range of pharmacogenetic (PGx) assays, ranging from low-throughput approaches that genotype single genetic mutations to high-throughput approaches to evaluate hundreds of genes simultaneously. Herein, we described a robust, high-throughput and flexible NGS-based assay for genotyping many pharmacogenes involved in drug metabolism and adverse drug reactions.

We selected a method called “Hi-plex”, for generating NGS libraries via one multiplex PCR amplification (Nguyen-Dumont et al. 2014). It uses automated primer design software and a simple, low-cost protocol for cost-effective and rapid performance. The original approach was slightly modified to increase read length from 100 bp to 200 bp and to include barcoded adapters. Sequencing was conducted on Ion Torrent PGM platform (Life Technologies) using the Ion 318 chip/Ion PGM 200 Sequencing Kit. Variants were called by Ion Reporter software.

Our current assay contains 54 amplicons for 21 pharmacogenes, with indexing for 48 samples. The Ion Torrent run summary shows that 48 barcodes were successfully detected in the run. Each sample yielded approximately 54,651 reads on average, with a mean read length of 208 bp. When mapped to the target regions, the on-target rate was 87.88%, with a mean of read depth per amplicon of 744x. The total number of variant calls generated by Ion Reporter is 4032, of which 3264 calls passed all the filtering criteria. Particularly, 84 variant sites that have rsID have been identified in our samples.

Our current assay format has identified a number of important pharmacogenetic mutations in 24 individuals. We also demonstrated the adaptability and flexibility of Hi-plex approach, which enables a customized set of genes and a variable number of samples. Other NGS platforms can be easily adopted by using compatible adapter sequences and defining suitable amplicon size.

Nguyen-Dumont, T., Pope, B. J., Hammet, F., Southey, M. C., & Park, D. J. (2013). *A high-plex PCR approach for massively parallel sequencing*. BioTechniques, 55(2), 69–74. doi:10.2144/000114052

Summary of Abstracts for the Poster Session (GD29-40)

No.	Title	Presenter	Institutions
GD29	Using Epigenetics to Identify the Fetal Cell of Origin for Childhood B – Cell Acute Lymphoblastic Leukaemia	A.A Alsaleh.	University of Otago, Dunedin, NZ.
GD30	Is Methylation a potential second hit mechanism in Polycystic Kidney Disease?	M.C. Bates	University of Otago, Dunedin, NZ.
GD31	The discovery of novel synthetic lethal compounds for the treatment of E-cadherin deficient tumours	Henry Beetham	University of Otago, Dunedin, NZ.
GD32	Flexible analysis of TCGA DNA methylation and gene expression data using UNIX tools	Mike Eccles	University of Otago, Dunedin, NZ.
GD33	Association analysis of the Beta-3 Adrenergic Receptor Trp64Arg (rs4994) polymorphism with urate and gout	T. Fatima	University of Otago, Dunedin, NZ.
GD34	Trialling the MinION™ nanopore DNA sequencer for direct measurement of human telomere length	Sarah Jodczyk	University of Otago, Christchurch, NZ.
GD35	Genome-wide methylation analysis identifies a core set of hypermethylated CpG islands in CIMP-H colorectal cancer	T. McInnes	University of Otago, Dunedin, NZ.
GD36	Predicting altered methylation patterns in early pre-eclampsia	Suzan Momani	University of Otago, Dunedin, NZ.
GD37	Multimodal assessment of oestrogen receptor status, expression and signalling activity in breast tumours	Anita Muthukaruppan	University of Auckland, Auckland, NZ.
GD38	Replication of association of the apolipoprotein A1-C3-A4 cluster with gout risk: evidence for a causal role in gout	H. Rasheed	University of Otago, Dunedin, NZ.
GD39	RNA-Seq analysis of gene expression changes caused by mood stabilizer drugs in a serotonergic cell line	Priyanka Sinha	University of Otago, Christchurch, NZ.
GD40	A Next Generation Sequencing PGx Panel For Predicting Adverse Drug Reaction And Unusual Reponse	Kim Ton	University of Otago, Christchurch, NZ.