Animal Genetics: QMB Abstracts

AG1: Ancient DNA analyses in the Pacific - implications for human settlement history

Matisoo-Smith, E.A., Gosling, A., Collins, C.

Department of Anatomy and Allan Wilson Centre, University of Otago, Dunedin, NZ.

We have recently sequenced several complete ancient mitochondrial genomes from Pacific populations which are providing surprising results regarding population histories including high levels of variation and evidence of population replacements. These data will be presented and the implications for reconstructing population histories in the region discussed.

AG2: Understanding the evolution of island gigantism - a genomic approach

Knapp, M.[1], Prost, S.[2], Haile, J.[3], Scofield, P.[4], Bunce, M.[5], Gilbert, M.T.P.[6]

[1]Department of Anatomy, University of Otago, Dunedin, NZ, [2]Department of Integrative Biology, University of California, Berkeley, USA, [3]School of Archaeology, University of Oxford, UK, [4]Canterbury Museum, NZ, [5]Department of Environment and Agriculture, Curtin University, Australia, [6]Centre for GeoGenetics, Natural History Museum of Denmark, University of Copenhagen, Denmark.

Evolution on islands has produced extraordinary forms of adaptations including flightless birds, such as the New Zealand Kakapo, and dog sized elephants, such as the extinct Sicilian Pygmy Elephant. Understanding the genetic basis of such environmental adaptation is of fundamental importance for the study of evolutionary biology. Among the most noticeable environmental adaptations is island gigantism. While this phenomenon is well known[1], we know little about the genetic processes involved in the evolution of island gigantism. One of the most spectacular examples of island gigantism is New Zealand's extinct Haast's Eagle (*Harpagornis moorei*). This iconic representative of New Zealand's unique bird fauna descended from a lineage of very small eagles and, after arriving in New Zealand, rapidly evolved into the largest known raptor in the world. Based on genetic divergence, the age of the most recent common ancestor of Haast's Eagle and its two closest relatives, the Australasian Little Eagle (*Hieraaetus morphnoides*) and the Northern Hemisphere Booted Eagle (*H. pennatus*) was estimated to be between 0.7 and 1.8 million years[2], yet Haast's Eagle is as much as 15 times the size of these two closest relatives. This suggests that, in adapting to the New Zealand environment, Haast's Eagle went through the largest size increase (relative to time) known of any vertebrate species in the world. Using complete genome data from Haast's Eagle and its closest relatives we aim to identify the functional genomic basis of this rapid size increase.

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AG3: Sex and speciation: Did sex chromosome turnover and rearrangement trigger mammalian divergence?

Jennifer A. Marshall Graves[1,2,3]

[1]School of Life Science, La Trobe University; [2]Research School of Biology, Australian National University; [3]Institute of Applied Ecology, University of Canberra, Australia

Humans and other therian mammals share a sex chromosome pair composed of a highly conserved X and a small Y chromosome, which progressively degenerated and specialised. The male-dominant *SRY* gene on the Y diverged from its X-borne partner *SOX3* about 190 MYA, the time that therians diverged from prototherian mammals (monotremes such as platypus). In reptiles, and even monotremes, this XY pair is represented by autosomes. Platypus and echidna share a bizarre $X_1X_2X_3X_4X_5Y_1Y_2Y_3Y_4Y_5$ system with homology to the bird ZW, suggesting that a mammalian ancestor may have had a bird-like ZW system.

I propose that the evolution of the sex determining *SRY* gene and definition of a novel XY chromosome pair in therian mammals ~190 MYA imposed a reproductive barrier with the ancestral population of mammal-like reptiles, and triggered the speciation event that led to the evolution of therian mammals. Meanwhile translocation with autosomes stabilised the ancestral system in monotremes. Hybrids between animals with the ancient system and the new *SRY* system, or the translocated monotreme system, would have had high frequencies of sex reversal, intersex development, and infertility, promoting divergence of prototherian and therian mammals. I also propose that more recently (~166 MYA), Robertsonian fusion of the therian XY pair with an autosome posed a reproductive barrier that promoted divergence of eutherian (placental) mammals and marsupials.

Several modern rodent lineages have variant sex determining systems (including complete loss of the Y), suggesting that rodents are undergoing a new explosion of speciation driven by Y chromosome degradation and sex chromosome turnover.

This theory conflicts with the long-prevailing paradigm that speciation results from accumulation of small mutational differences in isolated populations, but receives support from the many groups of fish and reptiles in which closely related species have different sex determination mechanisms, and interspecies hybrids are infertile.

AG4: Using genomics to manage adaptive potential in threatened populations

Santure, A.W.[1], Lee, K.D.[1], Ewen, J.G.[2], Brekke, P.[2]

[1]School of Biological Sciences, University of Auckland, Auckland, NZ, [2]Institute of Zoology, Zoological Society of London, London, UK.

For endangered species, one of the most promising applications of genomics is to understand the genetic basis of adaptation in the wild. For example, knowledge of genetic variants that increase or decrease aspects of fitness will be an important tool to help decide which individuals should be translocated to found new populations. This will maximise evolutionary potential of the new population while reducing risk from deleterious variants. In this talk, I will give an overview of our work to date in developing a genomic toolkit in the threatened New Zealand hihi (stitchbird; *Notiomystis cincta*), and the work we have planned to understand the genetic basis of important traits in the population. While discovering genes of large effect would help us understand selection and evolution in the wild, we believe it is likely that most traits will be influenced by many genes of small effect. However, borrowing from genomic prediction, we predict that we can use whole genome information to accurately identify high value individuals for breeding programs and translocations, thus maximising the evolutionary potential of threatened species.

AG5: Genetic Gains in New Zealand Sheep and Future Challenges

Mark Young

B+LNZ Genetics, PO Box 39-085, Harewood, Christchurch 8542, NEW ZEALAND

In the last 20 years genetic gain in New Zealand sheep has accelerated as more accurate evaluations and expanded descriptions of genetic merit became available. Compared to 1995 animals, maternal sheep in 2014 are ahead by $15.29 per ewe lambing for the NZ Maternal Worth index based on Reproduction, Lamb Survival, Lamb Growth & Adult Size, carcass Meat Yield & Wool production. The equivalent figure for meat sheep is $7.85 per lamb born for the NZ Terminal Worth index based on Lamb Survival, Lamb Growth & Meat Yield.

Genomic tools based on SNP technology have the potential to accelerate genetic gain further but we must address challenges due to economics, data availability and selection objectives. However, SNP tools can add value right away, at minimal cost, by sire paternity verification. Sheep breeding place high selection pressure on males and statistical evaluations focus on sire families. Errors in sire paternity of c.10% (Ovita breeder meetings, 2013) can be addressed immediately and cost effectively in all ram breeding flocks by using SNP based parentage tests.

More accurate breeding values (BVs) can be produced by combining SNP, performance and pedigree data. These are being introduced for the larger populations of maternal type in NZ. It is limited to these by the need to calibrate SNP predictions against large numbers of sires that have BVs proven by progeny performance. At present, many of the traits likely to benefit most from predictions of genetic merit using SNP data (manifest late, in one sex, costly to assess or assessed post-mortem) lack sufficient phenotypic data for calibration e.g. ewe longevity, meat qualities or Facial Eczema Tolerance. Genomic technologies are poised to add considerable value by bringing such traits into our selection programmes once this data deficiency is addressed.

An urgent requirement for NZ sheep breeding is new selection objectives. Existing objectives focus on the more easy to measure traits and change in one direction is implicit for all traits. Application of existing technology to current objectives has caused some traits to have got to, sometimes passed, trait optimums in some flocks. Commercial farmers, ram breeders and meat companies say selection to reduce carcass fatness has led to instances where lines of lambs carrying less fat than is needed for high meat quality, ewes carry less body condition (partly fatness) than is needed when feed intake cannot meet their needs, and too many lambs per ewe which can be a liability in large-scale flocks run under extensive conditions. Ways to bring traits with intermediate optimums into selection objectives are urgently needed.

Defining appropriate selection objectives for the next 20-30 years is imperative. Ram breeders need to begin assessing “next generation” traits now, to exploit the potential of genomics and maintain economic genetic gain.

AG6: Characterising inbreeding depression using runs of homozygosity in Jersey cattle populations in Australia and the USA

Jennie E. Pryce[1,2], Jeremy T. Howard[3], Mekonnen Haile-Mariam[1], Christian Maltecca[3]

1Department of Economic Development, Jobs, Transport and Resources and Dairy Futures Cooperative Research Centre, 5 Ring Road, Bundoora Victoria, 3083, Australia

2La Trobe University, Bundoora, Victoria 3086, Australia

3Department of Animal Science, North Carolina State University, Raleigh, NC 27695-7627, USA.

Inbreeding reduces fitness by increasing the number of homozygous deleterious recessives affecting traits related to fitness such as survival, disease resistance, fertility etc. Runs of homozygosity have been used to quantify the extent of inbreeding depression across the whole genome and at specific sites along the genome in Jersey cattle from Australia and the USA. For example we have found genomic regions that when inbred have an unfavourable impact on calving interval (a measure of fertility) by 1 genetic standard deviation. There is also evidence to suggest that variation in selection intensity exists across the genome, which could result in detectable differences in patterns of genome-level homozygosity arising across countries. This could due to differences in sire choices because of variation in environment, management practices, nutrition or selection objectives. The challenge is now to develop tools that can be used to control inbreeding using genomic information, such as mating plans and utilize differences in genome-level homozygosity that exist across countries to maintain the genetic diversity within the Jersey breed.

AG7: Discovery of thermoregulatory mutations in cattle

Mathew Littlejohn

Abstract Not Available

AG8: The value of genomic selection in a small population

Amer, P.R.[1], Hely, F. S.[1], Miller, S.[2]

[1]AbacusBio Limited, PO Box 5585, Dunedin, NZ, [2]Agresearch, Invermay Agricultural Centre, Puddle Alley, Private Bag 50034, Mosgiel 9053, NZ.

This paper will describe simulation results of accuracy of genomic selection in a small structured population with a commercial progeny test. The work will also show how selection index theory can be used as an alternative means of predicting accuracies of genomic selection in the context of a small population, and how this compares with predictions from theoretical equations. Some economic considerations will also be addressed. The conclusion is that useful accuracies can be achieved with 1-2 thousand new animals genotyped per year in a specific context where current options for genetic progress are limited, provided genomic testing costs are not too high.

AC9: Genetic and Environmental Control of DNA Methylation in Humans

McRae, A.

Centre for Neurogenetics and Statistical Genomics, The Queensland Brain Institute, The University of Queensland QBI Building (#79), St Lucia, QLD 4072, Australia

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.

AG10: Telling the story of single nucleotide polymorphisms in human growth and disease

William Schierding,1 Wayne Cutfield1,2, and Justin M. O'Sullivan1,2*

1Liggins Institute, University of Auckland, Grafton, Auckland, NZ, 2 Gravida: National Centre for Growth and Development, University of Auckland, Auckland, New Zealand

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.

AG11: Obesity and type-two diabetes: the role of epigenetics in disease, and the potential of these mechanisms as biomarkers of disease.

Donia Macartney-Coxson [1], Miles Benton [1,2], Angela Jones[1], Ronald D. Hagan[3], Alice Johnstone[1], Richard Stubbs[4], Michael A. Langston[3]

1Institute of Environmental Science & Research, Wellington, NZ. 2Genomics Research Centre, Institute of Health and Biomedical Innovation, Queensland University of Technology, QLD, Australia. 3Department of Electrical Engineering & Computer Science, University of Tennessee, Knoxville, USA. 4The Wakefield Clinic, Wellington, NZ.

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.

AG12: DNA Methylation in Patients with a Strong Family History of Early Onset Coronary Heart Disease

Pearson, J.F.[1], Cameron, V. A.[2]

1Biostatistics and Computational Biology Unit, University of Otago, Christchurch, NZ, 2Department of Medicine, Christchurch Heart Institute, University of Otago, Christchurch, NZ.

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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AG13: Circular RNA: A new enigma in genome function

Marjan E. Askarian-Amiri [1,2], Herah Hansji [1,2], Debina Sarkar [1,2], Euphemia Leung [1,2], Stefan K. Bohlander[2], Graeme J. Finlay[1,2], Bruce C. Baguley[1]

[1]Auckland Cancer Society Research Centre, [2]Department of Molecular Medicine and Pathology, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand

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.

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AG14: How the genome folds: now inside the loop

Erez Lieberman Aiden

The Centre for Genome Architecture; Department of Molecular and Human Genetics, Baylor College of Medicine; Department of Computer Science, Rice University

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.

AG15: Optimisation of sequencing effort for relatedness estimation using genotyping-by-sequencing

Dodds, K. G., McEwan, J.C., Brauning, R., Clarke, S.M.

AgResearch, Invermay Agricultural Centre, Private Bag 50034, Mosgiel 9053, NZ.

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.

AG16: Tumour Transcriptomes: Going beyond expression profiling

Davidson N.M.[1], Majewski, I.J.[2], Ekert, P.[1], Oshlack, A.[1]

[1]Murdoch Childrens Research Insititute, Parkville, Australia, [2]Walter and Eliza Hall Institute, Parkville, Australia

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.

AG17: Genomic host control of the rumen microbiome

S.J. Rowe[1], G.R. Wood[1], S. Ganesh[2], C. Pinares-Patiño[2], K.G.Dodds[1], M.R. Kirk[2], S.M. Hickey[3], J.C. McEwan[1], P.H. Janssen[2], and S. Kittelmann[2]

[1]AgResearch, Invermay Agricultural Centre, Mosgiel, NZ, [2]AgResearch, Grasslands Research Centre, Palmerston North, NZ, [3]AgResearch, Ruakura Research Centre, Hamilton, NZ.

The rumen microbiome plays a key role in the production of methane, a critical greenhouse gas and a waste product from ruminant digestion. Marker gene sequence data generated from 520 rumen samples from 260 sheep were used to estimate the relative abundance of bacteria in the rumen at two time-points. Sheep were measured for methane emissions in respiration chambers. Rumen microbial community (RMC) structure was determined by assigning bacteria sequences to 54 bacterial taxa. Heatmaps and simplex plots were used to visualise relationships between RMC and methane emissions. Results indicated that the bacterial microbiome data was broadly two-dimensional, with two dominant ruminotypes. To reduce the dimensionality of the data, and facilitate comparing RMC between individuals, correspondence analysis of a Bray-Curtis dissimilarity matrix was applied. The first and second co-ordinates (CA1, CA2) were used as intermediate phenotypes to determine heritability and repeatability of RMC. Preliminary results show that heritability was moderate ~0.24 (s.e. 0.10) and 0.13 (0.09) for CA1 and CA2 respectively. Repeatabilities were 0.51 (0.05) for CA1 and 0.45 (0.05) for CA2. For CA1 and CA2, genetic correlations with total methane emission (g CH4 / day) were 0.58 (0.42) and 0.77 (0.44) respectively. For methane emissions per unit of feed ingested (g CH4 /kg dry matter intake), genetic correlations were 0.06 (0.32) and 0.9 (0.35) respectively. Findings are consistent with previous analyses suggesting that CA2 divides low methane yielding animals into two groups with differing RMC. Results suggest that RMC is under host genetic control and could be a useful predictor of ruminant methane emission. RMC may also be a potential predictor for other traits related to rumen stoichiometry and feed intake. The next steps are to determine the association of RMC with methane and rumen fermentation products together with individual markers or regions of the host genome.

AG18: Neutrophil dysfunction during the transition period

Crookenden, M.A.[1], C.G. Walker[1], A. Heiser[2], J.J. Loor[3], K.M. Moyes[4], J.K. Kay[5], S. Meier[5], A. Murray[6], V.S.R. Dukkipati[6], M. Mitchell[7], J.R. Roche[5]

[1]DairyNZ, Private Bag 92019, Auckland Mail Centre, Auckland 1142, New Zealand
[2]AgResearch, Hopkirk Research Institute, Palmerston North 4442, New Zealand
[3]Department of Animal Sciences, University of Illinois, Urbana 61801
[4]Department of Animal and Avian Sciences, University of Maryland, College Park, MD
[5]DairyNZ, Private Bag 3221, Hamilton 3240, New Zealand
[6]Institute of Vet, Animal and Biomedical Sciences, Massey University, Palmerston North 4442, New Zealand
[7]University of Queensland, Centre for Clinical Research, Herston, Queensland 4029, Australia

There is a high incidence of infectious and metabolic disease in the transition period. During this time the immune system is modulated, bringing about a natural period of observed immunosuppression. The effect of parturition on neutrophil function is of particular interest, therefore we hypothesized that neutrophil dysfunction occurs around the transition period, as evidenced by altered gene expression. To determine this, we extracted neutrophils from peripheral blood of 45 cows at five time points: one wk pre-calving (-1 wk), day of parturition (d0), and post-calving at wk 1, 2, and 4. Key pathways of neutrophil function were investigated by qRT-PCR. The 'bovine immune panel' (BIP) included 96 targets for detection with Fluidigm® 96.96 integrated fluidic circuits and the Biomark™ HD system. Data analysis was performed using SAS 9.3; the effect of time was analyzed using a mixed model approach to repeated measures ANOVA and Tukey's t-test for pairwise comparisons between weeks. Data was submitted for pathway enrichment analysis using the protein analysis through evolutionary relationships tool. The top 3 pathways enriched over the transition period from gene targets on the BIP included; inflammation signalling (14-fold enrichment, $P < 0.001$), apoptosis signalling (19-fold enrichment, $P < 0.001$), and interleukin/cytokine signalling (18-fold enrichment, $P < 0.001$). Biological processes enriched at -1 wk, d0, and 1 wk post-calving included the immune system process, response to stimulus, and the immune response. The immune response process was most highly enriched (8-fold enrichment, $P < 0.05$) at 1 wk post-calving. No biological processes were enriched at 2 wk and 4 wk post-calving. Results indicate that the gene expression profile of neutrophils is altered over the transition period. This provides insight into neutrophil activity around parturition, which may play a vital role in the increased susceptibility to disease during this time.

AG19: Towards embryo-based genomic selection of cattle

Oback, B.[1], Couldrey, C.[1], Hyndman, D.L.[2], Auvray, B.[2], Fisher, P.J.[2], Oback, F.C.[1], Popovic, L.[1], McGowan, L.T.1 and Wells, D.N.1

1Reproductive Technologies, AgResearch Ruakura, Hamilton, New Zealand
2Centre for Reproduction and Genomics, AgResearch Invermay, Mosgiel, New Zealand

Cattle breeding has begun to shift from progeny- to genetic-based selection. Juvenile and adult animals have been genotyped with genome-wide single nucleotide polymorphism (SNP) arrays to predict individual breeding values based on agronomically important traits. We aim to genomically select at the preimplantation embryonic stage. Increasing the intensity of genomic selection prior to embryo transfer will i) accelerate the rate of genetic gain and ii) reduce cost associated with producing animals of low-genetic value. Using gametes from elite parents for in vitro fertilisation (IVF), we select the best embryo genotypes within one week. Here we summarize key elements of our embryo-based genomic selection platform and demonstrate its feasibility for predicting calf genotype and generating animals.

IVF embryos were produced by fertilising abattoir or ovum pick-up eggs from elite cows with sex-sorted or non-sorted sperm. Trophectoderm biopsies from high-quality blastocysts were taken before cryo-preserving the embryos. DNA from biopsies (~10-15 cells) was whole-genome amplified and genotyped on bovine Illumina SNP BeadChips. Genotype quality was determined by comparing call rates, replication error rate (including allelic dropout, false heterozygotes and opposite homozygotes), P-C heritabilities and gender calling between the biopsies and corresponding calves. Genotypic data were analysed using linear models and embryo survival data with Fisher's exact test. Overall, biopsied, vitrified and thawed embryos developed to term at rates similar to fresh control embryos. Genotyping quality from amplified DNA was inferior to non-amplified parental DNA obtained from blood. However, following imputation with parental genotypes, the frequency of highly accurate genotypes increased yielding very high concordance (≥95%) between embryo and calf genotypes in 12/14 cases. No obvious correlation between morphological quality of the embryos, or corresponding biopsies, and quality of genotyping was observed. In summary, we show that it is possible to genotype embryos from biopsies without compromising genetic accuracy, precision or embryo viability.

AG20: Genetic variation is associated with mastitis in dairy cows

Walker, C.G.[1], Sheahan, A.J.[2], Williamson, J.H. [2], Lacy-Hulbert, S.J.[2], Turner, S-A.[2]

[1]DairyNZ, Auckland, New Zealand, [2]DairyNZ, Hamilton, New Zealand.

Inflammation of the mammary gland (mastitis) significantly affects productivity of dairy cows. This study identified genetic variations associated with mastitis in heifers.

The presence of *Staphylococcus aureus* or *Streptococcus uberis* was determined by bacteriology on quarter fore-milk samples, collected aseptically from 2,963 heifers across 14 herds. Samples were collected at calving, mid lactation, dry off, and on detection of clinical mastitis (CM). Somatic cell counts (SCC) were also determined on herd test samples, collected on four occasions. Phenotypes, such as sub-clinical infection at calving, were derived.

The genotype of 39 candidate SNPs was determined using Sequenom MASSarray, and compared with the mastitis phenotypes using the R packages GeneticsPed and GenAbel. The polygenic function in GenAbel was used to fit a kinship matrix and the covariates. The residuals of the traits were then used to test for association with genotypes.

Seven SNPs were significantly associated with six mastitis phenotypes and explained 2.7 – 11% of trait heritability. Two SNPs were significantly associated with both subclinical mastitis at calving and overall incidence, and explained 6 and 8% of the genetic variation in those phenotypes respectively. One SNP was associated with incidence of CM due to *S. aureus* and explained up to 11% of the genetic variation. Another SNP was associated with presence of *S. aureus* and explained almost 6% of variation in this phenotype. Two SNPs were associated with incidence of CM due to *S. uberis* with each explaining 3% of the genetic variation in the trait. Another SNP was associated with the incidence of one or more herd test SCC greater than 150,000 cells/mL, and explained 4% of variation. Although several variants were identified that were associated with mastitis phenotypes, further research is needed to establish the economic benefit of including these in selection indices.

AG21: Application of Genotyping-by-Sequencing (GBS) in Atlantic Salmon (*Salmo salar*).

Anderson, R.M.[1], Dodds, K.G.[1], McEwan, J.C.[1], Brauning, R.[1], Van Stijn, T.C.[1], Kristjánsson T.[2], Clarke, S.M.[1]

[1]AgResearch Ltd, Invermay Agricultural Centre, Mosgiel 9053, New Zealand
[2]Stofnfiskur, Staðarberg 2-4, Hafnarfjörður IS-221, Iceland

Advances in next generation sequencing technology have made genotyping-by-sequencing (GBS) a viable and cost effective alternative to more traditional methods for studies of genetic diversity, parentage assignment and genomic selection in livestock and aquaculture species.

High-throughput GBS methodology produces SNP genotypes that are supported by varying depth of sequence reads. The cost effectiveness of the method is determined by the number of samples and proportion of the genome assayed within a lane of sequencing. Selecting the most appropriate combination to sequence will depend on the read depth required to validate the SNP genotypes for use in the intended downstream application.

Here we present results from Atlantic Salmon (*Salmo salar*) using GBS with the restriction enzymes ApeKI and PstI and the subsequent use of PstI for genetic diversity, parentage assignment and genomic selection studies. This work demonstrates how this approach can be implemented at low cost in a non-traditional species that contains a partially duplicated genome.

AG22: Adventures in next-generation sequencing

Taylor. J

University of Missouri, 920 East Campus Drive, Columbia, MO 65211-5300

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.

AG23: Mutation in MRPS34 Compromises Protein Synthesis and Causes Mitochondrial Dysfunction.

Richman, T.R.[1], Ermer, J.A.[1], Davies, S.M.K.[1], Perks, K. L.[1], Viola, H.[2,3], Hool, L.[2,3], Shearwood. A-M.J.[1], Hool, L.C.[2,3], Rackham, O.[1,4] ,Filipovska, A.[1,4]

[1]Harry Perkins Institute of Medical Research, Centre for Medical Research, QEII Medical Centre, The University of Western Australia, Nedlands, Western Australia, Australia [2] School of Anatomy, Physiology and Human Biology, The University of Western Australia, Crawley, Western Australia, Australia [3] Victor Chang Cardiac Research Institute, Darlinghurst, New South Wales, Australia [4] School of Chemistry and Biochemistry, The University of Western Australia, Crawley, Western Australia, Australia.

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.

AG24: Investigation of the influence of *DGAT1* K232A on gene expression in the bovine lactating mammary gland

Fink, T.[1], Lopdell, T.[1,2], Littlejohn, M.D.[2],

[1]School of Biological Sciences, University of Auckland, Auckland, NZ, [2] Livestock Improvement Cooperation, Hamilton, NZ,

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.

References

1. Grisart B, Farnir F. *Genetic and functional confirmation of the causality of the DGAT1 K232A quantitative trait nucleotide in affecting milk yield and composition*. Proc Natl Acad Sci. 2004;101. Available: http://www.pnas.org/content/101/8/2398.full.pdf+html&gt

2. Littlejohn MD, Tiplady K, Lopdell T, Law T A, Scott A, Harland C, et al. *Expression variants of the lipogenic AGPAT6 gene affect diverse milk composition phenotypes in Bos taurus*. PLoS One. 2014;9: e85757. doi:10.1371/journal.pone.0085757

AG25: 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.

Summary of Abstracts for the Poster Session

No.	Title	Presenter	Institutions
AG26	Determining the association of CD18 with susceptibility to pneumonia in NZ ruminants	McRae, K.M	AgResearch, Invermay Agricultural Centre, Mosgiel, NZ.
AG27	Assessment of Illumina BovineSNP50 to Sequence Imputation Accuracy in Beef Cattle	**Dr. Duc Lu[1]**, Dr. Stephen Moore[2], Dr. Mehdi Sargolzaei[3], Dr. Flavio Schenkel[3], Dr. Honghao Li[3], Dr. Paul Stothard[4], Dr. Manhong Ye[5], Dr. Stephen Miller[1,3,4]	1 - Invermay Agricultural Centre, AgResearch Ltd., Dunedin, New Zealand, 2 - University of Queensland, St. Lucia, Australia, 3 - University of Guelph, Guelph, Canada, 4 - University of Alberta, Edmonton, Canada, 5 - Yangzhou University, , China
AG28	Prediction of genomic breeding values for carcass and meat quality traits in a multi-breed sheep population using a HD SNP chip	**Mr. L.F. Brito[1,2]**, Dr. S.P. Miller[1], Dr. M.A. Lee[3], Dr. K.G. Dodds[1], Dr. F.S. Schenkel[2], Dr. N.K. Pickering[4], Dr. W. Bain[1], Dr. J.C. McEwan[1], Dr. S. Clarke[1]	1 - AgResearch / Invermay Agricultural Centre, Mosgiel, New Zealand, 2 - University of Guelph, Guelph, Canada, 3 - University of Otago, Dunedin, New Zealand, 4 - Focus Genetics, Napier, New Zealand
AG29	A pipeline for genetic analysis of the rumen microbiome and associated methane emissions	**Dr. Graham Wood[1]**, Dr. Suzanne Rowe[1], Dr. Sandra Kittelmann[1], Dr. Peter Janssen[1], Dr. Siva Ganesh[1], Mr. Arjan Jonker[1], Dr. Ken Dodds[1],	1 - AgResearch, Mosgiel, New Zealand

		Mr. John McEwan[1]	
AG30	Application of Genotyping-by-Sequencing (GBS) in Livestock	**Miss. Tracey Van Stijn[1]**, Dr. Rudiger Brauning, Dr. Ken Dodds, Dr. Suzanne Rowe, Mr. John McEwan, Dr. Shannon Clarke	1 - AgResearch Limited, Dunedin, New Zealand
AG31	A method for assessing sperm penetration in single cell bovine embryos	**Ms. Michelle French[1]**, Ms. Robin McDonald[2], Dr. Sara Edwards[1], Mrs. Anita Ledgard[2], Mr. Martyn Donnison[2], Dr. Susanne Meier[3], Dr. Debbie Berg[2]	1 - AgResearch Limited, Dunedin, New Zealand, 2 - AgResearch Limited, Hamilton, New Zealand, 3 - DairyNZ Limited, Hamilton, New Zealand
AG32	Determining the association of CD18 with susceptibility to pneumonia in NZ ruminants	**Dr. Kathryn McRae[1]**, Dr Suzanne Rowe[1], Ms Hayley Baird[1], Dr Shannon Clarke[1]	AgResearch, Invermay Agricultural Centre, Mosgiel, New Zealand
AG33	A linkage study of sheep keratin and keratin-associated protein genes with wool traits	**Mrs. Hannah Henry[1]**	AgResearch Limited, Dunedin, New Zealand
AG34	Designing a Methylation Chip for Sheep	**Dr. Rudiger Brauning[1]**, Dr. Christine Couldrey[2], Mr John McEwan[1], Dr. Shannon Clarke[1]	1 - AgResearch, Mosgiel, New Zealand, 2 - LIC, Hamilton, New Zealand
AG35	Genotyping-by-Sequencing application in Chinook Salmon	**Miss. Hayley Baird[1]**, Miss Rachel Ashby[1], Dr Jane	1 - AgResearch Limited, Dunedin, New Zealand,

		Symonds[2], Mr Jon Bailey[3], Dr Shannon Clarke	2 - NIWA, Ruakaka, New Zealand, 3 - The New Zealand King Salmon Co Ltd, Nelson, New Zealand
AG36	AG21: Application of Genotyping-by-Sequencing (GBS) in Atlantic Salmon (Salmo salar).	**Rayna Anderson,** M.1, Dodds, K.G.1, McEwan, J.C.1, Brauning, R.1, Van Stijn, T.C.1, Kristjánsson T.2, Clarke, S.M.1	[1]AgResearch Ltd, Invermay Agricultural Centre, Mosgiel 9053, New Zealand [2]Stofnfiskur, Staðarberg 2-4, Hafnarfjörður IS-221, Iceland