Perturbation of Small non-coding RNA Biogenesis in Human Brains of ALS Disease

Dr Rehab F. Abdelhamid
1Department of Neurology, Osaka University Graduate school of Medicine, Suita, Japan, 2Department of Statistical Genetics, Osaka University Graduate School of Medicine, Suita, Japan

Amyotrophic lateral sclerosis (ALS) is a debilitating, non-curable neurodegenerative disease. The adult onset starts with muscle weakness and the progression of the disease causes respiratory disorder leading to death usually within 3~5 years of the onset. Several studies have focused on understanding the metabolism of RNA and its implication in disease processes, but abnormal RNA biogenesis is still unknown. Here we report the perturbation of small ncRNA biogenesis in postmortem sporadic ALS Human Brain associated with TDP-43 aggregation.

Keywords: Amyotrophic lateral sclerosis (ALS), TAR DNA binding protein (TDP-43), small non-coding RNA, neurodegenerative disease.

Acknowledgements: This work was supported by the Grant-in-Aid for Scientific Research (C) [16K09690 to S.N.], grants from Japan Foundation for Neuroscience and Mental Health and Strategic Research Program for Brain Sciences (to S.N.)
Genomic HLA as a Predictive Marker for Overall Survival in Non-Small Cell Lung Cancer (NSCLC) Patients Post Immunotherapy

**Abed A**, **Calapre L**, **Chopra A**, **Khattak A**, **Millward M**, **Gray E**
1 Linear Clinical Research, Nedlands, Western Australia, Australia
2 Edith Cowan University, Joondalup, Western Australia, Australia
3 Institute for Immunology and Infectious Diseases (IIID), Murdoch University, Murdoch, Western Australia, Australia
4 St John of God Hospital, Subiaco, Western Australia, Australia
5 Department of Medical Oncology, Fiona Stanley Hospital, Murdoch, Western Australia, Australia
6 Department of Medical Oncology, Sir Charles Gairdner Hospital, Nedlands, Western Australia, Australia

**Background**: We aimed to assess the effect of HLA homozygosity in the overall survival (OS) of patients with advanced NSCLC treated with immunotherapy. **Method**: We collected blood from 170 patients treated with immune checkpoint inhibitors. High quality DNA was extracted from white blood cells and used for high resolution HLA-I/II typing, to derive HLA alleles and HLA class I supertypes possession in the cohort. Tumour PDL1 status, pre-treatment neutrophil to lymphocyte ratio (NLR) and sex were recorded. Each of those variables were correlated with OS independently and in a multivariate analysis, controlling for interaction effects. **Results**: Our data matured for 156 patients and PDL1 status results were available for 106 patients only. We found that homozygosity at one or more HLA-I loci has an unfavourable statistical trend towards shorter progression free survival (PFS) (HR=1.6, P=0.06) and OS (HR=1.7, P=0.08), comparing to homozygosity at one or more HLA-II loci. This effect was more pronounced in the multivariate analysis, controlling for PD-L1 status, sex and NLR, with HLA-I homozygosity showing a HR=2.6, P=0.02 for PFS and a HR=3.7, P=0.02 for OS. The interaction test showed that there is no statistically significant interaction between HLA-I/II and PDL1. Finally, the presence of HLA-B44 supertype had a positive trend association with improved survival, while HLA-B62 was associated with short survival. **Conclusions**: Our analysis suggest that PFS and OS of NSCLC patients treated with immune checkpoint inhibitors is moderately affected by homozygosity at HLA-I loci and possession of the HLA-B62 or HLA-B44 supertypes.

**Keywords**: Genomic HLA, Non-small cell lung cancer, Immunotherapy, Supertypes, Survival, Homozygosity

**Acknowledgements**: This work was supported by Melanoma Research Group at Edith Cowan University and a grant from the Fiona Stanley Hospital young investigator grant. We thank patients and staff at Sir Charles Gardner Hospital and Fiona Stanley hospital.
Analysis of genome-wide association data identifies shared loci and molecular genetic mechanisms for endometriosis and depression

Mr Emmanuel Adewuyi1, Professor Dale Nyholt1
1Queensland University Of Technology, Brisbane, Australia

Evidence from population studies indicates endometriosis and depression often co-occur. However, conflicting evidence exists, and the aetiology and biological mechanism(s) underlying their comorbidity remain unknown. Utilising genome-wide association study (GWAS) data, we investigate the relationship between endometriosis and depression. Single nucleotide polymorphism (SNP) effect concordance analysis (SECA) found a significant genetic overlap between endometriosis and depression. Linkage disequilibrium score regression (LDSC) estimates a positive and highly significant genetic correlation between the two traits. A meta-analysis of endometriosis and depression GWAS identified 20 independent genome-wide significant loci (P < 5 × 10⁻⁸), of which eight are novel. Mendelian Randomisation analysis suggests a causal effect of depression on endometriosis. Combining gene-based association results across endometriosis and depression identified 22 genes with a genome-wide significant Fishers’ combined P-value (FCP_{gene} < 2.75 × 10⁻⁶). Genes with nominal gene-based association (P_{gene} < 0.05) were significantly enriched across endometriosis and depression, and genes overlapping the two traits with P_{gene} < 0.1 and FCP_{gene} < 0.05 were significantly enriched for the biological pathways ‘cell-cell adhesion’, ‘inositol phosphate metabolism’, ‘Hippo-Merlin signalling dysregulation’ and ‘gastric mucosa abnormality’. Our study confirms the comorbidity of endometriosis and depression, identifies shared genetically controlled biological mechanisms in their co-occurrence.

Keywords: causal-relationship, comorbidity, depression, endometriosis, gene-based study, genetic overlap, Genome-wide association studies, LDSC, Mendelian Randomisation, molecular genetics
Identification and CRISPR-Mediated Activation of Novel Enhancer Regions for the Cardiac Muscle α-Actin Gene

Miss Georgina A. Allan¹, Dr Rhonda L. Taylor¹, Dr Joshua S. Clayton¹, Prof Alistair R.R. Forrest¹, Dr Gina Ravenscroft¹, Dr Kristen J. Nowak², Prof Nigel G. Laing¹

¹Harry Perkins Institute of Medical Research and Centre for Medical Research, University of Western Australia, Queen Elizabeth II Medical Centre, Nedlands, Australia, ²Office of Population Health Genomics, Public and Aboriginal Health Division, East Perth, Australia

Mutations in the skeletal muscle α-actin gene (ACTA1) cause a range of congenital myopathies, which often lead to death within the first year of life. Current treatment for the ACTA1 diseases is symptomatic rather than curative, necessitating the development of effective therapies. Upregulation of a highly conserved and functionally equivalent alternative isoform of ACTA1, cardiac muscle α-actin (ACTC1), has the potential to compensate for the defective ACTA1 gene. In mouse models, delivery of ACTC1 post-natally ameliorates the clinical phenotype of both recessive and dominant ACTA1 disease. The regulatory mechanisms controlling ACTC1 expression are largely unknown but may be fundamental to achieving therapeutic levels of ACTC1 upregulation. We aimed to identify enhancers for ACTC1 and target these regions with a CRISPR-based activator, dCas9-VPR, to upregulate ACTC1 expression. Interrogation of ENCODE and Hi-C data revealed two putative novel enhancer regions for ACTC1. In Hu5/E18 muscle cells, deletion of these regions with CRISPR-Cas9 significantly reduced ACTC1 mRNA expression, confirming their roles as enhancers. In human embryonic kidney cells, simultaneously targeting the ACTC1 promoter and enhancers with dCas9-VPR increased ACTC1 mRNA expression up to 911-fold. However, upregulation at the mRNA level did not translate to a detectable increase in protein abundance. We propose that cell-type-specific chromatin architecture in kidney cells precludes optimal activation of ACTC1. Thus, further work should be performed in the muscle cell context. This study provides novel insights into the regulatory landscape surrounding ACTC1 and lays the foundation for further investigation of CRISPR-based approaches to ACTC1 upregulation as a therapy for ACTA1 disease.

Keywords: Actin, enhancers, upregulation therapy, CRISPR-Cas9, dCas9-VPR

Acknowledgements: We thank Kin Tung Tam and Hamid Alinejad-Rokny for generating the Hi-C data (unpublished).
HLA Class I allele nucleotide variations in a Middle Eastern population from the United Arab Emirates

Ms Halima Alnaqbi1, Dr Guan Tay1,2,3,4,5, Dr Habiba Alsafar1,2,3
1Center for Biotechnology, Khalifa University of Science and Technology, Abu Dhabi, United Arab Emirates, 2Department of Biomedical Engineering, Khalifa University of Science and Technology, Abu Dhabi, United Arab Emirates, 3College of Medicine and Health Sciences, Khalifa University of Science and Technology, Abu Dhabi, United Arab Emirates, 4Division of Psychiatry, Faculty of Health and Medical Sciences, the University of Western Australia, Nedlands, Western Australia, Nedland, Australia, 5 School of Medical and Health Sciences, Edith Cowan University, Joondalup, Western Australia., Joondalup, Australia

Background and Aims: The human Major Histocompatibility Complex (MHC) on chromosome 6 is characterized by remarkably high levels of polymorphism. Members of the Human Leukocyte Antigens (HLA) multigene families are located within this region. These are typed to match transplant recipients to donors. Although functionally relevant, they only represent a small portion of the 300+ genes in the MHC. Since some non-HLA genes encode products that are important to the immune response, a matching strategy that considers these is necessary. Ancestral Haplotype (AH) is a term used to describe conserved population-specific MHC haplotypes, a continuous 3+ megabases sequence, that has been derived with little change from common ancestors. Consequently, genes of the MHC are physically linked and matching AHs consider non-HLA genes. It has subsequently been revealed that AHs contain stretches of several hundred kilobases that are devoid of recombination. In between, are areas of relatively low levels of polymorphism where recombination events are known occur. These genome stretches have been referred to as “polymorphic frozen blocks (PFB)”. An AH of the MHC carries a specific set of PFBs and fragmentation of these AHs have given rise the distribution of MHC haplotypes seen in the contemporary population. The MHC sequences of the UAE and regional populations are not well defined. Studying the variants of the MHC genes and defining the combinations of these (ie. AHs), will improve the clinical applicability of MHC matching.

Methods: To develop an appreciation for the nature of variation found in the MHC of Middle Eastern populations, 1,000 UAE consenting nationals provided samples. These were genotyped using the Illumina Omni5 Exome Bead Chip v.4.0.1. After data pre-processing and QC, 2.3 million (50%) SNPs were retained. All MHC SNPs were extracted, analyzed and visualized using the Integrative Genome Visualization tool.

Results and Conclusions: Genome data suggests that the MHC region in Middle Eastern populations is also highly polymorphic. The pattern of comparative nucleotide diversity with troughs and peak is consistent with previous studies. Regions of low nucleotide diversity were identified at anticipated locations, at the junctions between PFBs including the intersect between α and β where potential recombination hotspots are known to exist. Interestingly, other regions with low levels of polymorphism were also found within previously defined PFBs suggesting high coverage genome data has revealed other potential sites of recombination. These postulated regions of recombination events can be confirmed by segregation analysis in family studies.

Keywords: MHC, HLA, UAE, Middle East
Thyroid Cancer in the UAE: Epidemiological Factors And Molecular Characterization

Fatima Y. Alshamsi1, Sarah Al Ali1, Tamader Al Hoqani1, Amna Ahli1,2, Rashed Saeed Alrahbi3, Habiba Alsafar1,2,4*

1 Department of Biomedical Engineering, Khalifa University, Abu Dhabi, United Arab Emirates.
2 Department of Genetics and Molecular Biology, College of Medicine and Health Sciences, Khalifa University, Abu Dhabi, United Arab Emirates.
3 Oncology Department, Mafraq Hospital, Abu Dhabi, UAE.
4 Center for Biotechnology, Khalifa University, Abu Dhabi, United Arab Emirates.

In 2019 Abu Dhabi Department of Health reported that thyroid cancer is the 3rd most prevalent cancer in Emirati population. Published studies show that the incidents are to rise up to 920 thyroid cancer incidents by 2040. Many genes were found to be associated with the risk of developing thyroid cancer including BRAF and VAV3 genes. RAF protein, produced by the BRAF gene is involved in signaling pathways that determine the growth of cells, but its mutation causes the development of human cancer. The SNP rs3748093 has been reported to have an effect on the level of expression of the BRAF gene. We have conducted a pilot study, to investigate the association of two SNPs; rs3748093 in BRAF and rs4915076 in VAV3 with Thyroid cancer among UAE population.

A total of 1,182 Emirati subjects were recruited from UAE. The Two SNPs were genotyped using Taqman Assay. The allele frequencies were calculated and compared with other ethnic groups (Caucasian, African, East and South Asian, and Ashkenazi Jewish). The genotype frequencies of the SNPs in the Emirati population were as follows: rs3748093 (TT=97.5%, AT=2.5%, AA=0.0%) and rs4915076 (TT=79.9%, TC=19.2%, CC=0.9%). The major allele for both SNPs was the T allele with major allele frequency in rs3748093 and rs4915076 being 98.7% and 89.5%, respectively. The resultant allele frequencies of rs3748093 were found to be significantly different from Caucasian, African, East and South Asian populations and close to Ashkenazi Jewish population. However, the allele frequencies of rs4915076 were found to be similar to all studied populations except for Caucasian, Africans and East Asians. The significance was determined using chi-squared test, and p values less than 0.0001 were considered significant. The frequency distribution of both variants forms a framework for further gene-disease association studies with thyroid cancer. To further investigate the Emirati-specific genetic causes of thyroid cancer, future undergoing studies include performing microarray analyses on both healthy Emirati individuals and subjects affected by thyroid cancer.

**Keywords**: Thyroid, cancer, SNP, UAE, BRAF, RAF, variants

**Acknowledgement**: We gratefully acknowledge the contribution of participating individuals whose cooperation made this study possible. This study was supported by research incentive funds from Dubai Expo awarded to Dr. Habiba Al Safar. Al Mafraq oncology department has also shown great interest in supporting the research project, and providing us with the necessary samples to proceed with this study.
A Genetic Variant of \textit{VKORC1} Is Nominally Associated with Poor Response to Warfarin Among Filipinos

Aimee Yvonne Criselle L. Aman\textsuperscript{1,2*}, Elmer Jasper B. Llanes\textsuperscript{3}, Richard Henry P. Tiongco II\textsuperscript{3}, Eva Maria C. Cutiongco – de la Paz \textsuperscript{1,2}, Jose B. Nevado, Jr.\textsuperscript{1}, Jose Donato A. Magno\textsuperscript{3}, Deborah Ignacia D. Ona\textsuperscript{3}, Felix Eduardo R. Punzalan\textsuperscript{3}, Paul Ferdinand M. Reganit\textsuperscript{3}, Lourdes Ella G. Santos\textsuperscript{4}, Jaime Alfonso Aherrera\textsuperscript{3}, Lauro L. Abrahan IV\textsuperscript{3}, Charlene F. Agustin\textsuperscript{3}, Adrian John P. Bejarin\textsuperscript{1,2}, Rody G. Sy\textsuperscript{3}

\textsuperscript{1}Institute of Human Genetics, National Institutes of Health, University of the Philippines; \textsuperscript{2}Philippine Genome Center, University of the Philippines, Diliman, Quezon City; \textsuperscript{3}Section of Adult Cardiology, Department of Medicine, University of the Philippines – Philippine General Hospital;

Anticoagulants like warfarin are important in the management of coagulable states such as atrial fibrillation and valvular heart diseases. Despite the presence of new oral anticoagulants in the market, warfarin remains as a popular choice in the Philippines due to its low cost and availability. Its effectiveness depends on the maintenance of prothrombin time, which must be achieved within a relatively narrow therapeutic index to avoid either bleeding or increased risk of thromboembolic events. The required dose for warfarin varies from patient to patient, and evidence suggests that response to warfarin is determined by genetics. It is the aim of this study to identify variants that may be associated with poor warfarin response among Filipinos. A total of 85 unrelated Filipinos on warfarin were enrolled in this cross-sectional study that looked at 23 genetic variants associated with warfarin response. Genotyping using DNA from blood samples was done using customized Illumina GoldenGate microarray chips. Candidate variants and clinical data were correlated with poor response to warfarin using chi-square and logistic regression analysis. An intronic variant of \textit{VKORC1} (vitamin K epoxide reductase multiprotein complex 1) exhibited nominal association with poor response to warfarin among Filipinos (AA and AG vs GG: adjusted OR 5.76, 95% CI 1.13, 39.86; p-value 0.0320) even after adjusting for age, sex, and use of warfarin potentiators. Vitamin K epoxide reductase is warfarin's target of action, and polymorphisms in \textit{VKORC1} have been found to affect warfarin dosing. The International Warfarin Pharmacogenetics Consortium (IWPC) recommended the use of a \textit{VKORC1} variant in a dose calculator used to aid clinicians in warfarin prescription. However, the variant used to develop the IWPC calculator is different from the one associated with poor warfarin response in this current study. The results of the current study suggest the need to develop a different dose calculator to determine initial warfarin dosage among Filipinos after further validation studies.

**Keywords:** warfarin, \textit{VKORC1}, pharmacogenetics, Filipinos

**Acknowledgements:** This study is funded by the Philippine Council for Health Research and Development – Department of Science and Technology under the Grants-in-Aid (GIA) Program.
**Cell type and cortex-specific RNA editing in single human neurons informs neuropsychiatric disorders**

Dr Brendan Ansell¹, Mr Simon Thomas¹, Mr Jacob Munro¹, Dr Saskia Freytag², Prof Melanie Bahlo¹

¹Walter & Eliza Hall Institute of Medical Research, Parkville, Australia, ²Harry Perkins Institute of Medical Research, Murdoch, Australia

Conversion of adenosine to inosine in RNA by ADAR enzymes occurs at thousands of sites in the human transcriptome, and is essential for healthy brain development. This process, known as ‘RNA editing’, is dysregulated in many neuropsychiatric diseases, but is little understood at the level of individual neurons. We examined the full-length nuclear transcriptomes of 3,055 neurons from six cortical regions of a neurotypical post-mortem female donor, and identified 40,861 high-confidence edited sites. The majority of sites were located within Alu repeats in introns or 3' UTRs, and were present in previously published RNA editing databases. We identified 15,784 putative novel RNA editing sites, 30% of which were also detectable in independently generated neuronal transcriptomes from unrelated donors. The strongest correlates of global editing rates were expression levels of small nucleolar RNAs from the SNORD115 and SNORD116 cluster (15q11), known to modulate serotonin receptor processing and to colocalize with ADAR2, one of three known RNA editing enzymes in humans. As expected, expression of DNA and RNA binding proteins were negatively associated with editing. We present evidence for dysregulated RNA editing in six rare genetic conditions; and report 117 differentially edited sites between cortical regions and neuronal subtypes. These results provide spatial and neurophenotypic context for 1,871 and 998 sites that are differentially edited in the brains of schizophrenic and autistic patients respectively, and a reference for future studies of RNA editing in single brain cells from these cohorts.

RNA editing, single-cell, neurogenomics, neurotranscriptomics, neuropsychiatry
Late-onset Pompe disease: rescue of acid alpha-glucosidase expression by splice modification

Dr May Aung-Htut1,2, Mrs Kristin Ham1, Dr Frederick Schnell3, Prof Sue Fletcher1, Prof Steve Wilton1,2
1Murdoch University, Murdoch, Australia, 2Perron Institute for Neurological and Translational Science, Nedlands, Australia, 3Sarepta Therapeutics, Cambridge, USA

Recently, there has been an increase in the number of approvals for nucleic acid therapies to treat various diseases. Exondys 51, a splice modulating morpholino oligomer designed and evaluated in our laboratory, has been granted accelerated approval for the treatment of a subset of Duchenne muscular dystrophy patients in the United States. We are extending splice intervention therapy to Pompe disease, also known as glycogen storage disease II (GSDII), caused by a deficiency of the enzyme acid alpha-glucosidase. GSDII is an autosomal recessive disorder, and affected individuals are unable to degrade glycogen stored within lysosomes, leading to an accumulation of glycogen in tissues, mainly muscles. Clinically, GSDII may range from severe/infantile to a milder late-onset adult form. The most common GAA mutation associated with the latter is IVS1-13T>G, found in over two-thirds of the adult-onset GSDII patients. The consequence of this mutation is the production of mostly non-functional GAA isoforms due to aberrant exon 2 splicing during pre-RNA processing. Current enzyme replacement therapy for Pompe disease has drawbacks, including immune reaction and poor delivery to target tissues. Therefore, effective alternative therapies are needed.

We sought to enhance GAA exon 2 selection during pre-mRNA processing, using splice-switching antisense oligomers (AO) directed at splice silencer elements to promote recognition and retention of exon 2 in the mature GAA mRNA, thereby restoring enzyme function. Selection of AO sequences that promote GAA exon 2 inclusion in the mature transcript was carried out in fibroblasts derived from five adult Pompe patients. Phosphorodiamidate morpholino oligomers (PMOs) were transfected into patient cells and RT-PCR and acid-alpha-glucosidase enzyme assays were performed. Several PMO sequences showed an increase in the full-length amplicon, GAA expression, and activity. These data show that PMO mediated modification of GAA transcript could have therapeutic potential for a large portion of adult-onset Pompe patients.

**Keywords:** Pompe disease, antisense oligonucleotides, splice modification

**Acknowledgements:** This work was supported by Sarepta Therapeutics, Cambridge MA. A Strategic Infrastructure grant was given from the Perron Institute for Neurological and Translational Science.
Association of Genotypes and Haplotypes of Tumour Necrosis Factor –Alpha (Tnf-α) With Overweight/Obesity Related Phenotypes in Malaysian Population

Mrs Nur Ashikin Azemi1,2, Associate Professor Nik Ritza Kosai Nik Mahmood3, Dr Hazwanie Hashim2
1Women and Children Hospital Kuala Lumpur, Kuala Lumpur, Malaysia, 2International Medical University, Kuala Lumpur, Malaysia, 3Universiti Kebangsaan Malaysia Medical Centre, Kuala Lumpur, Malaysia

Obesity is classified as low-grade chronic inflammatory disease and genetic predisposition may play a role in the susceptibility to obesity development. Previous studies have shown that pro-inflammatory cytokine, TNF-α promoter region polymorphism was associated with overweight/obesity in various population. Therefore, this study aims to investigate the association of three single nucleotide polymorphisms of TNF-α (G-308A, C-863A and G-238A) and their haplotypes with the risk of overweight/obesity in Malaysian population. Classification of overweight/obesity followed the Malaysian Clinical Practice Guideline of Obesity (2004). Polymorphisms of TNF-α (G-308A, C-863A and G-238A) were evaluated on 105 overweight/obese subjects and 100 non-overweight/ non-obese subjects as controls through tetra-arms PCR. Chi square and logistic regression analysis were done using SPSS software while haplotype and linkage disequilibrium analysis were done using HaploView 4.2 software. The rare A alleles of TNF-α G-308A, C-863A and G-238A did not occur more frequently in overweight/obese individual as compared to controls. Similar results were obtained in allele frequency comparison among Chinese and Korean population for G-308A, G-238A and C-863A polymorphism. Although weak linkage disequilibrium was found between the markers tested, there were six haplotypes occurred in more than 1% of the population, with 308G/-863C/-238G as the most frequent haplotypes. This study recorded no association of TNF-α polymorphism with overweight/obesity in Malaysian population.

Keywords: Obesity, TNF-α, SNP, Haplotypes

Acknowledgements: This project is a collaboration between International Medical University and University Kebangsaan Malaysia Medical Centre and was funded by International Medical University [ID: BPI-1/13(27)2016]. My special thanks to Dr Roziana Ariffin, Head of Pathology Department, Women and Children Hospital Kuala Lumpur for allowing me the opportunity to further my studies.
VariantSpark: Generating Polygenic Risk Scores by Incorporating Higher-Order Epistasis Interactions

Dr Arash Bayat¹, Mr Piotr Szul², Mr Aidan O’Brien¹, Mr Rob Dunne², Mr Yatish Jain¹, Mr Brendan Hosking¹, Dr Denis Bauer¹

¹Health and Biosecurity, CSIRO, Australia, ²Data61, CSIRO, Kellyville, Australia

Body: Polygenic Risk Score (PRS) analysis has been accepted as a more accurate approach for identifying and combining risk indicators for complex diseases compared to single loci approaches. However, existing PRS models are limited to the additive effect of individual SNPs. As more evidence emerges of complex interactions between genomic loci, a more sophisticated PRS approach is required to adequately describe risk for some diseases. Here, we introduce VariantSpark, which can build risk models based on large number of SNPs that are able to capture both additive and complex effect. VariantSpark is available as a cloud-based solution, offering privacy and ownership preserving interactive analyses that are scalable to large cohorts. We benchmark the performance of VariantSpark against traditional (logistic regression) as well as standard PRS approaches on real and synthetic data.

Keywords: Polygenic Risk Score, Machine Learning, Cloud Computing
Low Pass Whole Genome Sequencing as a Method of Determining Copy Number Variations in Uveal Melanoma Tissue Samples

Aaron B Beasley¹, Jacqueline Bentel², Richard JN Allcock³, Tersia Vermeulen², Leslie Calapre¹, Timothy Isaacs⁴,⁵,⁶,⁷, Melanie R Ziman¹,³, Fred K Chen⁵,⁶,⁷, Elin S Gray¹,⁶

¹School of Medical and Health Sciences, Edith Cowan University, Joondalup, WA, Australia, ²Anatomical Pathology, PathWest Laboratory Medicine, Fiona Stanley Hospital, Murdoch, WA, Australia, ³School of Biomedical Sciences, University of Western Australia, Crawley, WA, Australia, ⁴Perth Retina, Subiaco, WA, Australia, ⁵Lions Eye Institute, Nedlands, WA, Australia, ⁶Centre for Ophthalmology and Visual Science, University of Western Australia, Crawley, WA, Australia, ⁷Department of Ophthalmology, Royal Perth Hospital, Perth, WA, Australia

Body: Different somatic copy number alterations are routinely used to accurately predict uveal melanoma patient prognosis. Many laboratories world-wide use multiplex ligation-dependent probe amplification to test these chromosome aberrations. This technique requires a relatively large amount of input DNA, often unattainable from small tumours using fine needle aspirate biopsies. If unsuccessful, no further biopsies are attempted. In this regard, a technique where small biopsies unsuitable for multiplex ligation-probe amplification would be beneficial to provide accurate prognostication to patients who would be deprived of this information. Herein we tested whole genome amplification of 1 ng of DNA, followed by low-pass whole genome sequencing to detect somatic copy number alterations associated with prognosis in uveal melanoma in 21 formalin-fixed-paraffin-embedded uveal melanoma samples. An unweighted Cohen’s κ was used to assess the agreement between both techniques for each individual chromosome arm (1p, 3p, 3q, 6p, 6q, 8p, and 8q) using the classifications “loss,” “neutral,” or “gain” for each arm. In our study we found that whole genome amplification followed by low-pass whole genome sequencing was able to detect somatic copy number alterations found in uveal melanoma. High concordance between multiplex ligation-dependent probe amplification and sequencing was found, with a Cohen’s κ of 0.856 (BCa 95% CI 0.770-0.94). Only 13 of 147 (8.8%) of chromosomes were discordant, with low-pass whole genome sequencing predominantly detecting more changes. Our study indicates that whole genome amplification followed by low-pass whole genome sequencing might be a suitable alternative to multiplex ligation-dependent probe amplification in cases where limiting DNA yields prevent the successful analysis of chromosomes associated with prognosis in uveal melanoma, and therefore provide patients with their prognosis in cases where they would otherwise go without.

Keywords: uveal melanoma, NGS, MLPA, WGA, LP-WGS

Acknowledgements: Supported by an Edith Cowan University Postgraduate Scholarship and a Cancer Council of Western Australia PhD Top Up Scholarship (A.B.). EG is supported by fellowships from the Cancer Research Trust and the Cancer Council of Western Australia. This study was funded by a Raine Medical Research Foundation Priming Grant and an Ophthalmic Research Institute of Australia Grant to EG.
Recurrent pregnancy losses and chromosomal imbalances: Genetic counselling and preimplantation genetic diagnosis indication using next-generation sequencing technology

Dr Nouha Bouayed Abdelmoula1, Dr Sonda Kammoun1, Dr Fatma Abid1, Mme Balkiss Abdelmoula1, Dr Khaled Trigui1, Dr Walid Smaoui1, Dr Jamel Feki1, Dr Mouna Rekik1, Dr Samir Aloulou1, Dr Saloua Ben Amor1

1UR17ES36, Medical University of Sfax, Sfax, Tunisia

Objective: During our practice as genetic counsellor at the Medical University of Sfax, we have seen many couples who are carriers of reciprocal or robertsonian translocations and who failed to conceive because of recurrent pregnancies losses of imbalanced embryos. Only few couples were able to experience preimplantation genetic diagnosis (PGD). Here, we describe the reproduction experience of a Tunisian couple for who cytogenetic analysis showed a maternal balanced reciprocal translocation t(13;20).

Patient(s): A Tunisian couple for who cytogenetic analysis was carried out after the birth of a first malformed baby. Result(s): The foetus was a survivor of a twin pregnancy complicated by an early loss of a conceptus at 15 weeks’ gestation. Prenatal ultrasound showed bilateral cleft lip/palate and high umbilical artery resistance indices. The pregnancy was sustained with a cesarean delivery. Perinatal cytogenetic analysis of the stillbirth showed an extra supernumerary marker chromosome that was compatible with a partial trisomy 13 and 20 due to a 3:1 segregation of the t(13;20) maternal translocation. During two years later, the couple experienced three early miscarriages. Consequently, they choose to conceive by using assisted reproductive technology (ART)-PGD cycles. Unfortunately, they failed to conceive either by using this reproductive alternative. Conclusion: PGD is an established option for chromosomal translocation carriers, but the success rates for having a child varies with the chromosomal rearrangement, the age of the female partner and the ART center. Nevertheless, results of PGD in couples with recurrent pregnancy losses (RPL) were controversial especially when using fluorescence in situ hybridization (FISH) assays and the routine implementation of PGD was pronounced as ineffective in reducing miscarriage rates at 2008. In Tunisia where PGD treatment is not available, such couples need to balance the financial and emotional burden of their treatment. Currently, next-generation sequencing (NGS) technology seems to be better in performing an extensive comprehensive chromosome screening/diagnosis and increases diagnostic accuracy when compared with molecular cytogenetic methods.

Keywords: Pregnancy loss, assisted reproductive technology, chromosomal translocation, preimplantation genetic diagnosis, next-generation sequencing

Acknowledgements: The full team of UR17ES36
The Use of Circulating Tumour DNA (ctDNA) for Molecular Profiling in Patients with Ovarian Cancer

Dr Leslie Calapre¹, Dr Tindaro Giardina², Mrs Anna Reid¹, Mrs Michelle Pereira¹, Dr Ashleigh McEvoy¹, Dr Colin Stewart²,³, Dr Benhur Amanuel¹,²,³, Dr Tarek Meniawy⁴,⁵, A/Prof Elin Gray¹

¹School of Medical and Health Sciences, Edith Cowan University, Joondalup, Australia, ²Anatomical Pathology, PathWest Laboratory Medicine, QEII Medical Centre, Nedlands, Australia, ³School of Biomedical Sciences, University of Western Australia, Crawley, Australia, ⁴School of Medicine, University of Western Australia, Crawley, Australia, ⁵Department of Medical Oncology, Sir Charles Gairdner Hospital, Nedlands, Australia

Biomarkers that can aid in the management of cancer patients is an important clinical requirement, more so for high grade serous ovarian cancer (HGSOC) as it is one of the deadliest gynaecological malignancies. Plasma-derived ctDNA has the potential to be a useful biomarker of treatment response in HGSOC patients. Mutations in TP53 are present in approximately 90% of HGSOCs, but are dispersed across all exonic regions of the gene. This underscores the need for a robust next generation sequencing (NGS) methodology for mutational analysis. In this study, we compared the suitability of the Accel (Swift Biosciences, Ann Arbor USA) and Oncomine (ThermoFisher, Massachusetts USA) panels for identification of TP53 mutations in plasma ctDNA of HGSOC patients (N=10). These two NGS panels differ primarily on the exonic coverage of TP53 (Accel = 100% vs Oncomine = 80%) and use of unique molecular identifiers (UMIs; Oncomine only). Of the 10 patients analysed for TP53 mutations using the Accel panel and the Erase-Seq bioinformatics pipeline, nine somatic nucleotide variants (SNVs) were identified across all exons of TP53 in 6 out of 10 patients (60%). Plasma ctDNA were sequenced to a median coverage of 33,563X with a range of 1,000X–62,081X, and limit of detection of 0.2% frequency abundance (FA). By contrast, the addition of UMIs in the Oncomine panel improved ctDNA detection in HGSOC patients, with 21 SNVs detected in plasma of all 10 patients analysed. Of note, there were 4 SNVs that overlapped between the two NGS panels. Overall sequence coverage using the Oncomine panel ranged between 21,021X-153,440X, with a median coverage of 69,773. Molecular coverage for each loci is approximately 1000X and limit of detection ranged between 0.03-0.86% FA. Orthogonal validation using droplet digital PCR of the 14 of the 21 SNVs found via Oncomine NGS respectively, confirmed 80% (12/15) of mutations identified by the panel. However, only five of the 8 mutations (63%) identified by the Accel panel were confirmed by ddPCR, including the four mutations that were also detected in both NGS panels. Overall, this proof-of-principle study demonstrated the utility of UMI-tagged NGS panel for TP53 mutation screening in ctDNA of HGSOC patients.

Keywords: Circulating Tumour DNA, High Grade Serous Ovarian Cancer, TP53, Next Generation Sequencing

Acknowledgements: We acknowledge and thank all the patients and their carers for their help in the study. We also extend our thanks to Dr Cristian Lonescu-Zanetti and Mr Jeff Jensen for their help with the Erase-Seq bioinformatics pipeline.
Rescue of Fibrillin-1 Microfibril Formation in Fibroblasts from Individuals with Marfan Syndrome

Miss Jessica Cale¹,²,³, Professor Sue Fletcher¹,², Professor Steve Wilton¹,²,³

¹Murdoch University, Murdoch, Australia, ²Centre for Molecular Medicine and Innovative Therapeutics, Murdoch, Australia, ³Perron Institute for Neurological and Translational Sciences, Nedlands, Australia

Marfan syndrome is one of the most common dominantly inherited connective tissue disorders, affecting 2-3 in 10,000 individuals, and is caused by one of over 2700 unique FBN1 mutations. Such mutations result in production of two different fibrillin-1 monomers that are unable to form functional microfibrils, resulting in destabilisation of the extracellular matrix. Disease management requires invasive surgical intervention and use of medications aimed at slowing disease progression, thus the need for new therapeutics. We aim to use short, synthetic nucleic acid sequences called antisense oligonucleotides to manipulate FBN1 exon selection during pre-mRNA splicing. FBN1 exon 59 harbours over 20 unique mutations, encodes one of 43 repeated motifs and its removal does not alter the reading frame, therefore we hypothesise that removing exon 59 will allow production of identical monomers capable of forming functional microfibrils. Antisense oligonucleotide sequences were optimised using 2'-O-Methyl modified bases on a phosphorothioate backbone (2'OMe PS), transfected into healthy control and patient fibroblasts. The most effective sequence was synthesised as a phosphoramidate morpholino oligomer (PMO), a chemistry shown to be safe and effective clinically. Transfected cells were assessed for fibrillin-1 expression and morphology via immunofluorescent staining. Exon 59 was skipped in ~45% of FBN1 transcripts in healthy cells transfected with the 2'OMe-PS antisense oligonucleotides, whereas PMO-59 induced exon 59 skipping in healthy (50%), c.7205 2A>G (90%) and p.Arg2414X (80%) patient cells after 10 days in culture. Immunofluorescent staining revealed a corresponding increase in fibrillin-1 microfibrils when target exon skipping was greater than 75%. In conclusion exon 59 can be efficiently removed from FBN1 pre-mRNA in fibroblasts from two different Marfan syndrome patients, resulting in increased microfibril formation. We show proof-of-concept that removal of mutation harbouring exons from FBN1 pre-mRNA allows the production of internally truncated but identical monomers capable of forming microfibrils, potentially reducing disease severity.

Keywords: Marfan syndrome, Antisense oligonucleotide, Molecular therapy, Alternative splicing
Evaluation of Novel Antisense Oligonucleotide-mediated Splice Modulation for Duchenne Muscular Dystrophy

Suxiang Chen1,2, Bao T. Le1,2, Madhuri Chakravarthy1,2, Kamal Rahimizadeh1,2, Tamer R. Kosbar1,2 and Rakesh N. Veedu*1,2
1Centre for Molecular Medicine and Innovative Therapeutics, Murdoch University, Australia, 2Perron Institute for Neurological and Body: Chemically modified antisense oligonucleotide (AO)-mediated exon skipping has been established as a successful strategy for the treatment of Duchenne muscular dystrophy (DMD). Eteplirsen, a fully phosphorodiamidate morpholino (PMO)-modified AO drug has been approved by the US FDA for the treatment of DMD while drisapersen, a fully 2'-O-methyl (2'-OMe) phosphorothioate (PS)-modified AO candidate was rejected based on safety issues. PMO oligos showed excellent safety profile, however, PMO is not compatible with other chemistries to synthesize mixmer AOs which make it more expensive and in addition, large scale production of PMOs is challenging due to difficult synthesis chemistry unlike 2'-OMe-PS chemistry. To overcome this, we envisioned the development of novel morpholino nucleic acid (MNA) to synthesise mixmer oligonucleotides in combination with 2'-OMe-PS nucleotides. In our study, we explored the scope of MNA/2'-OMe-PS mixmer AO (AO1) together with other modified AO candidates such as 2'-deoxy-2'-fluoro (2'-F)/2'-OMe-PS mixmers (AO2, AO3, AO4) and 2'-F/locked nucleic acid (LNA)-PS mixmers (AO5, AO6, AO7) to induce exon-23 skipping in mdx myotubes in vitro. The MNA/2'-OMe-PS mixmer, AO1 achieved a comparable exon-23 skipping to its fully 2'-OMe-PS AO control (~60%) at 400 nanomolar concentration. Furthermore, all of the 2'-F/2'-OMe-PS mixmers (AO2, AO3, AO4) and 2'-F/LNA-PS mixmers (AO5, AO6, AO7) induced higher exon-23 skipping (14%, 31%, 24%, 33%, 33%, 18%) in comparison with their fully 2'-OMe-PS AO control (13%) at extremely low concentration (2.5 nanomolar). Based on our preliminary results, AOs containing mixmer chemistries could be useful in achieving efficient splice modulation in cells.

Keywords: Duchenne muscular dystrophy, Antisense oligonucleotide, Chemical modification, Morpholino nucleic acid, 2'-deoxy-2'-fluoro.

Acknowledgements: RNV acknowledges the financial support from McCusker Charitable Foundation and Perron Institute for Neurological and Translational Science. SC thanks the funding from Perron Institute Top-Up Scholarship and International Tuition Fee Scholarship scheme from Murdoch University. BTL thanks the Murdoch International Postgraduate Scholarships scheme. MC thanks Greg and Dale Higham for financial support. The authors thank Prof. Steve Wilton and Prof. Sue Fletcher for providing H-2Kb-tsA58 mdx cells and guidance in cell culture protocols. The authors thank Prithi Raguraman for assistance towards experiments, Yanying An and Jessica Cale for help towards figure preparation and Kristin West for informative discussion.
The complex regulatory architecture of the Regulators of Complement Activation (RCA) gene cluster: enhancers, duplicated boundaries and more?

Ms Jessica Cheng¹, Dr Joshua Clayton², Dr Rafael Acemel³, Professor José Luis Gómez-Skarmeta³, Dr Rhonda Taylor², Professor John Harley⁴, Professor Elizabeth Quail¹, Associate Professor Daniela Ulgiati¹

¹The University Of Western Australia, Crawley, Australia, ²Harry Perkins Institute of Medical Research, Nedlands, Australia, ³Universidad Pablo de Olavide, Sevilla, Spain, ⁴Cincinnati Children’s Hospital Medical Center, Ohio, US

Body: Complement Receptor 2 (CR2/CD21) is an important B cell receptor which modulates antibody responses and is a known susceptibility gene for the complex autoimmune disease, Systemic Lupus Erythematosus (SLE). CR2 sits within a gene cluster called the Regulators of Complement Activation (RCA), which encodes five other immune modulators with close evolutionary relationships to CR2. These RCA members are also associated with SLE. Whether these genes are transcriptionally co-regulated or if SLE-associated variants in the RCA cluster affect expression of multiple genes is yet to be determined. We hypothesise that members of the RCA are transcriptionally co-regulated by common long-range elements such as chromatin looping and enhancers. Hence, we aimed to characterise the structural and regulatory organisation of the RCA gene cluster in the B cell lineage.

Using 4C-seq, we captured chromatin interactions between CTCF (a master regulator of chromatin looping) sites >400 kb apart across the RCA gene cluster in B cell lines. These data showed that while chromatin looping was extensive in this gene cluster it was constrained to two distinct regions, demonstrating that the RCA cluster spans two adjacent topologically associated domains (TADs). Remarkably, the inter-TAD boundary was located at a large segmental duplication within a gene (CR1) which is also associated with SLE.

Using 4C-seq maps and bioinformatic data, we mapped putative enhancers in the RCA cluster and functionally validated these using reporter gene assays and CRISPR deletion. We successfully isolated a functional enhancer which co-regulates the expression of two RCA genes (CR2 and CD55) in B cells, establishing for the first time that genes in the RCA cluster are transcriptionally co-regulated.

Here, we have shown that the RCA comprises a complex co-transcriptional network. Further work will focus on key SLE-associated variants in the RCA gene cluster, including the CR1 segmental duplication, to determine if this affects expression of multiple immunomodulatory genes in tandem, thereby amplifying its role in disease. Our work defines novel mechanisms which may exacerbate autoimmunity and highlights the importance of chromatin architecture in the genetic aetiology of complex diseases.

Keywords: TADs, enhancers, B cells, CTCF, Regulators of Complement Activation
The Role of Structural Variation in Type 1 Diabetes Development

Mr Mateusz Chilinski\textsuperscript{1,2}, Agnieszka Kraft\textsuperscript{1,2}, Sachin Gadakh\textsuperscript{1}, Kaustav Sengupta\textsuperscript{1}, Prof Dariusz Plewczynski\textsuperscript{1,2} \\
\textsuperscript{1}Centre of New Technologies, University of Warsaw, Warsaw, Poland, \textsuperscript{2}Faculty of Mathematics and Information Science, Warsaw University of Technology, Warsaw, Poland

Body: The poster presents the initial results of the ongoing research on the connection between 3D structure of human genome and its effects on the development of type 1 diabetes. The samples being analysed origin from two Polish families where the child developed diabetes. The methods used in the research consists of using short-read NGS data from Illumina and long-read Oxford-Nanopore data for the Structural Variants and SNP detections. Using the differences between reference genome and the samples of the families, the study shows de novo variants that are present only in the disease affected children and further analyses them in the context of their overlap with the CTCF binding motifs on chromatin domains borders, or chromatin loops anchors, resulting in the list of genes, which expression might have been altered by those SVs and SNPs.

Keywords: Structural Variants, Diabetes, Leukemia, 3D Genomics, New Generation Sequencing, Data Science, Computational Genomics, CTCF, CCD, SNP

Acknowledgements: This work has been supported by Polish National Science Centre (2014/15/B/ST6/05082), Foundation for Polish Science co-financed by the European Union under the European Regional Development Fund (TEAM to DP). The work was co-supported by grant 1U54DK107967-01 "Nucleome Positioning System for Spatiotemporal Genome Organization and Regulation" within 4DNucleome NIH program, and by European Commission as European Cooperation in Science and Technology COST actions: CA18127 "International Nucleome Consortium" (INC), and CA16212 "Impact of Nuclear Domains On Gene Expression and Plant Traits" (INDEPTH).
Hotspot mutation screening of Wilson disease in Thailand

Sermsiri Chitphuk¹, Boonyawish Kunakorn², Wasana Stitchantrakul¹, Manisa Boosabaratana³, Atchara Tunteeratum³, Donniphat Dejsuphong¹

¹Office of Academic Affairs, Research, and Innovation, Faculty of Medicine Ramathibodi Hospital, Mahidol University, ²Doctor of Medicine Program, Faculty of Medicine Ramathibodi Hospital, Mahidol University, ³Division of Medical Genetics, Department of Internal Medicine, Faculty of Medicine Ramathibodi Hospital, Mahidol University

Body: Mutation in ATP7B gene product, copper-transporting ATPase 2 protein, leads to defect in metabolism and deposition of copper in Wilson disease patients. Accumulation of copper in liver, brain and various internal organs contributes to hepatitis, liver cirrhosis and neurological defects. Wilson disease is inherited in an autosomal recessive pattern. ATP7B is the single causative gene and spans 21 exons. Molecular testing of carrier and patients suspected of Wilson disease requires whole gene DNA sequencing which is not cost effective and time consuming in Thailand. Mutation database has shown that hotspots of ATP7B mutation in exon 8, 12, 13 are the most prevalent in Asian population. We have developed PCR-RFLP technique for screening of Asian hotspot mutations of ATP7B gene in positions c.2333 G>T (p.Arg778Leu), c.2755 C>G (p.Arg919Gly), c.2975 C>T (p.Pro992Leu) and c.3443 C>T (p.Ile1148Thr). This screening method successfully detects mutations in Wilson disease patients and could be used as a screening test in carriers and patients in Thai population with clinical cost reduction and turnaround time in the country.

Keywords: Wilson disease, ATP7B, PCR-RFLP

Acknowledgements: This research was supported by Office of Academic Affairs, Research, and Innovation and Division of Medical Genetics, Department of Internal Medicine, Faculty of Medicine Ramathibodi Hospital, Mahidol University.
Micro-particles Amount In The Peripheral And Uterine Blood In Women With Atypical Hyperplasia And Endometrioid Endometrial Cancer.

**Olaf Chmura³, Barbara Zapala*¹, Marek Dziechciowski², Monika Piwowar³, Katarzyna Gawlik¹, Dorota Pawlica-Gosiewska¹, Krzysztof Skotniczny⁴, Bogdan Solnica¹, Kazimierz Pityński⁴

¹Department of Clinical Biochemistry, Jagiellonian University Medical College, Cracow, Poland
²Department of Gynecology and Oncology, University Hospital, Cracow, Poland
³Jagiellonian University Medical College, Cracow, Poland
⁴Department of Gynecology and Oncology, Jagiellonian University Medical College, Cracow, Poland
⁵Department of Bioinformatics and Telemedicine, Jagiellonian University Medical College, Cracow, Poland

**Body:** BACKGROUND: Endometrial cancer is one of the most common gynecologic malignancy in developed countries. We hypothesized that amount of circulating micro-particles in blood may be connected with the development of endometrial hyperplasia and endometrial cancer. The aim of this study was to measure the micro-particles amount in uterine venous blood and in peripheral venous blood in women with atypical endometrial hyperplasia and endometrioid endometrial cancer.

MATERIALS AND METHODS: By using flow cytometry (BD Canto II cytometer) we measure micro-particles amount in citrate plasma samples from peripheral and uterine venous blood of women with atypical hyperplasia of endometrium or endometrial cancer. We determined the amount of total (TF+), endothelial (CD144+) and monocytic (CD14+) micro-particles.

RESULTS: In this paper we showed higher micro-particle levels in women with atypical hyperplasia of endometrium or endometrial cancer in comparison to healthy women. In blood samples from uterine veins the circulating micro-particle levels were statistically significant different from peripheral blood samples. Very important was that we showed statistically significant differences between micro-particle levels in plasma samples from peripheral blood of women with both atypical hyperplasia of endometrium and endometrial cancer when compared to the control group of healthy women.

CONCLUSION: These results strongly suggested that the level of circulating micro-particles may be a sign of endometrial cancer development, however the detailed study is needed focusing on molecular processes passed through this small circulating molecules.

**Keywords:** Microparticles, Endometrial Cancer, Uterine blood, Atypical hyperplasia, Flow Cytometry

**Acknowledgements:** The study was financially supported by the Ministry of Science and Higher Education (grant No:N41/DBS/000266
Mate-pair Genome Sequencing Reveal Complexities in Karyotypic Simple Insertions

Dr Richard Choy, Zirui Dong, Matthew Hoi Kin Chau, Yvonne K Kwok, Cheung Sau Wai, Kwong Wai Choy, Zhang Yanyan, Yang Zhenjun

Department of Obstetrics & Gynaecology, The Chinese University of Hong Kong, Hong Kong, China, Zirui Research Institute, The Chinese University of Hong Kong, Shenzhen, China, The Chinese University of Hong Kong-Baylor College of Medicine Joint Center For Medical Genetics, Hong Kong, China, Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, United States

Background: Insertions are genomic rearrangements in which chromosome segments are inserted into non-homologous chromosomes or non-adjacent loci on the same chromosome. Phenotypic consequences of insertions can vary depending on the size and gene content of the inserted fragment and constitutes ~2% of nonrecurrent copy-number gains. Carriers of apparently balanced insertions are at risk (up to 50%) of passing derivative chromosomes to their offspring. Recently, genome sequencing (GS) showed advantages in detecting balanced/unbalanced structural rearrangements missed by conventional cytogenetics. Herein, we utilized mate-pair GS to detect the rearrangements, to delineate breakpoint junctions fine-mapped at nucleotide resolution of complex rearrangements and to better understand the molecular mechanisms of formation.

Methods: Mate-pair GS (70M read-pairs, paired-end 100bp) with large insert sizes (3~8-kb) was performed on 12 patient samples with simple insertions (3 breakpoints) by previous G-banded chromosome analyses; 7 cases with unbalanced insertions and 5 cases with balanced insertions. Detection of chimeric read-pairs with mapped distance >10-kb or to different chromosomes support rearrangement breakpoints and subsequent sanger sequencing pinpoint the breakpoints to nucleotide resolution. The orientations, rearrangements of the inserted segment(s) and the sequence features at the breakpoint junctions were investigated.

Results: Mate-pair GS not only detected all breakpoints identified by karyotype analysis in our cohort, but also revealed that complex rearrangements (including sub-microscopic rearrangements and inversions within the inserted segment) were involved in 9/12 (75%) of these apparently simple insertions. A total of forty-five breakpoints were detected among the 12 cases. These breakpoints resided on 12 of the 22 different autosomes. Sanger sequencing mapped the breakpoint of 33 (73%) junctions, while the other 12 were not pinpointed yet due to the large PCR products amplified. Sequence analysis at the breakpoints revealed that most junctions (17/33) had microhomologies between the proximal and distal sequences, while 11/33 and 5/13 junctions had blunt ends and short templated insertions (1-12bp), respectively. Involvement of repetitive elements e.g. short interspersed nuclear elements (SINEs) and long interspersed nuclear elements (LINEs) were observed in 5 and 16 junctions, respectively.

Conclusion: Mate-pair GS enables detection of underappreciated chromosome insertions. GS delineated and revealed (1) additional complexities including rearrangements and inversions of the inserted segments with pinpointed breakpoint-junctions, (2) the identification of microhomologies and short-templated-insertions further support that complex insertions may be generated through replication-based mechanisms and multiple template switches. The involvement of repetitive sequences may also contribute to genomic instability and facilitate these genomic rearrangements.

Keywords: chromosomal insertion, mate-pair genome sequencing, structural rearrangement

Acknowledgements: None
Ovine Congenital Progressive Muscular Dystrophy (OCPMD) is a Model of Nemaline Myopathy Caused by a Splice Site Mutation in TNNT1

Dr Joshua Clayton¹,², Ms Elyshia McNamara¹,², Mr Stefan Conijin³, Ms Hayley Goullee¹,², Ms Keren Muthsam⁴, Dr Gabrielle Musk⁴,⁵, Mr David Coote¹,², Dr James Kijas⁶, Dr Alison Testa¹,², Dr Rhonda Taylor¹,²,⁷, A/Prof Mandy O'Hara⁸, A/Prof David Groth⁹, Prof Coen Ottenheijm³, Dr Gina Ravenscroft¹,², Prof Nigel Laing¹,², A/Prof Kristen Nowak¹,²,⁷,¹⁰

¹Harry Perkins Institute of Medical Research, Perth, Australia, ²Centre for Medical Research, University of Western Australia, Perth, Australia, ³Department of Physiology, Institute for Cardiovascular Research, Amsterdam, Netherlands, ⁴Animal Care Services, University of Western Australia, Perth, Australia, ⁵School of Women's and Infants' Health, University of Western Australia, Perth, Australia, ⁶CSIRO Agriculture and Food, Queensland Bioscience Precinct, Brisbane, Australia, ⁷Faculty of Health and Medical Sciences, School of Biomedical Sciences, Perth, Australia, ⁸School of Veterinary and Life Sciences, Murdoch University, Perth, Australia, ⁹School of Biomedical Sciences, Curtin University, Perth, Australia, ¹⁰Western Australian Department of Health, Office of Population Health Genomics, Perth, Australia

Body: Ovine congenital progressive muscular dystrophy (OCPMD) is a naturally occurring large animal model first described in Merino sheep flocks in Queensland and Western Australia in the 1960s and 70s. The most prominent feature of the disease is a distinctive gait with stiffness of the hind limbs which can be seen as early as one month after birth. The disease is progressive and causes both dystrophic changes and abundant nemaline bodies specifically in slow muscle fibres. It was therefore never certain whether the disease is a dystrophy or a nemaline myopathy with dystrophic features. We performed whole genome sequencing on OCPMD sheep and identified a single base deletion at the conserved splice donor site (+1) of intron 13 of the slow myofibre-specific TNNT1 gene (KT218690 c.614+1delG). Examination of TNNT1 splicing by RT-PCR showed intron retention and premature termination which results in scrambling of the highly conserved 14 terminal amino acids. This change did not reduce TNNT1 protein levels or affect its localization but impaired its ability to modulate muscle contraction in response to calcium levels. Identification of the TNNT1 mutation demonstrates OCPMD is a large animal model of nemaline myopathy with dystrophic features which could be used for testing therapies.

Keywords: Congenital muscular dystrophy, nemaline myopathy, sheep, TNNT1, splicing

Acknowledgements: We gratefully acknowledge funding from the Australian Research Council (Future Fellowship FT100100734; KN), the Australian National Health and Medical Research Council Fellowship (Principal Research Fellowship APP1117510; NL), and the Australian National Health and Medical Research Council Fellowship (Career Development Fellowship APP1122952; GR). We sincerely thank the dedicated and professional staff at the University of Western Australia’s Animal Care and Veterinary Services.
Genetic and Epigenetic Signature Associated with Intracranial Aneurysm Phenotypes-Study from a South Indian Population

CM Shafeeqe1, Sanish Sathyan1, Linda Koshy Vaidyan1, Premkumar S2, Moinak Banerjee*1
1Human Molecular Genetics, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram; 2Department of Neurosurgery, Calicut Medical College, Calicut

Abstract: Intracranial aneurysm (IA) caused due to weakening of arterial wall accounts for 85% of Subarachnoid Hemorrhage. Genetic and expression studies have shown Extracellular matrix remodeling, inflammatory, hypertension and senescence pathways to be associated with IA. Still underlying biology is not completely elucidated mainly due to missing heritability as well as complexity of the phenotype. Understanding the epigenetic mechanism in combination with genetic changes is keen in understanding the complex phenotype like IA. One carbon metabolism (OCM) pathway control methyl group flow and play a critical epigenetic role via influencing methylation. Our study cohorts consist of 235 radiologically confirmed aneurysmal cases and 265 ethnically, age and sex matched controls from the Dravidian Malayalam speaking population of South India. RFLP, KASPar and Sanger’s di-deoxy termination method carried out for genotyping. Out of 24 variants screened, we could establish association for 12 variants from OCM pathway genes (MTHFR, MTR, MTRR, BHMT, CBS and DNMT1) with IA. Our in-silico observations with GTEx data for the associated alleles do indicate an altered expression of these genes, signifying risk alleles of associated variants having possible effect in the accumulation of Homocysteine (Hcy). Similarly, global DNA methylation using ELISA kit (Epigentek) showed hypomethylation in IA cases and that suggests a possible increased Hcy accumulation causing this effect. Using Mass Spectrometry, we found association of metabolites namely Acetylcarnitine, Malonylcarnitine, Arginine, Ornithine, Alanine, Valine and Proline with IA. Our study showed OCM metabolism playing an important role in IA pathogenesis at genetic and epigenetic level. This study also suggests a genotype-specific vitamin intervention in this disease management as well as possible biological signature of IA in this South Indian population.

Keywords: Intracranial Aneurysm, One Carbon metabolism, Metabolic Biomarker, Epigenetic Signature
Acknowledgements: Department of Biotechnology, Govt. of India for fellowship assistance.
Distinct Transcriptomic Profiles for Activated and Resting T Follicular Helper Cells Following a Successful Response to the Influenza Vaccine

Mx Jennifer Currenti, Dr Mark Pilkinton, Mr Christian Warren, Dr Silvana Gaudieri, Mr Ramesh Ram, Ms Rama Gangula, Dr Abha Chopra, Mr Shay Leary, Ms Rita Smith, Dr Spyros Kalams

1University of Western Australia, Crawley, Australia, 2Vanderbilt University Medical Centre, Nashville, USA, 3Institute for Immunology and Infectious Diseases, Murdoch, Australia

Influenza is associated with 291,000-646,000 deaths worldwide, disproportionately affecting individuals above 65 years of age, young children, pregnant women and individuals with chronic medical conditions. A vaccine against influenza is available yearly, but is not 100% effective. A predictor of successful seroconversion in older adults is an increase in activated circulating T follicular helper (cTfh) cells after vaccination. Therefore, it is important to understand the characteristics of an effective cTfh cell response in order to improve future vaccines. To assess this cTfh cell response, four healthy adults who showed an increase in activated cTfh to either the 2016-17 or 2017-18 standard dose vaccine at day 7 were selected. Peripheral blood mononuclear cells were single cell sorted for activated (CD38+ICOShi) and resting (CD38-ICOSlow) cTfh cells. Single cell RNAseq analysis revealed a number of up-regulated immune-related genes, such as TIGIT, GZMB/K, BCL-6, TGFB1 and STAT5A, in activated cTfh cells. As many individuals receive consecutive influenza vaccines, we also sought to assess the transcriptomic profile of the cTfh memory response to the 2017-18 vaccine in one of the four subjects at 28 days after vaccination in 2016 and 2017 using CITE-seq. Cells were stimulated overnight with either the egg eluent or the 2017-18 vaccine. No activated cTfh cells were observed after stimulation with egg eluent, with a greater proportion of activated cTfh cells observed in the 2017 compared to the 2016 sample, suggesting that pre-existing memory responses may be present, and then boosted by subsequent vaccination. Cellular immune responses are important for the efficacy of influenza vaccination, and transcriptomic profiling of activated and resting cTfh cells from these conditions is underway to understand the features of a robust cTfh response to yearly vaccines.

Keywords: T follicular helper cells, influenza vaccine, CITE-seq
The association of a variant near FGF5 with poor response to calcium channel blockers among Filipinos

Dr Eva Maria Cutiongco De La Paz1, 2, Dr Rody Sy2, Dr Jose Jr. Nevada1, Dr Elmer Jasper Llanes2, Dr Jose Donato Magno2, Dr Deborah Ignacia Ona2, Dr Felix Eduardo Punzalan2, Dr Paul Ferdinand Reganit2, Dr Lourdes Ella Santos2, Dr Richard Henry II Tiongco2, Dr Jaime Alfonso Aherrera2, Dr Lauro IV Abrahan2, Dr Charlene Agustin2, Dr Aimee Yvonne Criselle Aman1, 3, Dr Adrian John Bejarin1, 3

1 National Institutes of Health, University of the Philippines Manila, Manila, Philippines, 2 Department of Medicine, University of the Philippines, Manila, Philippines, 3 Philippine Genome Center, University of the Philippines, Diliman, Quezon City, Philippines

One of the most common drugs used for hypertension among Filipinos are calcium channel blockers (CCBs). CCBs prevent the contraction of vascular smooth muscle cells by blocking calcium ion influx, inducing vasodilation that leads to a decreased total peripheral resistance and reduced blood pressure. It is a highly efficacious drug class, but genetic variation has been shown to affect response to calcium-channel blockers among different populations. This replication study aimed to determine the association of selected genetic variants with poor response to this class of antihypertensive drugs among Filipinos. One hundred eighty-one hypertensive participants on calcium-channel blocker therapy were included in an unmatched case-control study: 66 cases and 115 controls. Genotyping using DNA from blood samples was done. To determine association of genetic and clinical variables with poor response to medication, chi-square analysis and logistic regression analysis were used. From the initial 96 variants associated with CCB response and hypertension, a variant found upstream of FGF5 is associated with poor blood pressure-lowering response based on additive effect (CT genotype: adjusted OR 3.41, p=0.001; TT genotype: adjusted OR 6.72, p<0.001) when adjusted for type 2 diabetes mellitus status. The T risk allele frequency of this variant is 21%, which is lower than the allele frequencies among East Asians (39%), admixed Americans (27%), Europeans (27%) and South Asians (25%), and higher than Africans (4%). While there were no prior studies associating this variant with CCB response, it has been associated with hypertension in general. It is possible that the poor response to CCBs among participants carrying the risk allele may be a result of an unregulated FGF5 expression which impacts vascular tone. The modulation of intracellular calcium ion concentrations may be the linking mechanism for FGF signaling, angiotensin II as a vasoconstrictor, and the effect of CCBs on blood pressure. Further studies to investigate the role of this variant may provide further evidence of association with poor response to CCBs prior to clinical application.

Keywords: FGF5, calcium channel blockers, hypertension, Filipino

Acknowledgements: This study was funded by the Philippine Council for Health Research and Development – Department of Science and Technology through the Grants-in-Aid (GIA) Program.
Loss Of Chromosome Y (LOY) In Peripheral Blood Cells Of Aging Men – Longitudinal Variation And Methods For Detection

Mr Marcus Danielsson¹, Jonatan Halvardson¹, Hanna Davies¹, Behrooz Torabi Moghadam¹, Jonas Mattisson¹, Edyta Rychlicka-Buniowska¹,², Janusz Jaszczyrski³, Julia Heintz¹, Lars Lannfelt⁴, Vilmantas Giedraitis⁴, Martin Ingelsson⁴, Jan P. Dumanski¹,², Lars A. Forsberg¹,⁵

¹Dept. of Immunology, Genetics and Pathology and Science for Life Laboratory, Uppsala University, Uppsala, Sweden, ²Faculty of Pharmacy, Medical University of Gdansk, Gdansk, Poland, ³Department of Urology, Maria Sklodowska-Curie Memorial Cancer Centre and Institute of Oncology, Kraków Branch, Kraków, Poland, ⁴Department of Public Health and Caring Sciences, Division of Molecular Geriatrics, Uppsala University, , Uppsala, Sweden, ⁵Beijer Laboratory of Genome Research, Uppsala University, , Uppsala, Sweden

Men have a shorter life expectancy than women but the biological mechanisms behind this sex bias are poorly understood. Mosaic loss of chromosome Y (LOY) is the most common somatic mutation and it is male specific. Men with LOY carry a fraction of blood cells without chromosome Y, due to its loss from hematopoietic progenitor cells during lifetime. LOY was long viewed as a neutral event associated with normal aging, but recent studies challenge this view. LOY has been associated with increased risk for all-cause mortality, various forms of cancer and Alzheimer’s disease, as well as other common human diseases. Here, we present data from serially studied men with up to 22 years of follow up time. A pronounced intra-individual variation in changes of frequency of LOY in blood over time was observed. In some individuals, the frequency of LOY clearly progressed over time and in other men, the frequency was constant or showed other types of longitudinal development. LOY can be estimated from SNP-array data by the median Log R Ratio of probes in the male specific part of chromosome Y (mLRRY). A formula to transform mLRRY-values to percentage of LOY is presented. This formula was derived using measurements from matched samples analyzed using SNP-array, WGS and ddPCR targeting the AMELX/AMELY genes. The methods described can be applied for additional discoveries of LOY associated diseases.

Keywords: Somatic mosaicism, loss of chromosome Y (LOY), genotyping arrays, whole genome sequencing (WGS), droplet digital PCR (ddPCR)
Pineoblastoma is uniquely tolerant of mutually exclusive loss of DICER1, DROSHA or DGCR8

Dr Leanne de Kock1, Dr Barbara Rivera2,3,4, Dr William D. Foulkes3,4,5

1Harry Perkins Institute of Medical Research, QEII Medical Centre and Centre for Medical Research, University of Western Australia, Perth, Australia, 2Program in Molecular Mechanisms and Experimental Therapy in Oncology (Oncobell), IDIBELL, Hospitalet de Llobregat, Barcelona, Spain, 3Gerald Bronfman Department of Oncology, McGill University, Montreal, Canada, 4Lady Davis Institute, Segal Cancer Centre, Jewish General Hospital, Montreal, Canada, 5Research Institute of the McGill University Health Centre, Montreal, Canada

DICER1 syndrome is a pleiotropic tumour predisposition syndrome characterised by a distinctive constellation of neoplastic and dysplastic lesions, which are generally rare and affect children and young adults. Germline pathogenic variants in the DICER1 gene are the underlying cause of the syndrome. The predisposing alterations are most often loss-of-function (LOF) in nature; the second somatic events are predominantly missense alterations involving one of five amino acid residues in the RNase IIIb domain – so-called “hotspot” mutations – which interfere with catalytic cleavage of one strand of a microRNA hairpin precursor. In 2012, we reported a single case of a child with a pineoblastoma and a LOF germline DICER1 pathogenic variant. Unlike all previously studied DICER1-related tumours, the second hit in the pineoblastoma was complete loss of the wild-type chromosome 14q. In follow-up studies, 5 pineoblastomas with DICER1 mutations for which tumour tissue was available also exhibited LOF DICER1 pathogenic variants paired with either another LOF variant or loss of heterozygosity (LOH). Three recent papers confirm our earlier observations: 22 additional pineoblastomas were found to exhibit biallelic DICER1 inactivation.

To answer the question of whether the mechanism of DICER1 alteration in pineoblastoma is truly distinct from other DICER1 syndrome-related tumours, we used a catalogue of DICER1 alterations reported in the literature as of December 2019 to compare the prevalence of LOH and biallelic inactivation in DICER1 syndrome tumours.

Loss of one or both DICER1 alleles was documented in 9% (59/668) of tumours. The tumours were comprised of 9 different types. Prevalence of allele loss ranged from 2–67%, being most prevalent in pineoblastomas (33/49, 67%), followed by DICER1-related central nervous system (CNS) sarcomas (8/35, 23%) (p<0.0001). In total, 32/350 (9%) tumours exhibited biallelic inactivation of DICER1, 30 of which were pineoblastomas.

DICER1-related pineoblastomas predominantly exhibited complete DICER1 loss (30/49 cases, 61%), often bearing one LOF alteration coupled with LOH. Biallelic inactivation of two other microRNA processing genes, DROSHA and DGCR8, have been identified in 31 and 6 pineoblastomas respectively, which too perturb microRNA production.

These findings illustrate that although allele loss and biallelic inactivation of DICER1 are infrequent mechanisms of alteration in DICER1 syndrome-related tumours, they do occur with increased prevalence in certain tumour types, particularly pineoblastoma. An appreciable fraction of pineoblastomas are driven by microRNA processing alterations that cause global microRNA dysregulation. The pineoblastoma cell-of-origin thus appears uniquely capable of tolerating complete or near-complete absence of microRNAs generated through the canonical pathway.

Keywords: Pineoblastoma; microRNA processing genes; DICER1; allele loss; inactivation.
Understanding Differences In Tolerances to Missense Variants Between Populations At The Sequence And Structural Level

Mr Elston Dsouza$^{1,2}$, Mr Michael Silk$^{1,2}$, Dr. Moshe Olshansky$^2$, A/Prof David Ascher$^{1,2}$

$^1$Department of Biochemistry and Molecular Biology, University of Melbourne, Parkville, Australia, $^2$Computational Biology and Clinical Informatics, Baker Heart and Diabetes Institute, Melbourne, Australia

Advances in genomic sequencing technologies have the potential to revolutionize personalized medicine, especially due to decreasing the time and cost of sequencing large populations at scale. This has led to exponential increases in the amount of data available, but over 60% of this data originates from people of European ancestry. Given that heterogeneity exists between ethnicities, particularly with regards to disease risk and molecular evolution, this European ancestral bias has complicated the potential for effective and widespread translation of approaches developed using this data. To address this discrepancy, we aim to identify regions in genes across the human genome that vary in selective pressure between different ethnic populations. Using whole genome datasets from gnomAD v2 and UK Biobank, we classified coding sequences from 184,648 individuals into 8 distinct ethnic populations. Next, the selective pressure acting on each ethnic population was measured at both the gene and protein residue level using the Missense Tolerance Ratio (MTR); a direct unbiased estimate of purifying selection acting on a given region. Genes under differing selective pressures between ethnic populations featured significant deviations from neutral MTR scores. A statistical post-hoc analysis and multivariate outlier detection identified 132 canonical genes that exhibited unique selective profiles across different ethnicities. Furthermore, a STRING analysis revealed a strong network of protein-protein interactions between these genes. Intolerant regions inside genes with the highest variability across different populations were mapped onto experimental protein structures to better understand the structural and mechanistic consequences of missense variants. Our method allows us to screen for differentially-selected genic regions across ethnic groups. Taking into account these differences may provide an improvement over existing in-silico machine learning models that predict the severity of missense variants; paving the way for ethnicity-based drug development and surveillance measures.

**Keywords**: Population genomics, structural biology, missense intolerance, genetic disease, protein structure and function

**Acknowledgements**: UK BioBank, gnomAD, Ensembl
Machine Intelligence Wide Tool For Automatic Combinatorial Biomarkers Design In High-Dimensional Data

Claudio Durán*, Aldo Acevedo1, Graeme Eisenhofer2,3, Massimo Alessio4, and Carlo Vittorio Cannistraci1,5,*
1Biomedical Cybernetics Group, Biotechnology Center, Center for Molecular and Cellular Bioengineering, Center for Systems Biology Dresden, Technische Universität Dresden, Dresden, Germany. 2Institute of Clinical Chemistry and Laboratory Medicine, Faculty of Medicine, Technische Universität Dresden, Dresden, Germany. 3Department of Internal Medicine III, Faculty of Medicine, Technische Universität Dresden, Dresden, Germany. 4Proteome Biochemistry Unit, IRCCS-San Raffaele Scientific Institute, Milan, Italy. 5Complex Network Intelligence Lab, Tsinghua Laboratory of Brain and Intelligence, Tsinghua University, Beijing, China. *

High throughput omic data are a cornerstone of the era of precision medicine. Indeed, the biomarker extraction is a vital process for the detection and comprehension of diseases and their subtypes, which serves for the increase of health care quality in terms of diagnosis, prognosis and treatment response. A computational tool termed CoBiD (Combinatorial Biomarkers Design), which integrates a range of machine learning and feature selection techniques for automatic design of biomarkers is presented. CoBiD is oriented to physicians and biological scientists with limited knowledge of programming. It integrates phases for the design of combinatorial biomarkers starting from the data pre-processing, feature selection, classifiers training, performance evaluations, and report with candidate biomarkers. CoBiD was tested in four biomedical omic datasets concerning distinct diseases, nevertheless we focused here in the diabetes data of single cell genomics. 635 cells were extracted from 9 individuals in adult and infant stages. Four adult individuals were diagnosed with diabetes, one with type 1 diabetes, and three with type 2 diabetes. The results for the analysis (Table I) demonstrate that CoBiD can obtain successfully great results with an ‘easy to use’ framework and that can be applied to a broad type of data-scenarios, including data with imbalance number of classes, missing values, etc. Finally, CoBiD can serve as a powerful resource in biomedical studies which aim to biomarkers design.

Table I. Validation performance of the RF models with and without confounding adjustment.

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<tr>
<th></th>
<th>RF Predicted</th>
<th>RF-Conf Predicted</th>
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</thead>
<tbody>
<tr>
<td>N</td>
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<td>T2D</td>
</tr>
<tr>
<td>True</td>
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<td></td>
</tr>
<tr>
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| Abbreviations: RF: random forest; Conf: confounding factor adjustment; N: healthy control patient; T1D: type 1 diabetes patient; T2D: type 2 diabetes patient; CM-based: confusion matrix-based performance metrics; S: score based performance metrics; AUC: Area under the curve; AUCp: AUC using confusion matrix metrics; AUPR: Area under the precision recall curve; MCC: Mathew’s correlation coefficient.

Keywords: Biomarkers Design, Machine Learning, Diabetes, Biomedical Tool, Single Cell Genomics
Expanded Preconception Carrier Screening – Results from a WA Pilot Program

Ms Samantha Edwards¹, Mr Royston Ong¹, Ms Georgina Hollingsworth¹, Ms Karen Harrop², Dr Sarah Moore³, Dr Benjamin Kamien²,⁴, A/Professor Nicholas Pachter²,⁵, Mr Mark Davis⁵, Ms Karen Carpenter⁵, Dr John Beilby⁵, Professor Nigel Laing¹,⁵
¹Centre for Medical Research, The University of Western Australia, Harry Perkins Institute of Medical Research, Nedlands, Australia, ²Genetic Services of Western Australia, King Edward Memorial Hospital, Department of Health WA, Subiaco, Australia, ³Rural Clinical School, University of Western Australia, Nedlands, Australia, ⁴UWA Medical School, University of Western Australian, Nedlands, Australia, ⁵Department of Diagnostic Genomics, PathWest Laboratory Medicine, WA Department of Health, Nedlands, Australia

Body:
**Background:** Expanded preconception carrier screening (EPCS) assesses the chance a couple will have a child affected with a recessive genetic condition regardless of family history. Next generation sequencing technologies make screening for hundreds of conditions simultaneously affordable. This pilot study aims to determine the requirements for successful implementation of a public health system EPCS program in Western Australia.

**Methods:** 225 couples planning a pregnancy are being screened for 425 severe genetic life limiting and/or chronic recessive conditions with onset in infancy or early childhood. Couples are recruited from the Perth and Busselton regions through general practitioner, clinical genetic and private genetic counselling services. Couples receive a ‘high-risk’ or ‘low-risk’ result and individual results are not reported. Eight known high-risk couples were sequenced as positive controls and the data underwent blind analysis to validate the laboratory processes.

**Outcomes:** In the first 131 couples sequenced, 354 pathogenic variants were identified indicating that each participant is a genetic carrier of at least one severe recessive condition. Of 8 known high-risk couples, 5 were correctly identified and 3 were incorrectly assessed as low risk, highlighting inadequate sharing of rare variant data in reference databases as a major issue. Analysis identified 7 new high-risk couples indicating a population frequency of around 1 in 25, higher than previous publications would suggest. 13 couples reported pregnancy following sample collection and before testing was complete, emphasising the need for a reliable service with rapid result delivery. Health professional evaluation demonstrated increased knowledge and confidence with adequate support.

**Keywords:** Carrier screening, preconception, next generation sequencing, population frequency
Molecular Testing as Second Tier Test for Newborn Screening of Congenital Adrenal Hyperplasia

Ms Terence Diane Fabella1, Dr. Eva Maria Cutiongco- de la Paz1,2, Dr. Cheryll Calalo1,2, Dr. Carmencita Padilla1,2

1Institute of Human Genetics, National Institutes of Health, University of the Philippines, Manila, Manila, Philippines, 2Department of Pediatrics, University of the Philippines College of Medicine-Philippine General Hospital, Manila, Philippines, 3VU University Medical Center Amsterdam, Amsterdam, The Netherlands

Body: Congenital Adrenal Hyperplasia (CAH), an autosomal recessive disorder, is due to deficiency of the enzymes involved in adrenal steroidogenesis. More than 90% of all cases result from a 21-hydroxylase (21-OH) (cytochrome P450c21) enzyme deficiency involving two genes, the active gene (CYP21A2) and a pseudogene (CYP21A1P). Severely affected newborns, if not treated, result to death or a female with ambiguous external genitalia. Thus, newborn screening for CAH is deemed necessary for early detection and treatment. However, the newborn screening uses an immune assay test that measures the concentration of 17-OHP through the use of an antibody and fluorescent tag which also lead to cross-reactivity with other analytes. False positive results can occur especially among very sick newborns. As of December 2018, the Philippine Newborn Screening (NBS) Program reports a CAH prevalence of approximately 1 in 15,560 newborns. Our study aimed to do confirmatory testing via CYP21A2 genotyping of CAH newborns screened through NBS Program.

Dried blood samples (DBS) of four hundred twenty (n=420) newborns screened with CAH due to high levels of 17-OHP were included in the study. DNA samples were extracted and a second tier molecular testing via the Multiplex Ligation-dependent Probe Amplification (MLPA) with a panel of 8 common mutations in the CYP21A2 gene and fragment analysis techniques were done. Result of fragment analysis was analyzed using Cofalyser. Net.

Of the 420 newborns, 17.6% had a mutation in the CYP21A2 gene. The deleterious I2G mutation causing the salt wasting CAH phenotype was the most common mutation detected. The additional second-tier test using molecular technique, MLPA, detects heterozygous I2G as the most common mutation identified in samples tested. The heterozygous I2G state suggests that detection of other CYP21A2 mutations were probably missed using the MLPA technique alone. Future goal of the project is to confirm mutations detected via MLPA using DBS samples and identify other CYP21A2 and CYP21A1P mutations in samples tested via the sequencing analysis.

Keywords: Congenital Adrenal Hyperplasia, CYP21A2, newborns, Filipinos, MLPA

Acknowledgements: MRC Holland, Philippine Genome Center
Title: Rapid Diagnosis of Rare Genetic Disease in Paediatric Patients

**Vanessa S Fear\(^1\)*, Catherine Forbes\(^1\), Genevieve Syn\(^1\), Alexia Weeks\(^1\), Sarra Jamieson\(^1\), and Timo Lassmann\(^1\).

\(^1\)Telethon Kids Institute, University of Western Australia, Australia.

**Body:**

There are an estimated >400 million people living with a rare disease globally, with genetic variants the cause of approximately 80% of cases. Rare diseases are often serious, chronic diseases and potentially life-threatening. Accordingly they have been identified as a public health priority. The advent of Next Generation Sequencing (NGS) has allowed high speed, affordable sequencing, with whole exome sequencing implemented in WA as the rare disease diagnostic method of choice. However, disease diagnosis requires that the genetic variant is validated functionally in a living cell. This requirement for functional validation creates a major bottleneck, with generally >5 years to patient diagnosis, and multiple clinical specialist visits. During this time the possibilities for early childhood intervention may be lost.

We aim to revert the single nucleotide variant predicted to cause the disease in the patients’ own cells to restore gene function. Patient derived inducible pluripotent stem cells (iPSCs) are edited using CRISPR/Cas9 homology directed repair. Patient iPSCs are next used to model disease by inducing their differentiation into a mature cell, or tissue organoid, relevant to the patient phenotype. Differentiated cells are investigated by RNAseq. Differential gene expression and network analysis are employed to elucidate gene variant induced cellular perturbations. This methodology reveals gene variants that lead to disease, and has the potential to identify drug targets that may be suitable for patient intervention strategies.

We have patients with variants for diagnosis in both neural and cardiac cell differentiation. Gene targeting of variants with CRISPR/Cas9 and amplicon sequencing results indicate that we can edit living cells. In preliminary results we have successfully matured iPSCs into the different lineages.

The juxtaposition of genome editing and DNA/RNA analysis of iPSCs will fast-track rare disease diagnosis in paediatric patients.

**Keywords:** Genetic rare disease, CRISPR/Cas9 genome editing, inducible pluripotent stem cells, Amplicon sequencing, RNAseq.

**Acknowledgements:** We would like to thank McCusker Foundation, Ian Potter Foundation, Stan Perron Foundation for their support in this research.
Single-Nucleus Transcriptome Profiling of Mouse Oral Mucosa to Explore Cellular Diversity and Morphogenic Specification

Dr Simon Fox1, Dr Elena Denisenko1, Prof. Camile Farah2, Prof. Alistair Forrest1
1Harry Perkins Institute of Medical Research, Nedlands, Australia, 2Australian Centre for Oral Oncology Research and Education, Nedlands, Australia

A significant challenge in understanding oral mucosal biology and carcinogenesis is that the heterogeneity in cellular composition and functional states have not been well characterized at single-cell resolution. Here, we report a single-nuclei RNA-seq (snRNA-seq) transcriptomic study on mouse oral mucosal tissue from the dorsal tongue, which is composed of multiple cell types with distinct functions. We used snRNA-seq to obviate the technical difficulties of single cell isolation from tough complex tissues and reduce biases towards easily dissociated cell types, as well as minimizing dissociation induced artefacts in gene expression. By transcriptome profiling of over 27,000 nuclei we resolved distinct clusters of major and rare cell types within the oral mucosa and underlying tissue including epithelial, stromal, endothelial and different immune cell populations. We found significant heterogeneity within cell types including epithelial cells, which clustered into distinct populations of basal, replicating, differentiating and terminally differentiated cells. Furthermore, using marker genes within these clusters we identified subpopulations of cells associated with specific epithelial structures. Using computational pseudo-time analysis we examine transcriptional programs underlying epithelial renewal and morphogenic specification in our dataset. Our work both broadens and deepens the resolution of the cellular composition in oral mucosal tissue. These results provide an invaluable resource for studying oral epithelial biology and form a reference for further studies of human oral mucosa and carcinogenic mechanisms in this tissue.

Keywords: Oral mucosa, Tongue, Mouse, Single nuclei RNA-seq, Morphogenesis
Subtle Variation In mRNA Export Factor THOC2 Affects TREX Complex Protein Stability And Is The Cause Of Variable Neurodevelopmental Disturbance

Raman Kumar1, Elizabeth Palmer2, Alison Gardner1, Renee Carroll1, Siddharth Banka3, Dian Donnai4, Ype Elgersma5, Cynthia J Curry6, Alice Gardham7, Mohnish Suri8, Rishi Malla9, Lauren Brady10, Mark Tarnopolsky10, Dimitar N Azmanov11, Gareth Baynam12, Lauren Dreyer13, Melanie Leffler2, Michael Field6, Jozef Gecz4

1The University of Adelaide, Adelaide, Australia; 2GOLD Service, Waratah, NSW, Australia; 3University of Manchester, Manchester, UK; 4NHS Foundation Trust, Manchester, UK; 5Erasmus MC University Medical Center, Rotterdam, The Netherlands; 6University of California, San Francisco, CA, USA; 7Northwick Park Hospital, Harrow, UK; 8Nottingham University, Nottingham, UK and the Genomics England Research Consortium; 9UT Houston, Houston, Texas, USA; 10McMaster University Medical Centre, Ontario, Canada; 11PathWest, Nedlands, and University of Western Australia, WA, Australia; 12University of Western Australia, Perth, WA, Australia. 13Undiagnosed Diseases Program, Government of Western Australia, Perth, WA.

Body: Highly conserved TREX (Transcription-Export)-mediated mRNA export is emerging as a key pathway in neuronal development and differentiation. TREX subunit variants cause neurodevelopmental disorders (NDDs) by interfering with mRNA export from the cell nucleus to the cytoplasm. We have identified twenty missense, three splicing-defective (e.g., Exon35:c.4450-2A>G) and one deletion THOC2 (e.g., ∆Ex37-38) variant. Our data from ex vivo missense variant testing and patient-derived cell lines showed 9 of the 14 missense variants result in reduced protein stability (e.g., p.Leu438Pro), and all of splicing-defective and deletion variants lead to loss of small regions of C-terminal THOC2 RNA binding domain. A number of other TREX subunits (e.g., THOC1, THOC5, THOC7, THOC6) have also been associated with NDDs. Reduced stability of THOC2 variant proteins also affects stability of other NDD-associated THOC subunits. Along with the intellectual disability (ID) phenotype, persistent hypotonia, pre- and post-natal growth restriction, brain malformations, epilepsy and other congenital anomalies are frequent. THOC2 performs diverse and critical roles in development such as pluripotency maintenance, haematopoiesis and synapse development as well as gene expression, 3’ mRNA processing, stress responses, mitotic progression and genome stability. While elucidating precisely how TREX subunit variants cause NDDs continues to be a challenge, the current evidence suggests that subtle alterations to the canonical mRNA export pathway, otherwise essential for cellular life, can be compatible with life but lead to a range of developmental disabilities, neurodegeneration or cancer.

Keywords: mRNA export, mutation, neurodevelopmental disability, functional genomics, patient
Acknowledgements: Australian NHMRC
An Approach to Prioritizing Novel Disease Gene Candidates and Investigating their Role in Muscle Biology and Disease

Ms Hayley Goullée¹²³, Dr. Joshua Clayton²³, Dr. Rhonda Taylor¹²³, Prof. Nigel Laing²³, Dr. Gina Ravenscroft²³, Dr. Alistair Forrest²³

¹The University of Western Australia, Crawley, Australia, ²Harry Perkins Institute of Medical Research, Nedlands, Australia, ³UWA Centre for Medical Research, Nedlands, Australia

The advent of next generation sequencing drastically enhanced identification of recessive human disease genes, including neuromuscular disease genes. However, ~50% of neuromuscular disease patients remain without a genetic diagnosis following diagnostic screening. Small families with dominant conditions are particularly difficult to diagnose, as hundreds of rare coding variants may be identified. Many of these variants are found in genes with unknown function, and thus no clear association with the disease phenotype. Correct identification of the causative mutation requires methods for prioritization and functional assessment of candidate disease genes.

This study aimed to (1) compile a list of candidate muscle disease genes for investigation based on gene expression, and (2) develop methods to define their role in human skeletal muscle development and function. As disease genes tend to be highly expressed in the tissues where they cause a phenotype, we used the FANTOM5 gene expression database to identify genes with >100 fold enriched expression in human skeletal muscle compared to other tissues. Candidates with robust expression in primary human myoblasts and/or myotubes were selected for further investigation.

We next optimized a transfection protocol for CRISPR-mediated disruption of these candidates in primary human myoblasts and subsequent quantification of genomic editing using TIDE (Tracking of Indels by Decomposition). The final optimized protocol achieved up to ~93% editing, producing indels predicted to disrupt gene function. CRISPR-edited samples are currently being analysed using single cell and single nuclei RNA-sequencing to assess transcriptional changes caused by gene disruption. These data will allow us to identify affected biological pathways, and ultimately infer the function of each candidate.

In conclusion, we have developed an in vitro method of exploring the relationship between skeletal muscle-specific expression and muscle disease phenotype. This method could be applied to a wide variety of monogenetic diseases with suitable in vitro models. Overall, this streamlined method of investigation could ultimately accelerate the discovery of novel disease genes and elucidate the function of genes of poorly studied genes in skeletal muscle.

Keywords: Skeletal muscle, clinical genomics, neuromuscular disorders, CRISPR, novel disease genes
Why does the GJB1 5' UTR Mutation c.-103C>T Cause X-linked Charcot-Marie-Tooth Neuropathy?

Ms Bianca Grosz1,2, Professor John Svaren2,3,4, Dr Gonzalo Perez-Siles1,2, Professor Garth Nicholson1,2,5, Professor Marina Kennerson1,2,5

1ANZAC Research Institute, Concord, Australia, 2Sydney Medical School, Camperdown, Australia, 3Waisman Center, Madison, U.S.A, 4Department of Comparative Biosciences, Madison, U.S.A, 5Molecular Medicine Laboratory, Concord, Australia

This study aims to reassess the pathogenic mechanism of the GJB1 5' untranslated region (UTR) c.-103C>T variant [chrX:71,223,249 (hg38); NM_000166.5] that causes Charcot-Marie Tooth neuropathy type X1 (CMTX1). It was previously reported that this pathogenic variant disrupts an internal ribosomal entry site (IRES) in the GJB1 5' UTR, and bioinformatics analysis also suggests the GJB1 c.-103C>T variant may create a splice donor site. Bicistronic plasmids were generated where translation of firefly luciferase (FLuc) was cap-dependent, and translation of NanoLuc luciferase (NLuc) required the intercistronic GJB1 5' UTR to function as an IRES to initiate cap-independent translation. IRES activity was assessed by determining the ratio of NLuc:FLuc for bicistronic plasmids containing the wild type GJB1 5' UTR and pathogenic GJB1 c.-103C>T variant, and comparing this to NLuc:FLuc ratios from negative and positive IRES control vectors. There was no statistically significant difference in the NLuc:FLuc ratio of the negative control, wild type GJB1 5' UTR and GJB1 c.-103C>T variant when compared by One-Way ANOVA [F(3, 24)=0.245, p=0.864]. To assess splicing, both the wild type and GJB1 c.-103C>T coding regions were separately cloned into the exon trapping pSpliceExpress plasmid between two rat insulin exons. The pSpliceExpress vectors were transfected into HeLa cells, and reverse transcribed cDNA was prepared from extracted RNA and amplified using primers which annealed to the flanking rat insulin exons. The PCR products underwent Sanger sequencing, which revealed no difference in the final spliced sequence of the wild type GJB1 and GJB1 c.-103C>T transcripts. The results of these assays do not support IRES dysfunction or the creation of a splice donor site as the pathogenic mechanism for the GJB1 c.-103C>T variant. Further investigation is warranted to define the pathogenic mechanism of the GJB1 c.-103C>T mutation so a suitable therapeutic approach can be developed.

Keywords: Charcot-Marie-Tooth, Peripheral Neuropathy, Rare Disease, Neurogenetics
Investigating the Functional Impact of General Population Missense Variants In ZBTB18

Isabel A. Hemming1,2,3,4,*, Steven Blake3,4,5,6, Sarah J. Beecroft1,2, Mark Agostino4,5,6, and Julian I-T. Heng3,4,5,*

1Harry Perkins Institute of Medical Research, QEII Medical Centre, Nedlands, Western Australia, Australia; 2Centre for Medical Research, The University of Western Australia, Crawley, Western Australia; 3Sarich Neuroscience Research Institute, Nedlands, Western Australia, Australia; 4Curtin Health and Innovation Research Institute, Curtin University, Bentley, Western Australia, Australia; 5School of Pharmacy and Biomedical Sciences, Curtin University, Bentley, Western Australia, Australia; 6Curtin Institute for Computation, Curtin University, Bentley, Western Australia, Australia.

Body: The development of neurons within the mammalian cerebral cortex is underpinned by the activities of DNA-binding transcription factors, which orchestrate the expression of downstream target genes vital for neural circuit assembly. One such transcription factor is the zinc finger repressor ZBTB18 (a.k.a. RP58, ZNF238, C2H2-171, TAZ1). ZBTB18 is involved in the production, maturation, and synaptic integration of neurons within the brain. Mutations in ZBTB18 are associated with human brain developmental disorders in a dominant (heterozygous) fashion. Recently, a study demonstrated that heterozygous missense variants can alter the capacity for ZBTB18 to mediate sequence-specific DNA-binding and regulate transcriptional signalling, successively influencing neurodevelopment [1]. Interestingly, three-quarters (77.8%, 14/18) of ZBTB18 missense variants associated with human neurodevelopmental disorders lie within the zinc finger-encoding region. In contrast, we find that this region is depleted (18.4%, 30/163) for ZBTB18 missense variants identified within the 141,456 general population samples of the Genome Aggregation Database (gnomAD v2.01) [2]. At the same time, some gnomAD ZBTB18 missense variants are within the zinc finger-encoding region. A third (36.7%, 11/30) of these zinc finger variants are removed from a gnomAD subset population that excludes individuals who are cases in case-control studies for neurological disorders. We hypothesised that some of the ZBTB18 zinc-finger missense variants identified in gnomAD would impact ZBTB18 function. An in silico consensus prioritisation approach was undertaken to rank each variant within this region by their consensus impact score, established using publically available prediction tools. Through this approach, we selected and subsequently investigated a subset of variants to find half significantly influenced ZBTB18-mediated DNA binding and transcriptional signalling. Thus, ZBTB18 missense variants have the capacity to influence the functions of ZBTB18, with potential consequences for human brain development and neuronal homeostasis.


Keywords: ZBTB18, gnomAD, missense, brain development,

Acknowledgements: The authors would like to thank the Genome Aggregation Database (gnomAD) and the groups that provided exome and genome variant data to this resource. A full list of contributing groups can be found at https://gnomad.broadinstitute.org/about. In addition, Isabel A. Hemming would like to acknowledge Daniel G. MacArthur, along with Laurent C. Francioli and Konrad J. Karczewski from the MacArthur Laboratory, for their assistance in understanding the gnomAD database.
NATMI: A network analysis toolkit for multicellular interactions in single-cell transcriptome data

Mr Rui Hou¹, Dr Elena Denisenko¹, Ms Huan Ting Ong², Dr Jordan A. Ramilowski³, Prof Alistair R. R. Forrest¹,³
¹Harry Perkins Institute of Medical Research, Nedlands, Australia, ²Ear Science Institute Australia and Ear Sciences Centre, Nedlands, Australia, ³RIKEN Center for Integrative Medical Sciences, Yokohama, Japan

Body: Recent developments of high throughput single-cell sequencing technologies have made it cost-effective to profile thousands of cells from a complex sample. Examining ligand and receptor expression patterns in the cell types identified from these datasets allows to predict cell-to-cell communication at the level of niches, tissues and organism-wide. Here, we developed NATMI (Network Analysis Toolkit for Multicellular Interactions), a Python-based toolkit for multi-cellular communication network construction and network analysis of single-cell gene expression data. NATMI uses most up-to-date manually curated ligand-receptor interaction lists with literature support and user supplied gene expression tables with cell type labels to predict and visualize cell-to-cell communication networks. We demonstrate the utility/usage NATMI by interrogating intercellular communication in the Tabula Muris dataset and identifying known intercellular relationships and revealing differences in predicted cellular communities when comparing intercellular communication mediated by secreted factors to plasma-membrane ligands. We also confirm our previous predictions from bulk data that autocrine signalling is a major feature of cell-to-cell communication networks and for the first time ever show a substantial potential for self-signalling of individual cells through hundreds of co-expressed ligand-receptor pairs. Lastly, we identify age related changes in intercellular communication between the mammary gland of 3 and 18-month-old mice in the Tabula Muris dataset. NATMI and our updated ligand-receptor lists are freely available to the research community.

Keywords: Single-cell transcriptomics, Ligand, Receptor, Network, Visualization

Acknowledgements: We would like to acknowledge and thank Prof. A. Swarbrick, Dr. S. Wu and Dr. R. Tothill for their feedback on NATMI.
Composite selection signals in purebred dogs

Mr Victor Wei Tse Hsu¹, Dr. Mehar Khatkar¹, Associate Professor Peter Williamson¹
¹University Of Sydney, Darlington, Australia

Composite selection signals (CSS) have demonstrated that the locus or interesting regions can be localized in multi-breed populations. The CSS method combines three signals based on population structure, selected or derived allele frequency, and extended haplotype homozygosity (ancestral and convergent sweeps). Here, we investigated the application of CSS to canine SNP data to assess previously known signatures or identify novel regions from various purebred dogs breed comparisons. In addition, we tested the use of CSS to reveal regions associated with canine disease, using lymphoma susceptibility as an example. Multiple studies have identified a predisposition to lymphoma dogs belonging to the Mastiff clade, including Bullmastiffs. To gain a more comprehensive insight into lymphoma predisposition, we performed a population-based study using the CSS method to analyze selected regions for potential impact on lymphoma incidence. 364 Bullmastiffs were used as a target group with a number of comparative reference groups derived from single or combined breed data. A SNP dataset of gray wolves, previously reported in a European study, was used as a source of ancestral alleles. Using the ancestral or convergent sweeps, clusters of signatures of selection were detected at 101 regions on nine canine autosomes. A gene ontology and pathway analysis of genes in regions identified by CSS revealed 89 candidate genes with enrichment for lymphoma-associated ontologies. The most significant signals were related to the regulation of lymphocyte migration. The CSS is a useful tool for cross-species for identifying potential candidate genes under selection.
Characterizing Stargardt Disease-Causing Mutations to Identify Gene Lesions Amenable to Splice Intervention Therapies

Di Huang1,2,  Jennifer A. Thompson4, Samuel McLenachan2, Shang-Chih Chen2, Dan Zhang2, Mary Attia2, Sukanya Arunachalam2, Terri L. McLaren2,4, Tina M. Lamey2,4, John N. De Roach2,4, May Thandar Aung-Hlut1,3, Abbie Adams1, Sue Fletcher1,3, Steve Wilton1,3, Fred K. Chen2,5,6*.

1Centre for Molecular Medicine and Innovative Therapeutics, Murdoch University, Murdoch, Western Australia, Australia, 2Centre for Ophthalmology and Visual Science (incorporating Lions Eye Institute), The University of Western Australia, Nedlands, Western Australia, Australia, 3Centre for Neuromuscular and Neurological Disorders, The University of Western Australia and Perron Institute for Neurological and Translational Science, Nedlands, Western Australia, Australia, 4Australian Inherited Retinal Disease Registry and DNA Bank, Department of Medical Technology and Physics, Sir Charles Gairdner Hospital, Nedlands, Western Australia, Australia, 5Department of Ophthalmology, Royal Perth Hospital, Perth, Western Australia, Australia, 6Department of Ophthalmology, Perth Children’s Hospital, Nedlands, Western Australia, Australia

Purpose: Stargardt disease (STGD1, OMIM: 248200) is frequently caused by missense, frameshifting or nonsense mutations in the ATP-binding cassette transporter gene (ABCA4). However, some pathogenic variants result in aberrant splicing leading to exon skipping or pseudo-exon inclusion. Although ABCA4 is a photoreceptor- and retinal pigment epithelium-specific protein, ABCA4 transcripts have also been detected in fibroblasts. We examined ABCA4 mRNA in fibroblasts from 66 STGD1 patients to investigate the types and frequency of aberrant splicing.

Methods: Patient-derived fibroblasts were propagated, total RNA was extracted and ABCA4 transcripts were analysed by reverse transcription polymerase chain reaction (RT-PCR) to evaluate transcript structure. Antisense oligonucleotides (AO) sequences targeting splicing motifs were designed to enhance production of the correctly spliced mature ABCA4 transcript. The AOs were transfected into patient-derived fibroblasts and the ABCA4 transcript was analysed 72 hours after transfection.

Results: Exons 1-12 of the ABCA4 transcript could not be amplified from fibroblast RNA. However exons 13-50 could be readily amplified. Of 77 different ABCA4 variants identified primarily by next generation sequencing of relevant ocular genes and targeted Sanger sequencing, 60 variants (78%) were located beyond exon 12. Six ABCA4 variants (ABCA4 c.5461-10T>C, c.4773+3A>G, c.5835+1G>A, c.6031_6044delins, c.4919G>A, and c.5197-4C>A), carried by 17 STGD1 patients (10% of variants located beyond exon 12), affected pre-mRNA processing, with aberrantly spliced ABCA4 isoforms detected. Mature ABCA4 mRNA transcripts in carriers of the c.5461-10T>C allele were missing exon 39, or exons 39 and 40. An AO designed to weaken the intronic splice silencers was able to increase the correctly spliced ABCA4 transcript 1.5-fold compared to that from untreated patient fibroblasts.

Conclusions: Patient-derived fibroblasts are useful for identifying ABCA4 splicing variants affecting exons 13-50. We showed that splice-modulating AOs may have therapeutic potential for STGD1 patients carrying the ABCA4 c.5461-10T>C mutation. Future work to characterize retina-specific splice variants will require retinal pigment epithelium and photoreceptors derived from human induced pluripotent stem cells.

Keywords: Stargardt disease (STGD1), ABCA4 gene, transcript analysis, splicing defects, antisense oligonucleotide

Acknowledgements:
PhD Scholarship is awarded by the Perron Institute and Murdoch university
Telethon-Perth Children’s Hospital Grant
Macular Degeneration Foundation Australia
NHMRC Career Development Fellowship (MRF1124163)
NHMRC Centre of Research Excellence (GNT1116360)
Miocevich Family
Integration of Molecular Chemistries Supporting a Full-Length mRNA Sequencing Library Preparation Method on a Microfluidic Circuit

Jean Huang, Julie Alipaz, Joel Brockman, Sangpen Chamnongpol, Brian Fowler, Jennifer A. Geis, Tom Goralski, Christopher Kubu, Raphael Kung, Benjamin Lacar, Charles Park, David A. King

Fluidigm Corporation, 7000 Shoreline Court, Suite 100, South San Francisco, CA 94080 USA

RNA sequencing (RNA-seq) provides more precise measurement of transcript levels and their isoforms than other expression profiling methods. We developed a highly cost-effective microfluidics-based workflow and chemistry to generate RNA-seq libraries. This method automates solid-phase capture of polyadenylated RNA, reverse transcription, and index PCR within a compact nanoscale integrated fluidic circuit (IFC) on our Juno™ instrument. The workflow will support simultaneous processing of up to 48 samples and will include reagents necessary to generate full-length, random-primed RNA-seq libraries from as little as 10 ng of total RNA, while preserving strandedness information.

To determine assay robustness, we conducted an internal analytical validation study using 3 different operators on 6 different instruments with 3 different reagent and 3 different IFC lots. Over 900 samples were sequenced comprising ~5 billion total paired-end 75 bp reads. To further assess assay robustness, we performed two additional studies. First, we used 20 different tissue types with varying RIN scores (RNA Integrity Number; Agilent). The different tissue samples were correlated with their RINs against number of genes and transcripts detected. Secondly, we conducted a dilution series of UHRR that included input RNA amounts below our minimum of 10 ng. After a one-click script to initiate the workflow on our instrument, a bead affinity column was formed, polyadenylated RNA was enriched, and template RNA was converted into cDNA, which was then amplified with sample indexes. Finally, we pooled, purified, quantified, and sequenced harvested libraries on an Illumina® HiSeq® 2500 System to an average sequencing depth greater than 50 million reads per sample. The various input amounts were assessed for percent of reads mapped to the transcriptome (RefSeq). Also, average gene-level Pearson’s correlation at all comparison input amounts was calculated.

Results herein have shown excellent mapping rates of >80% with low-percent rRNA reads of 98% in all conditions. Also, our additional studies demonstrated our kit is robust to input amounts below 10 ng and supports RNA with RIN numbers less than 7. We also provided clear evidence of differential isoform expression. In conclusion, RNA-seq library preparation can be streamlined using automated microfluidics technology, enabling solid-phase enrichment and multistep chemistries. This workflow reduces tedium and simplifies the library preparation process. Overall cost is reduced as well due to nanoliter volumes utilized on the IFC.

**Keywords:** Transcriptomics, RNA-seq, Automation, New Technology
Generation and Transcript Profiling of iPSC, Retinal Organoids and RPE in RCBTB1-Related Retinopathy

Ms Zhiqin Huang1,2, Dr. Samuel McLenachan1,2, Dr. Dan Zhang1,2, Dr. Jennifer A. Thompson3, Dr. Saumya Shekhar Jamuar4,5, Dr. Terri McLaren1,2, Dr. Tina Lamey1,3, Dr. Enid Chelva6, Dr. John De Roach1,3, Dr. Choi Mun Chan7, Dr. Fred K. Chen1,2

1The University of Western Australia, Nedlands, Australia, 2Lions Eye Institute, Australia, 3Australian Inherited Retinal Disease Registry and DNA Bank, Sir Charles Gairdner Hospital, Australia, 4Genetics service, Department of Paediatrics, KK Women’s and Children’s Hospital, Australia, 5Paediatric Academic Clinical Programme, Duke-NUS Medical School, Singapore, 6Medical Technology and Physics, Sir Charles Gairdner Hospital, Australia, 7Medical Retina Department, Singapore National Eye Centre, Australia

Body: Purpose: Mutations in the RCC1 and BTB domain-containing protein 1 (RCBTB1) gene have been identified in various inherited retinal disorders however, no transcript profiling in retinal cells has yet been reported in RCBTB1-related retinopathy. Herein, we identified compound heterozygous mutations in RCBTB1 from a Singaporean-Chinese female patient and her asymptomatic sibling through next generation sequencing. To assess the effects of these mutations on pre-mRNA splicing, we characterized RCBTB1 transcripts in induced pluripotent stem cells (iPSCs), neural retinal organoids (NRO) and retinal pigment epithelium (RPE) derived from the proband patient.

Methods: Proband DNA was genetically analyzed using a 537-gene NGS Vision panel and targeted Sanger sequencing was used for confirmation of detected variants and cascade family testing (performed by Molecular Vision Laboratory, Oregon, USA). The patient-derived iPSC line LEIi011-A was differentiated into NRO and RPE. Expression of retinal and RPE markers in iPSC-derived cells were confirmed by ICC and qRT-PCR. To characterize RCBTB1 transcripts in iPSCs, NRO and RPE derived from the proband, qRT-PCR, TA cloning and Sanger sequencing were performed.

Results: Panel testing revealed biallelic frameshifting mutations, c.170delG (p.Gly57Glufs*12) and c.707delA (p.Asn236Thrfs*11) in RCBTB1, which segregated with disease. The iPSC-derived NRO and RPE presented typical morphological features and expressed retinal markers. RCBTB1 mRNA expression was significantly reduced in patient-derived iPSCs (p=0.02), NRO (p=0.01) and RPE (p=0.01) compared to that in healthy controls. Moreover, PCR amplification and sequencing of RCBTB1 mRNA from patient-derived iPSCs, NRO and RPE demonstrated expression of aberrantly spliced transcripts. Exon 3 mutation (c.170delG) resulted in the production of two aberrant RCBTB1 transcripts: one transcript with a deletion of 713 bp (lacking exons 5-9) predicted to disrupt the open reading frame causing a premature stop codon in NRO, the other with a deletion of 649 bp (in-frame deletion of exons 5-8) in NRO and RPE. No transcript with exon 7 mutation (c.707delA) was detected in NRO or RPE, suggesting it was subjected to nonsense-mediated mRNA decay.

Conclusions: Novel splice-isoforms of RCBTB1 were identified in patient-derived NRO and RPE, indicating that the pathogenic mechanism for these two disease-causing RCBTB1 variants involves their aberrant impact on splicing.

Keywords: RCBTB1, frameshifting mutation, aberrant splicing, retinal organoid, RPE

Acknowledgements: We would like to thank the Lee and Low family for their generous donations supporting this work. This work was also supported by funding from the National Health & Medical Research Council, Australia (Centre of Research Excellence and Career Development FellowshipsAPP1116360, APP1142962, FKC), the Government of Western Australia (Department of Health, New Independent Researcher Infrastructure Support Award, FKC), and Retina Australia (Australian Inherited Retinal Diseases Registry and DNA Bank).
Formation Relationship of Secondary DNA Structures at Different Cell Cycle Stages in a Human Breast Cancer Cell line

Miss Kelly Irving\(^1\), Miss Jessica King\(^1\), Mr Diwei Ho\(^1\), Dr Cameron Evans\(^1\), Prof. Killugudi Swaminathan Iyer\(^1\), Dr. Nicole Smith\(^1\)

\(^1\)University of Western Australia, Crawley, Australia

**Body:** Oncogenes are genes that cause gain-of-function effects critical for cancer progression. Once altered from their proto-oncogenic state, oncogenes code for substrates associated with cellular proliferation and/or apoptosis. They are of significant interest when investigating cancer therapeutics due to their cancer-specific expression. The promoter regions of oncogenes often contain guanine (G) and cytosine (C)-rich regions which have a propensity to form non-canonical secondary DNA structures, G-quadruplexes (G4) and i-motifs (iM) respectively, when rendered single-stranded. Both G4 and iM secondary structures occur in the genome of human cells, being more abundant during interphase when the cells are most active and undergoing DNA replication and transcription. It has been demonstrated that targeting G4 structures in the promoter region of oncogenes with small molecules can decrease or silence oncogene expression, while targeting iM structures have been associated with enhanced gene expression. Given the G-rich and C-rich strands are complementary, it is unclear whether G4/iM formation at a given loci is mutually exclusive or if they form concomitantly. Given these structures are altered with cell cycle progression, we examined the formation relationship between these structures at each cell cycle stage in a human breast cancer cell line, MCF7, using immunofluorescence with a G4-specific antibody (BG4) and iM-specific antibody (iMab). We further assessed what effect inducing and stabilising G4s has on iM formation and vice versa and the extent of colocalization of G4 and iM structures in unsynchronised, G1 (when iMs are most prevalent) and S (when G4s are most prevalent) arrested cells.

**Keywords:** Secondary DNA Structures, G-quadruplex, i-motif,

**Acknowledgements:** Daniel Christ (institute), Prof. Shankar Balasubramanian (institute), Dr Zoë Waller (institute), Prof. Laurence Hurley (institute), CMCA, NHMRC, ARC, NBCF
A Homozygous Nonsense Mutation in C11ORF80 Causes Infertility in a Consanguineous Pakistani Family

Presenting author: Nazish Jabeen, First Author: Yuying Jiao, Nazish Jabeen, Xiaohua Jiang, Suixing Fan, Co-authors: Huan Zhang, Hanwei Jiang, Hui Ma, Asim Ali, Ranjha Khan, Yang Li, Jianqiang Bao, Beibei Zhang, Jianze Xu, Fazal Wahab, Ghulam Murtaza, Qamar Zaman, Muhammad Aslam, Ihsan Khna, Ayesha Yousaf, Yuanwei Zhang, Corresponding Author: Qinghua Shi*

Address: Hefei National Laboratory for Physical Sciences at the Microscale, The First Affiliated Hospital of USTC, USTC-SJH Joint Center for Human Reproduction and Genetics, The CAS Key Laboratory of Innate Immunity and Chronic Diseases, School of Life Sciences, CAS Center for Excellence in Molecular Cell Science, University of Science and Technology of China, Collaborative Innovation Center of Genetics and Development, Collaborative Innovation Center for Cancer Medicine, Hefei 230027, Anhui, China.

Meiotic recombination between homologous chromosomes, initiated by programmed DNA double-strand break (DSB) formation, is essential for normal progression of meiosis and successful generation of gametes. Here, we report four infertile siblings born to a consanguineous marriage, among which three brothers had non-obstructive azoospermia and one sister was diagnosed with idiopathic infertility. An autosomal recessive mutation in TOP6BL was identified, co-segregating with infertility phenotype, in this family, and investigation of one male patient revealed failure in programmed DSB formation prior to pachytene. Mouse models carrying similar mutations phenocopied the DSB formation failure, meiotic defects, and infertility of the patients. Our study thus illustrates how failed with programmed meiotic DSB formation causes infertility in both males and females.

Keywords: Infertility; Spermatogenesis; Azoospermia; Topoisomerase

Acknowledgements: We thank all the participants for their cooperation.
Investigating the Splice-altering Effect of the $ABCA4$ c.768G>T Mutation in STGD1 Using Patient-derived Induced Pluripotent Stem Cells and Retinal Organoids

Luke Jennings$^{a,b}$, Dan Zhang$^{a,b}$, Shang-Chih Chen$^b$, Sang Yoon Moon$^{a,b}$, Tina M. Lamey$^{a,c}$, Jennifer A. Thompson$^c$, Terri L. McLaren$^{a,c}$, John N. De Roach$^{a,c}$, Fred K. Chen$^{a,b,c,d,*}$, Samuel McLenachan$^{a,b}$

$^a$ Centre for Ophthalmology and Visual Sciences, The University of Western Australia, Nedlands, Western Australia, Australia, $^b$ Lions Eye Institute Australia, Nedlands, Western Australia, Australia, $^c$ Australian Inherited Retinal Disease Registry and DNA Bank, Department of Medical Technology and Physics, Sir Charles Gairdner Hospital, Perth, Western Australia, Australia, $^d$ Department of Ophthalmology, Royal Perth Hospital, Perth, Western Australia, Australia

**Body:** Autosomal recessive Stargardt disease (STGD1) is the most common inherited retinal disease (IRD) in Australia and is caused by over 900 different mutations in the $ABCA4$ gene. The $ABCA4$ (NM_000350.2) c.768G>T mutation was reported to cause STGD1 by altering the pre-mRNA splicing of $ABCA4$'s exon 6 region, leading to the selection of a cryptic splice donor site resulting in the inclusion of a 35-nucleotide pseudo-exon in the mature mRNA. Induced pluripotent stem cells (iPSC) reprogrammed from somatic patient cells provide an in vitro disease modelling platform for IRDs such as STGD1. Patient-derived iPSCs can be differentiated into retinal organoids (RO) to investigate the pathophysiological mechanism of mutations in retinal-specific genes like $ABCA4$. To establish an in vitro STGD1 model, iPSCs generated from a STGD1 patient carrying the $ABCA4$ c.([768G>T];c.6079C>T) mutations were characterised for pluripotency by immunocytochemical staining, qRT-PCR and embryoid body differentiation. Patient iPSCs were differentiated into ROs and characterised for retinal gene expression by qRT-PCR. The reported splice alterations within $ABCA4$ pre-mRNA resultant from c.768G>T were investigated in patient-derived ROs by PCR. Patient-derived iPSCs were confirmed as pluripotent, expressing similar levels of OCT4, NANOG, SOX2 and KLF4 as a control iPSC line, and spontaneously differentiating into embryoid bodies expressing markers of the ectoderm, mesoderm and endoderm lineages. Patient iPSC-derived ROs were confirmed to be of a retinal lineage. ROs expressed retinal progenitor cell markers, and demonstrated laminated neuroepithelial tissue and darkened patches of developing retinal pigmented epithelial tissue. $ABCA4$ mRNA expressed from the c.768G>T allele displayed inclusion of a 35 nucleotide pseudo-exon, however this transcript was only detected in patient-derived ROs after treatment with the translation inhibitor cycloheximide, indicating it is subject to nonsense mediated decay. This work demonstrated the successful establishment of a patient-derived RO disease model for STGD1, and verification of the reported pre-mRNA splice-altering effect of the $ABCA4$ c.768G>T mutation. Such ROs provide a model for future screening of novel therapeutics aimed at restoring correct splicing to $ABCA4$ pre-mRNA.

**Keywords:** Induced pluripotent stem cells, retinal organoids

**Acknowledgements:** This work was funded by the Australian National Health and Medical Research Council (MRF1142962 and GNT1116360), the Telethon-Perth Children’s Hospital Research Fund (Australia) and the Macular Disease Foundation Australia as well as generous donations from the Saleeba, Miocevich and McCusker families.
Analysis of the transcriptome of sorafenib-resistant hepatocellular carcinoma cells provides insight into overcoming therapeutic resistance

**Dr Tasnuva Kabir**

Ms Shelby Margolius

Ms Rikki Brown

Dr Andrew Woo

Ms Kirsty Richardson

Ms Dianne Beveridge

Dr Samuel Beck

Ms Lisa Stuart

Professor Peter Leedman

1 Harry Perkins Institute of Medical Research, Nedlands, Australia

2 Centre for Medical Research, The University of Western Australia, Crawley, Australia

3 MDI Biological Laboratory, Kathryn W. Davis Center for Regenerative Biology and Medicine, USA

Patients with advanced hepatocellular carcinoma (HCC) inevitably acquire resistance to sorafenib, the primary targeted therapy, and succumb. Little is known of the key molecular pathways that promote development of the resistant phenotype. Double-stranded RNAs (dsRNAs), such as microRNAs (miRs), are playing an increasingly important role in cancer development, metastasis, and as potential therapies. Many miRs are aberrantly expressed in HCC. For example, microRNA-7 (miR-7) expression is decreased in HCC and it acts as a tumour suppressor. To better understand the mechanisms of acquired sorafenib resistance, we generated multiple sorafenib-resistant HCC cell lines. Here, we aimed to uncover the drivers of the resistance in order to identify new pathways that could be targeted therapeutically, potentially by specific miRNAs.

The whole genome transcriptome of Huh-7 HCC cells and its sorafenib resistant subline Huh-7/SR1 were profiled using RNA-sequencing. Gene expression of caveolin (CAV-1), the driver of the caveolar mediated endocytosis pathway, and Axl were significantly dysregulated in sorafenib-resistant HCC cells. CAV-1 and Axl are both recognised mediators of therapeutic resistance in other cancers, including breast, prostate, head and neck, ovarian and lung, but not known to be associated with HCC.

Depletion of CAV-1 using siRNAs reduced survival and spheroid formation of Huh-7/SR1 cells. These cells required CAV-1 to rearrange the cytoskeleton which facilitated their adhesion to matrix proteins, migration and invasion in vitro (as assessed by atomic force microscopy). The Huh-7/SR1 cells required concomitant activation of the GAS6/Axl and AKT pathway for CAV-1 to mediate its function. Interestingly, we found that miR-7 is a potent direct inhibitor of CAV-1 expression as well as the GAS6/Axl/AKT axis in sorafenib resistant cells. Further, miR-7 was also able to completely overcome sorafenib-resistance of the HCC cells and restore sensitivity to the drug.

Taken together, these data demonstrate that sorafenib-resistant HCC cells sustain their survival and mesenchymal phenotype by up-regulating expression of CAV-1 and caveolar mediated endocytosis, and by signalling through the GAS6/Axl/AKT pathway. Further, they identify miR-7 as a potent inhibitor of CAV-1 and Axl/AKT signalling, suggesting that miR-7 replacement therapy may represent a new approach to treat sorafenib-resistant HCC.
A General, Flexible Framework for Integrative Gene Expression Analyses

Dr Bogumil Kaczkowski¹, Dr Erik Arner¹

¹RIKEN Center for Integrative Medical Sciences, Yokohama, Japan

High-throughput gene expression profiling technologies are widely used due to advances in technology and lowering cost. However, the interpretation of the high-dimensional data often remains a challenge. Here, we propose a general, flexible framework for integrative analysis where the newly generated experimental data (such as differential expression results) are intersected with prior biological knowledge such as pathways, gene ontologies, published lists of genes (molecular signatures), or combination thereof. In the basic one-by-one implementation, the results are similar to those obtained by Gene Set Enrichment Analysis (GSEA) or Gene Ontology (GO) enrichment. However, we extend the framework in a way that makes it possible to simultaneously analyze and integrate multiple sources of prior knowledge in a general and flexible way. Additionally, by overlapping FANTOM5 promoter regions with ChIP-seq peaks from the ReMap and ChIP-Atlas databases, we generated gene signatures for over 10 thousand ChIP-seq experiments.

Keywords: gene expression, transcription factors, integrative analysis.

Acknowledgements: IMS-001 (IMS Kofu-kin) Research Grant from MEXT to the RIKEN Center for Integrative Medical Sciences. Kakenhi Grant-in-Aid for Young Scientists (B) (17K18366) to BK. Incentive Research Projects for Individual Germinating Research from RIKEN to BK.
Meta-Analysis of DMD Pseudoexons Reveals Distinct Initiation Pathways and Interrelations with Predicted Recursive Splice Sites

Niall P Keegan1,2,3*
1Murdoch University, 2CMMIT, 3Perron Institute

The DMD gene is the largest in the human genome, with a total intron content exceeding 2.2Mb. In the decades since DMD was discovered there have been numerous reported cases of pseudoexons arising from exon-like sites in the DMD introns of some individuals, either as the result of mutations or as low-frequency errors of the spliceosome. In many genes, including DMD, exon-like sites within introns can also function as ratchet points for the recursive splicing of those introns.

To investigate a potential overlap between DMD pseudoexons and recursive splice sites, a database of 58 DMD pseudoexons was collated from the literature and cross-referenced against a published list of predicted recursive splice sites (pRSSes) in DMD. This analysis found matches for 12 out of 47 possible pseudoexon acceptor sites and 14 out of 44 possible pseudoexon donor sites. Unexpectedly, a strong correlation was also observed between pRSS-pseudoexons and pseudoexons induced by distal mutations.

Although DMD pseudoexons exhibited great diversity in their characteristics and origins, this study found that all 58 could easily and meaningfully be categorised solely by the proximity of their inducing mutations. Should these categories apply equally well to reported pseudoexons in other genes, they may prove useful in discovering new recursive splice sites in those genes. Finally, the fact that multiple DMD mutations have been observed to convert distal pRSSes to pseudoexons implies the presence of recursive splice silencer elements at the mutation sites.

Keywords: DMD, Pseudoexons, Recursive Splicing

Acknowledgements: This research was funded by a Commonwealth Government Research Training Program Scholarship. I would also like to thank my supervisors, Profs. Sue Fletcher and Steve Wilton, and my colleagues at CMMIT and Perron for their feedback and guidance.
Genetic Variability and the Clinical Course of Parkinson’s Disease and Efficacy of Levodopa Treatment

Mr Jan Koper¹, dr. Barbara Zapała², dr. Agnieszka Spychałowicz³, dr. Monika Piwowar⁴, dr. Sylwia Czekalska⁵, Ms. Maria Hadasik¹, dr. Magdalena Zawada², Ms. Adrianna Wąsińska¹, Mr. Olaf Chmura¹

¹Jagiellonian University Medical College, Cracow, Poland, ²Department of Clinical Biochemistry, Jagiellonian University Medical College, Cracow, Poland, ³The Center of Movement Organ Rehabilitation “KRZESZOWICE” SP ZOZ, Cracow, Poland, ⁴Department of Bioinformatics and Telemedicine, Jagiellonian University Medical College, Cracow, Poland, ⁵Department of Hematology Diagnostics, The University Hospital, Cracow, Poland

Background:
Parkinson’s disease is the second most common neurodegenerative disorder in the world with Levodopa being the gold therapeutic standard. The response to levodopa treatment may be varied, possible from the fact that the genetic variability may determine the response to the treatment.

Aim:
The aim of this work was to investigate the impact of genetic variants in the genes coding monoamine oxidase B (MAOB), dopamine receptor D2 (DRD2) and DOPA decarboxylase (DDC) on the observed differences in the clinical course of Parkinson’s disease and the effects of levodopa treatment in the diagnosed patients. Patients diagnosed with Parkinson’s disease were included into the study group (126 patients: women and men aged 39 to 95). The whole peripheral blood was drawn from the patients and protected, then the genetic material in the form of DNA was extracted and the genotyping of single nucleotide polymorphisms (SNP) was performed using the TaqMan probes.

Results:
It was stated that rs2283265 and rs1076560 genetic variants of the DRD2 gene determine more frequent presence of dementia and higher patients’ scores in the II and III part of the UPDRS (p < 0.05). Furthermore, they do not affect the presence of the levodopa treatment complications and the need of deep brain stimulation in the patients diagnosed with Parkinson’s disease (p > 0.05). It was also demonstrated that the genetic variants of rs1799836 of the MAOB gene and rs921451 of the DDC gene do not affect the clinical course of Parkinson’s disease and the effects of levodopa treatment in diagnosed patients (p > 0.05).

Conclusion:
We believe that genetic sequencing may in future serve as an tool to assess the future course of PD.

Keywords: Parkinson’s Disease, Levodopa, SNP, Dopamine, Dementia
Genetic Legacy of the Last Hunter-Gatherer Group in Borneo

Dr Pradiptajati Kusuma1,2, Dr Guy S Jacobs2,3, Prof Murray P Cox4,5, Prof Herawati Sudoyo1, Prof J. Stephen Lansing6
1Eijkman Institute for Molecular Biology, Jakarta, Indonesia, 2Complexity Institute, Nanyang Technological University, Singapore, 3Department of Archaeology, University of Cambridge, Cambridge, UK, 4School of Fundamental Sciences, Massey University, Palmerston North, New Zealand, 5Te Pūnaha Matatini, New Zealand Centre of Research Excellence for Complex Systems, Auckland, New Zealand, 6Santa Fe Institute, Santa Fe, USA

Body: The behavioral repertoire of our species evolved during the Pleistocene, at a time when our ancestors lived as hunter-gatherers. Consequently, the study of the few remaining populations of hunter-gatherers has high priority for understanding the evolutionary basis of human behavior. This is especially true in island Southeast Asia, an understudied hub of human cultural and biological evolution, where rapid development poses particular threats to hunter-gatherer subsistence. On the island of Borneo, with a deep archaeological record of hunter-gatherers living in rock shelters, the Punan are the largest and most diverse extant hunter-gatherer group. However, anthropological interest has been muted – both because most communities have long been resettled into sedentary villages, and because, phenotypically Asian and speaking Austronesian languages, the Punan have repeatedly been presented as descendants of migrating Neolithic farmers who recently reverted to a hunter-gatherer way of life. Here we present genetic, data from a particularly remarkable group, the Punan Batu Sajau (Cave Punan Sajau), which is inconsistent with the recent reversion hypothesis. This community have maintained a substantially mobile lifestyle, living in temporary camps and sometimes, as was traditional, under rock shelters. Our results argue for an urgent reassessment of the importance of the Indonesian hunter-gatherers in our understanding of human genetic diversity and evolution.

Keywords: Cave Punan, Hunter-Gatherer, Borneo, Genetics, Population

Acknowledgements: Methods used for human subject research were approved by the Research Ethics Commission of the Eijkman Institute for Molecular Biology (Jakarta). Funding were provided by the National Geographic Research Grant and Singaporean Ministry of Education Tier 1 Grant (to PK), NTU Presidential Post-doctoral Grant (to GSJ), the Leakey Foundation and the US National Science Foundation (to JSL), and a Indonesian Ministry of Research and Technology Block Grant (to PK and HS). The authors thank Steven Kuhn, Thomas Mita, Datuk Karim, Ahmad Arif, Safarina G. Malik, Eijkman Institute sampling team, and lastly, the Cave Punan community.
Functional Genomics For Genomic Diagnostics

Prof Nigel Laing1,2, Dr Gianina Ravenscroft1, Associate Professor Bruce Bennetts3, Professor John Christodoulou4, Professor Jozef Gecz5, Professor Ian Smyth6, Dr Zornitza Stark4, Professor Patrick Tam7, Professor Hamish Scott8, Professor Kathryn North4, Professor Andrew Sinclair4

1Harry Perkins Institute of Medical Research, University of Western Australia, Nedlands, Australia, 2Diagnostic Genomics, PathWest, WA Department of Health, Nedlands, Australia, 3Department of Molecular Genetics, The Children’s Hospital at Westmead, Westmead, Australia, 4Murdoch Children's Research Institute, Parkville, Australia, 5Faculty of Health and Medical Sciences, The University of Adelaide, Adelaide, Australia, 6Australian Phenomics Network, Monash University, Clayton, Australia, 7Children’s Medical Research Institute, University of Sydney, Westmead, Australia, 8Department of Genetics and Molecular Pathology Centre for Cancer Biology, a South Australia Pathology and University of South Australia Alliance, Adelaide, Australia

Body:

Functional genomics may be critical for interpreting variant pathogenicity in known and novel candidate disease genes. Functional genomics pipelines for novel gene discovery are relatively well defined. They can include ad hoc collaborations with researchers that have published on the candidate gene or protein. Alternatively, they can involve more systematic match-making programs between genomics and functional genomics research teams such as the Canadian Rare Diseases Models and Mechanisms Network or the Australian Functional Genomics Network. However, functional genomics for diagnostic laboratories is not well established. Genomic diagnostic laboratories analyse thousands of genes. The challenge for diagnostic laboratories is variants of uncertain significance (VUS), which may or may not cause the patient’s disease. Proving that a VUS causes the phenotype in the patient for thousands of genes is an impossible task for diagnostic laboratories. Increased data sharing may clarify pathogenicity of some VUS (“variants of undersharing”). Experimental diagnostic functional genomics would have to be established on a gene by gene basis as, in general, different genes require different assays. Different variant types in one gene, for example gain of function versus loss of function variants, may result in different diseases. The same variant type, for example loss of function, at different locations in a gene, may result in different diseases. As variant effects might differ with genomic background, assays could require patient-specific cells. With so many VUS to test, diagnostic functional genomics requires high-throughput analyses, but how they would work is unclear. Saturation mutagenesis is one method, but probably inappropriate for large genes. Cell-based assays are not foolproof: a changed biochemical readout does not necessarily mean disease relevance. VUS may therefore require whole animal models and extensive characterisation. Diagnostic laboratories, do not have the resources, personnel or know how to perform the necessary functional genomics. Research laboratories with expertise in the gene or protein in question will therefore have to play a major role. However, funding streams that support gene discovery functional genomics are usually unavailable for diagnostic functional genomics. Alternative funding mechanisms therefore need to be developed. It would be wasteful and inefficient for multiple groups to perform similar functional genomics assays for one variant. Diagnostic functional genomics should therefore have systematic national/international approaches. Diagnostic functional genomics is one of the grand challenges facing the entire genomics community, but there is currently no international body such as the Global Alliance for Genomics and Health focussed on it.

Keywords: Functional Genomics, Diagnostic Genomics, Disease Gene Discovery, Variants of Uncertain Significance, Grand Challenge

Acknowledgements: NGL is supported by Australian National Health and Medical Research Council (NHMRC) Fellowship APP1117510
Effect of Millimetre Waves on Genome Architecture in Primary Human Fibroblasts

Mr Nicholas Lawler¹,², Dr Cameron Evans¹, Dr Sergii Romanenko², Ms Nutan Chaudhari¹, Dr Mark Fear³, Prof Fiona Wood³, Dr Nicole Smith¹, A/Prof Vincent Wallace², Prof Killugudi Swaminathan Iyer¹

¹School of Molecular Sciences, University of Western Australia, Perth, Australia, ²Department of Physics, The University of Western Australia, Perth, Australia, ³Fiona Wood Foundation and Burn Injury Research Unit, The University of Western Australia, Perth, Australia

Body: Electromagnetic radiation interacts with the genetic material of living organisms via many frequency dependent mechanisms, causing effects including DNA damage and mutation. The effects of millimetre waves (MMWs), with wavelengths of 1 – 10 mm and frequencies of 30 – 300 GHz, on the genome and other biological processes are less understood than other frequencies as strong attenuation by atmospheric oxygen historically resulted in minimal natural exposure. Despite being non-ionising, MMWs have non-trivial effects on humans, with therapeutic applications utilising MMWs to treat a range of ailments emerging in several Eastern European nations. Additionally, multiple frequency bands within this range are being adopted in 5G mobile networking technologies, establishing the importance of investigating the biological effects from the context of both increased natural exposure and potential medical applications.

Previous research has shown that exposure to MMWs induces numerous biological responses, including modified biological membrane properties, gene expression and altered neuronal action potentials. However, the mechanisms describing these effects remain unknown. Initially attributed to the thermal effects of absorption, it has been shown that the changes in gene expression are distinct from the heat shock responses and are now thought to arise from unique interactions with the genome.

G-quadruplexes (G4) and i-motifs (iM) are sequence specific non-canonical DNA secondary structures occurring in the human genome within certain G- and C-rich regions, respectively. Both iM and G4 are over-represented in regulatory regions of the genome with implications in transcriptional regulation and genomic instability. Many proposed models for MMW interactions with the genome describe conditions that may alter formation of these structures, including the induction of resonances causing transient DNA single strandedness.

We have investigated the effects of MMWs on genomic architecture, focusing on G4 and iM, in primary human fibroblasts via immunocytochemistry with the structure specific BG4 and iMab antibodies. Simultaneously, we examined the effects of MMWs on collagen production at the mRNA and protein levels, as enhanced collagen expression is a key feature of fibroblast stimulation.

Keywords: Millimetre waves, DNA secondary structures, G-quadruplex, i-motif

Acknowledgements: Diwei Ho (UWA), Prof. Daniel Craig (Garvan) and Prof. Shankar Balasubramanian (Cambridge). NL acknowledges the support of the Forrest Research Foundation Scholarship.
Understanding ATRX Role In PML Nuclear Bodies In ATR-X Syndrome

Nayla León¹, Stefan Bagheri-Fam², and Vincent Harley¹

¹Sex Determination and Gonadal Development Laboratory, Hudson Institute of Medical Research, ²Department of Anatomy and Neuroscience, The University of Melbourne.

The ATR-X (alpha thalassemia, mental retardation, X-linked) syndrome is a severe developmental disorder affecting males caused by mutations in the chromatin remodelling gene ATRX. 80% of patients display genital abnormalities which include hypospadias and ambiguous genitalia. These patients have small poorly formed testes containing few seminiferous tubules and previously, we generated a mouse model to study these testicular defects, with Atrx specifically inactivated in the Sertoli cells (ScAtrxKO). ScAtrxKO mice develop small testes with discontinuous tubules due to a prolonged G2/M phase and apoptosis of Sertoli cells during fetal life.¹

Here, we investigate further the cellular and molecular mechanisms underlying the death of ATRX-deficient Sertoli cells. In each wildtype Sertoli cell nucleus, ~6 PML nuclear bodies (PML-NBs) are present. Intriguingly, in ScAtrxKO mice, a single PML-NB within each Sertoli cell nucleus was increased in size about 3-fold. Giant PML-NBs have been observed by others in the lymphocytes of ICF syndrome patients, in which a single giant PML contains hypomethylated DNA and undergoes specific chromosome 1, 9, and 16 breakage.²

Normally after mitosis, ATRX, DAXX and heterochromatin protein 1 (HP1) facilitate re-establishment of condensed heterochromatin within PML-NBs. In the wildtype testes, HP1 and DAXX co-localized with Sertoli cell markers, FOG2 and GATA4, at a single PML nuclear body per Sertoli cell. In contrast, in the ScAtrxKO giant PML-NB, DAXX and HP1 were no longer present, suggesting condensation could be affected. In ScAtrxKO testes, PH3 expression, a marker of late G2 phase and mitosis, was increased suggesting that chromosome condensation was affected at G2/M phase. The giant PML-NBs also co-localized with γ-H2AX, a marker of DNA double strand breaks. We propose that ATRX loss at a single PML-NB in Sertoli cells leads to a failure in chromatin compaction leaving the cell vulnerable to DNA damage and cell death.

Giant PML-NBs do not co-localize with minor satellite DNA, as CENPA marker is absent from these foci. One possibility is that GATA-rich sequences present uniquely on the Y chromosome bind to GATA4 in the PML-NBs. Future studies are directed at defining the precise protein and DNA components of these giant PML NBs.

Keywords: ATR-X syndrome, PML nuclear bodies, Disorders/Differences of sex development.

Morpholino Oligomer-Induced Dystrophin Isoforms to Map the Functional Domains of Dystrophin Protein

Dr Dunhui Li1, Mrs Abbie Adams1, Mr Russell Johnsen1, Prof. Sue Fletcher1, Prof. Steve Wilton1
1Centre for Molecular Medicine and Innovative Therapeutics, Murdoch University, Perth, Australia

Dystrophin is the cytoskeleton protein that maintains the sarcolemma stability during muscle contractions, deficiency of which causes the progressive muscle wasting disorder, Duchenne muscular dystrophy. Antisense oligonucleotide-mediated manipulation of pre-messenger RNA splicing has been approved of safety and efficacy to overcome disease-causing mutations and restore the dystrophin expression level in patients. This strategy is inspired by the genotype-phenotype correlations as indicated by the allelic milder Becker muscular dystrophy, where in-frame deletions of some of the dystrophin exons especially in the central rod domain result in internally truncated but semi-functional dystrophin protein. However, the paucity of Becker-causing mutations in the last third of the dystrophin gene makes the amenability of mutations in this region to exon skipping strategies undetermined. In this study, dystrophin isoforms were induced in vivo by intraperitoneal injection of the peptide-conjugated phosphorodiamidate morpholino oligomers in C57BL/10ScSn and C57BL/10ScSnmdx mice for the functionality evaluation to indicate any therapeutic potential. We demonstrate that the dystrophin expression level was decreased after the induction of dystrophin isoforms by skipping in-frame exon block exons 56+57, or 58+59, with severe dystrophic pathology being examined in the mouse muscle.

Keywords: Antisense oligonucleotide, Pre-mRNA splicing, Duchenne muscular dystrophy;

Acknowledgements: This work was supported by funding to S.D.W. and S.F. from the National Health and Medical Research Council (Grant # 1144791). D.L. receives a postgraduate scholarship from Muscular Dystrophy Western Australia. The authors thank Sarepta Therapeutics Inc. for generously providing the PPMOs. This work was conducted in Perth, Australia.
Exploring Candidate Causal Genes Of IgG N-glycosylation By A Combination Of Genome-wide, Transcriptome-wide And Phenome-wide Association Studies

Xingang Li\textsuperscript{1}, Hao Wang\textsuperscript{1,2}, Yahong Zhu\textsuperscript{3}, Manshu Song\textsuperscript{1,2}, Youxin Wang\textsuperscript{1,2}, Lijuan Wu\textsuperscript{2}, Yuxiang Yan\textsuperscript{2}, Xuehua Guo\textsuperscript{2} and Wei Wang\textsuperscript{1,2,4}*

\textsuperscript{1} School of Medical and Health Sciences, Edith Cowan University, Joondalup, Western Australia, Australia
\textsuperscript{2} Beijing Key Laboratory of Clinical Epidemiology, School of Public Health, Capital Medical University, Beijing, China
\textsuperscript{3} Beijing Lucidus Bio-information Technologies, Beijing, China
\textsuperscript{4} School of Public Health, Shandong First Medical University, Taian, China

Body: Up to date, genome-wide association studies (GWAS) have identified over 10 genetic loci strongly associated with IgG N-glycosylation. However, the suppositional causal genes and the underpinning mechanisms remain unclear. Here, leveraging the GWAS summary statistics of 8,090 Europeans and large-scale expression quantitative trait loci (eQTL) data from Genotype-Tissue Expression (GTEx v7, 53 tissues), we first performed a linkage disequilibrium score for specific expression of genes (LDSC-SEG) investigation and identified 27 types of tissue to be significantly enriched with IgG N-glycosylation. We then conducted transcriptome-wide association studies (TWAS) on IgG N-glycosylation and identified 55 genes whose predicted expression levels are significantly associated with IgG N-glycosylation in 14 tissues. The significance of these 55 TWAS-significant genes is mostly explicated by the cis-regulation of gene expression. Pathway enrichment analysis demonstrated several IgG N-glycosylation related pathways such as asparagine N-linked glycosylation, N-glycan biosynthesis, and transport to the Golgi and subsequent modification. Through phenome-wide association studies (PheWAS), the most significant eQTLs were found to be correlated with autoimmune and neurodegenerative diseases such as systemic lupus erythematosus, inflammatory bowel disease and Parkinson’s disease, and the pathogenesis of these diseases has been reported to be involved with IgG N-glycosylation. Our study provides a list of potential susceptibility genes for further studies on the mechanisms of IgG N-glycosylation and its related diseases.

Keywords: IgG N-glycosylation, Genome-wide association studies (GWAS), Transcriptome-wide association study (TWAS), Phenome-wide association studies (PheWAS)

Acknowledgements: This work was supported by Australia-China International Collaborative Grant (NHMRC APP1112767-NSFC 81561128020), National Natural Science Foundation of China (NFSC 81773527 & 81573215), China Scholarship Council (CSC-201708110200) and Bioyong Industry Scholarship at Edith Cowan University.
Understanding functional drivers of liver metastasis in uveal melanoma

Dr Weitao Lin1,2, Dr Elena Denisenko2, Mr Rui Hou2, Mr Matthew Jones2, Clinical Associate Professor Benjamin A. Wood3,4, Professor Mayank Bhandari5, Clinical Associate Professor Byron Jaques6, Professor Michael Millward7,8, Professor Alistair R. R. Forrest2, Associate Professor Elin S. Gray1

1School of Medical and Health Sciences, Edith Cowan University, Joondalup, Australia, 2Harry Perkins Institute of Medical Research, QEII Medical Centre, the University of Western Australia, Nedlands, Australia, 3Department of Anatomical Pathology, PathWest, QEII Medical Centre, Nedlands, Australia, 4School of Biomedical Sciences, the University of Western Australia, Crawley, Australia, 5Fiona Stanley Hospital, Murdoch, Australia, 6Western Australia Kidney and Liver Transplant Service, Sir Charles Gairdner Hospital, QEII Medical Centre, Nedlands, Australia, 7School of Medicine and Pharmacology, the University of Western Australia, Crawley, Australia, 8Department of Medical Oncology, Sir Charles Gairdner Hospital, QEII medical Centre, Nedlands, Australia

Uveal melanoma (UM) is the second most common subtype of melanoma. Although the primary tumour within the eye is successfully controlled by either surgery or radiation, distant metastases develop eventually in up to 50% of UM patients. Most patients die within 12 months after diagnosis of metastases. The liver is initially the primary metastatic site in 95% of cases. However, the mechanisms for the development of metastases in the liver remain unclear. We propose to exploit Single-cell RNA sequencing (scRNA-seq) to uncover the transcription programing and cell-cell communication signalling that governs UM metastases in the liver.

Three metastatic tumours were collected from 2 UM patients in St. John of God Subiaco Hospital and Sir Charles Gairdner Hospital in Perth. The tumours were freshly dissociated into single cells using the tumour dissociation kit from Miltenyi Biotec as per manufacturers’ instruction. One out of three samples had a large number of single cells therefore part of the cells was cryopreserved. scRNA-seq libraries were prepared for both fresh and cryopreserved metastatic tumours using the 10X Chromium Single Cell 3’ Reagent Kits. Libraries were sequenced using the Illumina NovaSeq 6000. Raw data of scRNA-seq were process by Cell Ranger, followed by in-house pipeline. Seurat 3.0 was used for sample integration, normalisation, scaling, clustering and analysis.

The impact of cryopreservation on the single-cell library was evaluated by comparing cellular recovery of the libraries prepared from fresh and cryopreserved cells. The number of cells and the number of total genes detected were comparable in both libraries. Both libraries have recovered an equal number of cell clusters. Single-cell data were generated from 10,403 cells from 3 liver metastases. Different cell types were identified including melanoma cells, hepatocytes, hepatic stellate cells and endothelial cells. All three tumours contained a large proportion of immune cells, including T cells, B cells and macrophages. The expression of several immune checkpoint molecules was confirmed in melanoma and immune cells, including LAG-3, CTLA4 and CD86. Analysis of cell-to-cell communication between UM cells and liver cells is still ongoing.
Integrating RNA-seq with environment exposure for prioritization of diagnosis biomarkers with schizophrenia

Dr Jia-Hui Ma¹,², Dr Lai-Lai Yan¹, Ms. Ya-Qiong Liu¹, Ms. Qing Xie¹, Dr. Chung-Chau Hon², Dr. Jing-Yu Wang¹
¹Peking University, Beijing, China, ²RIKEN, Yokohama, Japan

Schizophrenia (SCZ) is a severe and complex mental illness, while the exact etiologies remain unknown. However, harmful environment exposure and altered genetic expression have been reported to contribute to the pathogenesis of SCZ. Currently, the diagnosis of SCZ largely relies on subjective cognitive assessment, because there are no widely accepted biochemical or genetic biomarkers for diagnosis. In this study, we collected blood samples from 20 drug naïve patients with SCZ and 20 healthy controls. We first quantified the level of trace elements and transcriptomic profile for each individual. Next, we identified significantly different trace elements and RNAs between SCZ patients and healthy controls, as well as the correlation of the severity of SCZ with respectively trace elements level and RNA expression. We further investigated the gene-environment interaction for SCZ risk by measuring the contribution of altered gene under different environment condition. We demonstrated that genetic variants associated with SCZ, which are also involved in brain functions and neuro-development pathways, are specific for excessive or deficient of some trace elements. By integrating such risk factors including both environment and genetic factors, we identified several panels of biomarkers that could diagnose SCZ and assess the severity of SCZ for different environment condition. These prioritized biomarkers would facilitate the identification of the role of environmental and gene interactions in SCZ, and shed lights on the diagnosis and evaluation of SCZ.

Keywords: Schizophrenia, Biomarker, Environment, Genetic

Acknowledgements: This research was supported by Peking University and RIKEN. We thank all the people involved in this project especially the volunteers donated the blood samples.
Novel *NSDHL* gene variant for Congenital Hemidysplasia with Ichthyosiform Erythroderma and Limb Defects (CHILD) Syndrome

**Ebner Bon G. Maceda¹,2, Lisa E. Kratz³, Veronica Marie E. Ramos⁴, Marie Abigail R. Lim¹, Mary Ann R. Abacan¹,2**

¹ Department of Pediatrics, Philippine General Hospital, University of the Philippines Manila  
² Institute of Human Genetics, National Institutes of Health, University of the Philippines Manila  
³ Biochemical Genetics Laboratory, Kennedy Krieger Institute, Baltimore, Maryland  
⁴ Section of Dermatology, Department of Medicine, Philippine General Hospital, University of the Philippines Manila

**Background:** Congenital Hemidysplasia with Ichthyosiform Erythroderma and Limb Defects (CHILD) syndrome is a rare malformation syndrome due to an inborn error of cholesterol metabolism characterized by unilateral limb reduction defect and ipsilateral ichthyosiform skin lesions, chondrodysplasia punctata and visceral anomalies.

**Objective:** This report aims to present a case of CHILD syndrome, through its clinical, radiologic, biochemical and molecular features and its management.

**Case Description:** We report a case of a 1 year 2 month old female with features of CHILD syndrome. Sterol analysis from skin flakes revealed increased levels of mono 4-alpha methyl sterols, as also seen in plasma. Large amount of 4 alpha-carboxy-4-methyl-cholesterol-8(9)-en-3beta-ol and the presence of 2 keto-sterols, which are usually below the limit of detection, were also noted. This sterol pattern is consistent with abnormal function of the 4 alpha-methylsterol-4-demethylase complex. *NSDHL* gene testing revealed the presence of a variant of uncertain significance, c.130G>A (p.Gly44Ser). This missense mutation currently is not included in population databases (ExAC no frequency). It has not yet been reported in literature in any individual with NSDHL-related condition. Parental studies showed that neither parent carries the NSDHL variant. This variant was then reclassified as likely pathogenic. Genetic counseling was done. The patient was started on keratolytic agents, emollients and ketoconazole.

**Conclusion:** This report highlights the role of clinical presentation, plasma/tissue sterol measurements and molecular studies in the diagnosis and management of CHILD syndrome.

**Keywords:** CHILD syndrome, cholesterol metabolism  
**Acknowledgements:** The authors would like to thank Invitae for facilitating the testing of the parents free of charge.
How do we tackle the problem of equity for minorities when developing Polygenic Risk Scores (PRS)?

Presenting Author: David A Mackey.
Institute name, Centre for Ophthalmology and Visual Science, University of Western Australia.

Body: There is a lack of racial and ethnic diversity in genomics studies and biorepositories. This may result in misclassification of benign variants as pathogenic in minorities. In addition polygenic risk scores (PRS) derived from other populations may not be as accurate in minorities. We need to establish biorepositories of ancestry-matched controls for variant interpretation as well as biorepositories of disease cohorts from ethnic minorities to validate PRS. Australia has limited genetic data on its Aboriginal population, but work is underway to attempt to address this issue. The majority of Australian’s are descended from Northern European populations, but Australia is now a multicultural population with many other ethnic minorities. What are the more common minorities and how do we address the issue of establishing control and disease cohorts for future genetic studies? National data on country of birth is available every five years since the first Australian census in 1901. Since World War two there was a large influx of immigrants from around the Adriatic (e.g. Italy, Former Yugoslavia and Greece) many of their Australian born children and grandchildren will have genetic heritage of these countries. More recently we have seen large numbers of immigrants from Vietnam, Philippines, Malaysia, South Korea, Indonesia as well as India and China. Whilst it would seem easy to recruit control members of these and other nationalities through local community organizations and foreign language media, as many of these and other populations have less than 100,000 members in Australia, collecting affected individuals with some diseases will be a challenge.

To address these challenges with translating genetics research for the eye disease glaucoma, we are proposing a multipronged approach. Firstly auditing and recruiting from the multicultural public hospital clinics; secondly engaging with ophthalmologists with targeted ethnic backgrounds in Australia; thirdly collaborating with former fellows and researchers in the eye clinics in the targeted home countries. This aims to build an adequate number of genotyped glaucoma-affected individuals from each nationality. To maximize the value of this approach we would hope to collaborate with other researchers. If many different disease cohorts are collected then these can be used as controls for the other diseases, an approach that was useful with the Wellcome Trust Case Control Consortia.

Keywords: Polygenic Risk Scores 1, Genome Wide Association Studies 2, Minority populations 3, Glaucoma 4,

Acknowledgements: National Health and Medical Research Council Practitioner Fellowship
Genome Wide Methylation Profiling Identifies Key Biological Pathways Deregulated in Indian Early and Late Onset Breast Cancer Patients by Epigenetic Deregulation

Shreshtha Malvia¹, Chintamani Chintamani², Ramesh Sarin³, Deepshikha Arora⁴, Sunita Saxena¹, Sarangadhara Appala Raju Bagadi*¹
1. ICMR-National Institute of Pathology, New Delhi, India
2. Department of Surgery, Safdarjung Hospital, New Delhi, India
3. Department of Surgery, Indraprastha Apollo Hospital, New Delhi, India
4. Department of Pathology, Indraprastha Apollo Hospital, New Delhi, India

Objective
To identify discriminating epigenetic signatures associated with early and late onset breast cancer

Method
In the present study biopsies from 36 histologically confirmed breast cancer patients, of which 19 biopsies were from early onset (ET) (age ≤40 yrs.) and 17 biopsies were from late onset (LT) (age ≥55 yrs.) were recruited for whole genome methylation profiling using Illumina methylation array. The patient samples were collected prior to any therapy. The differential methylation was validated in 740 breast cancer patients and 92 controls using TCGA database in silico tools MethHC.

Result
To identify the genes that are hypermethylated in early and late onset breast cancer patients, differential methylation analysis was done. The analysis showed several aberrantly methylated genes in ET and LT breast cancer; 6050 CpG sites differentially methylated in ET and 4966 sites differentially methylated in LT cancer. These differential CpG sites corresponded to 528 genes (327 hypermethylated, 201 hypomethylated) in ET and 525 genes (466 hypermethylated, 59 hypomethylated) in LT. Comparison of these genes showed 385 (42%) unique genes aberrantly methylated in ET, 382 (42.3%) genes unique to LT and 143 (15.7%) genes found methylated in both early and late onset tumours. Pathways such as apoptosis, cAMP signalling pathway, cell adhesion molecule pathway, cell cycle, cytokine-cytokine receptor interaction pathway were altered in ET while cAMP signalling pathway and cell adhesion pathways were altered in LT. Hierarchical clustering was done amongst breast tumour and controls, differentially methylated clusters could be identified amongst them. Network analysis was also done to identify the top methylated nodes in ET and LT breast cancer patients. Integration of methylation with gene expression data showed STAT5A, LIPE, ALDH1A2 genes getting hypermethylated and downregulated in ET while genes as LEP, EDNRB, FBLN2 were specifically getting hypermethylated and downregulated in LT tumours. They are known to function by deregulating lipid metabolism pathway thus disrupting energy homeostasis in breast cancer. In silico analysis of genes which were found differentially methylated was done using TCGA database with the help of online tools MethHC. The methylation of the genes, showed concordance with that of TCGA data.

Conclusion
Differential methylation analysis has identified several genes and the pathways getting deregulated in early and late onset breast cancer which holds potential to serve as biomarker. They need to be further validated in larger cohort of patients.

Keywords: epigenetic, differential methylation, early/late onset, breast cancer

Acknowledgement: The authors thank Department of Biotechnology (DBT), India, for funding the experimental work.
The Combination of Congenital Aniridia and Congenital Hearing Organ Malformation in One Clinical Portrait

Andrey V. Marakhonov*, Tatyana A. Vasilyeva¹, Vitaly V. Kadyshhev¹, Rena A. Zinchenko¹
¹Research Centre for Medical Genetics, Moscow, Russia

Aim: To determine the genetic cause of the phenotype in a patient with congenital aniridia and other congenital malformations.

Material and methods: The proband (girl, 4 months old) underwent clinical and paraclinical examination. A search for pathogenic variants was performed using next-generation sequencing (NGS) analysis and the detected changes were confirmed by direct Sanger sequencing. Written informed consent was obtained from the child’s parents.

Results: During the ophthalmologic examination, the following were revealed: bilateral congenital complete aniridia, stage 1 keratopathy, compaction of anterior cortical layers and lens stroma with dot dense blurring in the center; hypoplasia of optic nerve discs was seen on the eye fundus. Somatic examination revealed bilateral microtia of 2-3 degrees, conductive hearing loss of 3-4 degrees was diagnosed; congenital atresia of the anus with perineal fistula, doubling of the left kidney were also seen. The father has similar clinical changes in the eyes, somatically healthy. The NGS analysis revealed the previously described pathogenic variant in the PAX6 gene: NM_000280.4(PAX6):p.718.C>T, p.(Arg240*), inherited from the father, and de novo probably pathogenic novel variant in the HOXA2 gene NM_006735.3(HOXA2):c.124C>G, p.(Leu42Val) - both in the heterozygous state.

Conclusion: The combination of the two phenotypes in the proband is the result of changes in PAX6 and HOXA2 genes. In the described case the NGS analysis allowed us to perform differential diagnosis of the clinical portrait and to establish two nosological units of hereditary pathology in the proband. The variants in the PAX6 gene in the heterozygous state lead to the development of congenital aniridia (OMIM #106210), the variants in the HOXA2 gene in the heterozygous state are described in patients with congenital microtia and hearing loss/noise impairment (OMIM #612290). This example indicates the need to fix all details of the phenotype and then choose NGS analysis as a DNA diagnostic method for objective genetic counseling.

Keywords: “double trouble”, congenital aniridia, congenital microtia, NGS sequencing, hearing loss

Acknowledgments: The study was partly supported by RFBR grant 19-015-00122.
An Association Study of HLA-G Regulatory Region Polymorphisms with Recurrent Pregnancy Loss (RPL)

Ms Binata Marik, Mr Anshul Sharma, Dr Nutan Agarwal, Ms Tripti Grover, Ms Sushil Kumari, Dr. Arundhati Sharma

All India Institute of Medical Sciences (AIIMS), New Delhi, India

Introduction: Human Leukocyte Antigen-G (HLA-G), a nonclassical Class-I major histocompatibility complex (MHC) gene, is expressed on the cytotrophoblast cells of the placenta. It modulates maternal immune system during pregnancy and helps in the acceptance of the semiallogenic fetus. Polymorphisms in the HLA-G promoter and 3'-untranslated region (UTR) may impact HLA-G expression leading to pregnancy loss. Aim: To evaluate the association of HLA-G regulatory region polymorphisms with RPL. Methodology: A total of 106 couples with ≥3 consecutive idiopathic miscarriages were recruited in comparison to 86 couples who had normal pregnancies. Detailed clinical and family history was documented, and seven ml peripheral blood was drawn. Karyotyping was done to look for chromosomal abnormalities and only those patients with normal karyotype screened for the HLA-G polymorphisms using PCR followed by direct sequencing. Results: Chromosomal abnormalities were not found in the couples. HLA-G screening revealed the presence of -716T/G, -725C/G, -762C/T, -689A/G, -486C/A and -477 C/G polymorphisms in the HLA-G promoter. Among them, only -762C/T and -417T/G showed association with RPL with the frequency of CC homozygotes being higher in controls (31% and 41% respectively) than in patients (5% and 4% respectively). These two polymorphisms increase the risk of RPL because -762 C/T closely flank interferon response factor-1 (IRF-1) binding motif. Therefore, the ability of IRF-1 to bind to HLA-G promoter having -762 TT or TG genotype might be reduced resulting in decreased HLA-G expression and fetal loss. Also, -477 C/G SNP is located within tissue specific regulatory element which is important for trophoblast specific expression of HLA-G. A 14-bp insertion/deletion polymorphism at position +3741 in 3'-UTR of exon 8 was identified and the frequency of the heterozygous genotype was significantly higher in RPL patients (53%) as compared to controls (34%). The 14-bp insertion introduces an alternative splice site that removes 92-bp from the 3'-UTR modulating HLA-G expression. This polymorphism is associated with reduced concentrations of HLA-G mRNA and soluble HLA-G in serum. Genotype combination analysis revealed the presence of two combinations (14-bp insertion/insertion x -477GG x -762CT; 14-bp insertion/insertion x -477CG x -762TT) only in patients and not in controls suggesting that these three genotypes when present together might affect HLA-G expression and could cause fetal loss. Conclusion: This study shows a protective role of the CC genotypes of the two polymorphisms and also suggests that HLA-G polymorphisms either alone or in combination could be responsible for RPL.

Keywords: semiallogenic, fetus, promoter, 14-bp insertion/deletion
The Germline SequenceVariant rs2736100_C in TERT IncreasesMyeloproliferative Neoplasms Risk: ACase-Control Study and Meta-Analysis

Md Abdullah Al Maruf1, Kim Hung Leung1, Regina Kwarteng1, Chien Ling Huang1, Shea Ping Yip1*

1Department of Health Technology & Informatics, Faculty of Health & Social Sciences, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong SAR, China

Body: Genome-wide association studies have disclosed the association of thousands of germline variants with multiple types of cancers as well as other diseases. Several previous studies suggest that some single nucleotide polymorphisms (SNPs) of the TERT gene and myeloproliferative neoplasms (MPN) have an association. The C allele of the rs2736100 SNP located in the second intron of the TERT gene has been identified as a susceptibility factor for MPN and other cancers in multiple ethnicities. In this study, we investigated the association between rs2736100 polymorphism and MPN in Hong Kong Chinese people. We genotyped 192 MPN cases and 480 healthy controls using unlabeled probe melting curve analysis. Odds ratio (OR) and 95% confidence interval (CI) were calculated by logistic regression. A meta-analysis of ten potentially relevant studies was also performed after comprehensive literature search in four electronic databases including PubMed, EMBASE, Scopus and Google Scholar to elucidate this association by increasing statistical power. ORs and 95% CIs were estimated to assess the association between rs2736100 and MPN risk in selected case-control studies. A sensitivity analysis, a test of heterogeneity, a cumulative meta-analysis, and an assessment of bias were also performed. After conducting a single-marker analysis, we found a strong association of TERT rs2736100_C with Hong Kong Chinese MPN patients (P = 0.0003). The C allele of rs2736100 was found to be a risk allele (OR: 1.54, 95% CI: 1.22–1.97, P = 0.0007) after adjustment for age and sex. In meta-analysis part, the rs2736100 polymorphism was associated with an increased MPN risk in five genetic models: allelic model (C vs. A), OR: 1.55, 95% CI: 1.46–1.64, P < 1 × 10^{-10}; homozygous model (CC vs. AA), OR: 2.49, 95% CI: 2.20–2.82, P < 1 × 10^{-10}; heterozygous model (CA vs. AA), OR: 1.79, 95% CI: 1.60–2.01, P < 1 × 10^{-10}; dominant model (CC+CA vs. AA), OR: 2.02, 95% CI: 1.82–2.26, P < 1 × 10^{-10}; and recessive model (CC vs. CA+AA), OR: 1.67, 95% CI: 1.44–1.93, P < 1 × 10^{-10}. In conclusion, we demonstrated that TERT rs2736100_C is a predisposing factor for MPN in Hong Kong Chinese patients. Moreover, our results from meta-analysis confirm that the TERT rs2736100 polymorphism confers increased overall MPN risk.

Keywords: Myeloproliferative neoplasms, TERT, rs2736100, Case-control study, Meta-analysis

Acknowledgements: The study was supported by the grants from The Hong Kong Polytechnic University, Hong Kong.
Associative analysis of 62 genetic variants (SNPs) with the variability of cognitive function domains, defined by the MoCA (Montreal Cognitive Assessment) test in older people

A. V. Marusin*, O. A. Makeeva, K.V. Vagaitseva, A. V. Bocharova, V. A. Stepanov
Research Institute of Medical Genetics, Tomsk National Medical Research Center, Russia

Cognitive decline with age is an important social and medical problem that leads to a significant reduction the quality of life of the elderly. The aim of this work was the replicative association analysis of 61 SNPs with the variability in cognitive function, defined by the MoCA (Montreal Cognitive Assessment) test, in the elderly.

The study was performed on a population sample of the 708 elderly Russian individuals (181 men and 527 women). The mean age was 71.9±0.6 years (from 62 to 87 years). On the basis of the selected 62 markers were formed 2 multiplexed SNP panels and genotyping by MALDI-TOF mass spectrometry on Sequenom MassArray platform was performed.

The largest number of statistically significant associations with the cognitive domain of the test the Montreal scale of cognitive function scores determined by the method of generalized multiple regression was shown for the locus of the linked markers PVRL-TOMM40-APOE-APOC1 gene cluster on chromosome 19q13.32. Polymorphic variants in the APOE gene (rs429358, rs769449 and apoE isoforms) were associated with cognitive domains of attention, memory, and visuospatial abilities at the 5% significance level. For the PVRL2 gene (rs6857, rs6859), as well as TOMM40 (rs2075650, rs157580), APOC1 (rs4420638) genes, the relationships with memory domains and visuospatial abilities were also revealed.

The data obtained during the implementation of the project expand understanding of the neuropsychiatric diseases inheritance, as well as the biological processes underlying the decline in cognitive abilities in the elderly.

Keywords: domains of cognitive performance, genetic polymorphism, associations, elderly people

Acknowledgements: The reported study was funded by RFBR, project number 20-015-00397.
Genomewide Dysregulation of Genes Associated with LOY in Immune Cells found by Single-cell and Bulk RNA Sequencing

Jonas Mattisson¹, Jonatan Halvardson¹, Hanna Davies¹, Edyta Rychlicka-Buniowska², Behrooz Torabi Moghadam¹, Kazimierz Węglarczyk³, Karolina Bukowska-Strakova¹, Marcus Danielsson¹, Erin Oerton⁴, Vičmintas Giedraitis⁵, Lena Kilander⁶, Maciej Siedlar³, Alicja Klich-Rączka⁷, Janusz Jaszczyński⁶, Jarosław Baran³, Martin Ingelsson⁵, John R. B. Perry⁴, Janusz Rys⁹, Lars A. Forsberg¹⁰, Jan P. Dumanski¹¹

¹Department of Immunology, Genetics and Pathology and Science for Life Laboratory, Uppsala University, Uppsala, Sweden, ²International Research Agendas Programme, 3P Medicine Laboratory, Medical University of Gdańsk, Gdańsk, Poland, ³Department of Clinical Immunology, Institute of Paediatrics, Jagiellonian University, Collegium Medicum, Kraków, Poland, ⁴MRC Epidemiology Unit, School of Clinical Medicine, University of Cambridge, Cambridge, United Kingdom, ⁵Department of Public Health and Caring Sciences/Geriatrics, Uppsala University, Uppsala, Sweden, ⁶Department of Internal Medicine and Gerontology, Jagiellonian University, Collegium Medicum, Kraków, Poland, ⁷Department of Urology, Maria Skłodowska-Curie Memorial Cancer Centre and Institute of Oncology, Kraków Branch, Kraków, Poland, ⁸Department of Pathology, Maria Skłodowska-Curie Memorial Cancer Centre and Institute of Oncology, Kraków Branch, Kraków, Poland, ⁹The Beijer Laboratory, Uppsala University, Uppsala, Sweden, ¹⁰Faculty of Pharmacy and 3P Medicine Laboratory, International Research Agendas

Loss of chromosome Y (LOY) in leukocytes is associated with all-cause mortality and risk for diseases such as cancer, Alzheimer’s disease as well as other common human disorders. Men with LOY carry a fraction of peripheral blood cells without the Y chromosome due to its loss from hematopoietic progenitor cells during lifetime. For single cells, LOY is a binary event and the mutation manifests as a continuous mosaicism in bulk samples. Here we perform single-cell and bulk RNA sequencing in leukocytes, observing considerable variation in the rate of LOY across individuals and cell types. Our findings highlight a broad role for chromosome Y, challenging the view of it as a “genetic wasteland”. The Y chromosome harbor 64 protein coding genes that are involved in processes such as gene regulation, immune response and immune cell signaling. Remarkably, we observe that immune cells with LOY demonstrated a profound degree of transcriptional dysregulation impacting also ~500 autosomal genes. Genes displaying LOY Associated Transcriptional Effects (LATE) are preferentially involved in immune functions, such as LAG3, LY6E, IL2R1 and CD99, but also encode proteins with roles in other diverse biological processes. Overall, our results support the hypothesis that altered immune function in leukocytes is a mechanism that could directly link LOY in blood cells, with risk for disease in other organs. Men in the entire world live on average about five years shorter compared with women. As a male specific somatic mutation, LOY in immune cells could help explain this sex bias in longevity.

Keywords: LOY, Mosaic loss of chromosome Y, Single-cell RNA sequencing, scRNA-seq, Leukocytes
Development of A Paraspeckle Biosensor To Sense Cellular Stress

Mr Finn McCluggage\textsuperscript{1,2}, Dr Nicole Smith\textsuperscript{1}, Professor Charles Bond\textsuperscript{1}, Dr Liisa Hirvonen\textsuperscript{3}, Dr Max Nobis\textsuperscript{4}, Professor Paul Timpson\textsuperscript{4}, Assoc/Prof Archa Fox\textsuperscript{1,2,3}

\textsuperscript{1}School of Molecular Sciences, The University of Western Australia, Crawley, Australia, \textsuperscript{2}School of Human Sciences, University of Western Australia, Crawley, Australia, \textsuperscript{3}Harry Perkins Institute of Medical Research, Nedlands, Australia, \textsuperscript{4}The Garvan Institute of Medical Research, Darlinghurst, Australia

Body: Cellular stress responses are protective mechanisms employed by cells that are critical for the maintenance of cellular homeostasis. Disruptions of cellular stress responses are implicated in a range of diseases, such as cancer, viral infection and neurodegeneration. One common feature between many cellular stress responses is the increased formation of subnuclear bodies, termed paraspeckles. Paraspeckles are ribonucleoprotein bodies formed via interactions between the long noncoding RNA (IncRNA), NEAT1, and paraspeckle associated proteins. The rapid response of paraspeckles to stress and disease states suggests paraspeckles as a biological system for studying cellular homeostasis and the stress response. However, limited effective methodologies are available for visualising paraspeckles using live cell imaging. The aim of the study is to develop and validate a paraspeckle-specific fluorescent biosensor for live-cell imaging. Specifically, the study aims to generate a biosensor activated by protein-protein interactions monitored by Förster resonance energy transfer (FRET). Stable cell lines expressing essential paraspeckle proteins tagged with EGFP and mCherry will be generated. FRET between interacting tagged proteins will then be visualised via Time-Correlated Single Photon Counting (TCSPC) Fluorescent Lifetime Imaging Microscopy (FLIM). The benefit of the development of a biosensor to visualise paraspeckles is three-fold. Firstly, as a ubiquitous stress biosensor for the visualisation of stress responses across time. Secondly, for use in paraspeckle studies, to increase knowledge of paraspeckle function and structure. Finally, for live cell imaging of the NEAT1 IncRNA, to further develop understanding of IncRNA dynamics and function.

Keywords: Paraspeckles, FLIM-FRET, Biosensor, Cellular stress, NEAT1

Acknowledgements: Supported by the Australian Government Research Training Program Scholarship.
Antisense Oligonucleotide-Mediated Exon Skipping to Treat Spinocerebellar Ataxia Type 3

Mr Craig McIntosh\textsuperscript{1,2}, Dr May T. Aung-Htut\textsuperscript{1,2}, Prof. Sue Fletcher\textsuperscript{1,2}, Prof. Steve Wilton\textsuperscript{1,2}

\textsuperscript{1}CMMIT, Murdoch University, Murdoch, Australia, \textsuperscript{2}The Perron Institute for Neurological and Translational Science, Nedlands, Australia

Spinocerebellar ataxia type 3 (SCA3) is a devastating neurodegenerative disease, which is one of nine polyglutamine disorders. Although SCA3 is pathogenically heterogeneous, the main feature is progressive ataxia, which in turn affects speech, balance and gait of the affected individual. There is currently no cure, nor effective treatment strategy for affected individuals. SCA3 is caused by an expanded polyglutamine tract found in ataxin-3, resulting in conformational changes that lead to toxic gain of function. This expanded glutamine tract is located at the 5’ end of the penultimate exon (exon 10) of \textit{ATXN3}. This study aims to use antisense oligonucleotide (AO) mediated exon skipping to develop a therapeutic strategy for the treatment of SCA3.

Initial \textit{in vitro} data using 2’-O-methyl AOs in patient cells show that it is possible to create an internally truncated protein, missing the toxic CAG repeat contained in \textit{ATXN3} and still maintain normal function of the protein. Confirmatory data using the clinically relevant phosphorodiamidate morpholino oligomer (PMO) chemistry showed complementary positive results to 2’O-methyl data. Additionally, significant downregulation of both the mutant and wild-type protein was observed, allowing for a combination of benefits. However, PMO is widely considered to be a superior chemistry when compared to 2’-O-methyl, as they are chemically stable and have an excellent safety profile to date. Further data shows that PMO chemistry is longer lasting and significantly better tolerated by cells. Therefore, this study provides a possible therapeutic strategy to treat SCA3.

\textbf{Keywords:} spinocerebellar ataxia type 3; antisense oligonucleotides; exon skipping; ataxin-3; polyglutamine; phosphorodiamidate morpholino oligomer

\textbf{Acknowledgements:} All CMMIT staff and students
Oxford Nanopore Technologies and Cancer Whole Genome Sequencing: the COLO829 Truth Set

Liam McIntyre1, Marjan Mojtabavi Naeini1, Scott Wood1, Katia Nones1, Conrad Leonard1, John Pearson1, Nicola Waddell1 and Liam McIntyre*
1QIMR Berghofer Medical Research Institute

Body: Cancer genomics is paving the way for genomic guided precision medicine whereby treatments are selected based on a patient’s germline and tumour genomic information. Short paired read whole genome sequencing (WGS) has been the enabling technology to date, however, it is inherently limited by the sequencing by synthesis chemistry. Subsequently, there are several blind spots in short read WGS such as an inability to map to repetitive genomic regions and challenges in calling indels and other SVs larger than 75bp. Long read sequencing technologies, such as Oxford Nanopore Technologies (ONT), may overcome some of these short read limitations. Further, ONT WGS allows for individual genome assembly, phasing and simultaneous profiling of DNA methylation. However, ONT has inherent limitations too - a high level of sequence error makes somatic variant calling extremely challenging.

Here, we present a somatic consensus truth set derived from 6 technical replicates of short read sequence data (Illumina, n=3; BGI, n=3) for a melanoma cell line and matched germline sample and compare to one technical replicate whole genome sequenced by ONT. We also explore the ability of ONT to identify methylation changes. We discuss the challenges of utilising ONT WGS for somatic cancer profiling and evaluate strategies to combine short and long read technologies to derive the best possible variant data.

Keywords: Oxford Nanopore Technologies, whole genome sequencing, clinical genomics, epigenetics, genetic diagnosis

Acknowledgements: Kinghorn Centre for Clinical Genomics (KCCG) Sequencing Laboratory, Garvan Institute of Medical Research and the Australian Skin and Skin Cancer Research Centre (ASSC) Early Career Researcher grant.
Chromosomal Aberrations in Primary Amenorrhea Patients: Malaysian Experience

Farah Amalina M.Z.1, Chin L.K1, Roziana A.1
1Genetics Laboratory, Women and Children Hospital Kuala Lumpur.

Primary amenorrhea is defined by absence of menses when a female reach the age of 16, despite presence of normal growth and secondary sexual characteristics. Amenorrhea accounted about 20% of infertility cases. It was previously reported that 24.7% primary amenorrhea caused by abnormal karyotype consisting of numerical and structural chromosomal abnormality. The objective of this study is to evaluate frequency of chromosomal abnormalities seen in primary amenorrhea patients referred to Genetics Laboratory, Women and Children Hospital Kuala Lumpur, Malaysia. A retrospective study using patients’ data from 2016 to 2019 was carried out and Giemsa-stained conventional cytogenetics results were evaluated. Fluorescence in Situ Hybridization (FISH) was performed as confirmation study to detect presence of Sex-determining region Y (SRY) gene and chromosome X. Our study revealed that the most frequent chromosomal abnormality seen in primary amenorrhea was 45,X (Turner syndrome), XY female (Swyer syndrome), 45,X/46,XY (variant Turner syndrome) and structural chromosomal rearrangement in chromosome X. Patients with 46,XX were screened to exclude mosaicism. Presence SRY gene is crucial in the development of male sexual organs and presence of this SRY gene in female will exhibit development of female reproductive organs. This condition leads to under developed gonads and patients with this conditions were also called as 46,XY complete gonadal dysgenesis. These results highlighted the important roles of cytogenetic analysis and confirmation of structural chromosomal aberrations by FISH in primary amenorrhea which then will facilitate patient diagnosis alongside with patient management.

Keywords: primary amenorrhea, chromosome, SRY, XY female
Acknowledgements: Special thanks to residents of Genetics Laboratory, Pathology Department, Women and Children Hospital Kuala Lumpur
CRISPR/dCas9 Activates Endogenous Gene Expression in Patient Fibroblasts

Sang Yoon Moon\textsuperscript{1,2}, Dan Zhang\textsuperscript{2}, Carla Mellough\textsuperscript{1,2}, Shang-Chih Chen\textsuperscript{2}, Tina Lamey\textsuperscript{1,3}, Jennifer A. Thompson\textsuperscript{3}, Terri McLaren\textsuperscript{1,3}, John N. De Roach\textsuperscript{1,3}, Fred K. Chen\textsuperscript{1,2,3,4}, Samuel McLenachan\textsuperscript{1,2}

\textsuperscript{1} Centre for Ophthalmology and Visual Science, The University of Western Australia, Nedlands, Western Australia
\textsuperscript{2} Lions Eye Institute, Nedlands, Western Australia
\textsuperscript{3} Australian Inherited Retinal Disease Registry and DNA Bank, Department of Medical Technology and Physics, Sir Charles Gairdner Hospital, Perth, Western Australia, Australia
\textsuperscript{4} Department of Ophthalmology, Royal Perth Hospital, Perth, Western Australia

\textbf{Body:} The ability to differentiate induced pluripotent stem cells (iPSCs) into retinal cells holds promising potential to characterise the effect of genetic mutations. However, the time-consuming and costly nature of this process constrains high throughput implementation. The use of a catalytically inactivated Cas9 enzyme (dCas9) to directly activate endogenous gene expression in patient fibroblasts presents an alternative and more rapid approach to characterise mutations, by bypassing the need to utilise iPSCs and perform lengthy retinal differentiation experiments. Mutations in the Crumbs homolog 1 (CRB1) gene are associated with various forms of retinal dystrophy, including retinitis pigmentosa (RP). We hypothesise that treatment of human fibroblasts derived from a patient with CRB1-related RP with CRISPR/dCas9 will increase the expression of the disease-causing gene allowing transcriptional analysis.

Dermal fibroblasts were cultured from an RP patient carrying compound heterozygous mutations (c.1892A>G and c.2548G>A) in the CRB1 gene. The Edit-R CRISPRa transcriptional activation system with synthetic guide RNA was used to transcriptionally activate the CRB1 gene in these patient fibroblasts. Gene expression was analysed by qRT-PCR and gel electrophoresis using primers targeting the C-terminus. PCR products were purified and CRB1 transcripts confirmed by Sanger sequencing.

qRT-PCR analysis revealed successful upregulation of CRB1 in CRISPR/dCas9-treated patient-derived fibroblasts compared with controls. Gel electrophoresis confirmed activation of the CRB1 transcript by Sanger sequencing. We demonstrate the successful upregulation of CRB1 in patient fibroblasts using the CRISPR/dCas9 system for further characterisation. Activation of endogenous genes in fibroblasts will provide rapid access to mRNA and protein to determine the effect of mutant genes.

\textbf{Keywords:} CRISPR/dCas9, gene activation, mutation characterisation

\textbf{Acknowledgements:} This project was funded by the National Health and Medical Research Council of Australia (grants 1142962, 1116360 and 1188694), the Ophthalmic Research Institute of Australia (ORIA/Esme Anderson Grant) and generous donations from the Hogg family.
Investigating the Genetic Architecture of Osteoporosis Using Expression Quantitative Trait Locus Data from Human Osteoclasts

Benjamin H Mullin1,2, Jennifer Tickner2, Kun Zhu1,3, Jacob Kenny2, Shelby Mullin1,2, Suzanne J Brown1, Frank Dudbridge4, Nathan J Pavlos5, Edward S Mocarski6, John P Walsh1,3, Jiakie Xu2, Scott G Wilson1,2,6

1Department of Endocrinology & Diabetes, Sir Charles Gairdner Hospital, Nedlands, WA, Australia, 2School of Biomedical Sciences, University of Western Australia, Crawley, WA, Australia, 3Medical School, University of Western Australia, Crawley, WA, Australia, 4Department of Health Sciences, University of Leicester, Leicester, UK, 5Department of Microbiology and Immunology, Emory Vaccine Center, Emory University School of Medicine, Atlanta, GA, USA, 6Department of Twin Research & Genetic Epidemiology, King’s College London, London, UK.

Body: Osteoporosis is a systemic bone disease characterised by a reduced bone mineral density (BMD), which leads to increased bone fragility. The disease has a strong heritable component and genome-wide association studies (GWAS) have identified many genetic variants associated with low BMD. A recently published GWAS for estimated bone mineral density (eBMD) identified 1,103 independent genome-wide significant association signals. Most of the genetic variants underlying these signals are non-coding, suggesting that regulatory effects may drive many of the associations. Expression quantitative trait locus (eQTL) studies using disease-specific cell types have increasingly been integrated with the results from GWAS to identify genes through which the observed GWAS associations are likely mediated. We recently generated a unique osteoclast-specific eQTL resource using cells differentiated in vitro from 158 patients undergoing BMD scanning at Sir Charles Gairdner Hospital in Western Australia. To identify genes with a role in osteoporosis, we integrated the recently published eBMD GWAS association results with those from our osteoclast eQTL dataset. Sixty-nine significant cis-eQTL effects (+/- 1Mb) were identified for eBMD GWAS variants after correction for multiple testing, most of which were located in close proximity to their respective gene transcription start site. A significant proportion (34.8%) of these eQTL associations are potentially osteoclast-specific when compared with eQTL data from the 53 tissues of the GTEx V7 dataset. Co-localisation of eBMD GWAS and osteoclast eQTL association signals was detected for 19 of the 69 loci, implicating a number of genes including CCR5, CPE, RIPK3, IQGAP1 and FLCN. Summary-data-based Mendelian Randomisation analysis of the eBMD GWAS and osteoclast eQTL datasets identified associations between expression levels of 53 genes and the eBMD trait. Analysis using the GARFIELD software demonstrated significant enrichment of osteoporosis risk variants among high-confidence osteoclast eQTL across multiple GWAS P-value thresholds (P=7.79×10^{-18}-3.25×10^{-40}). Mice lacking one of our genes of interest, the apoptosis/necroptosis gene RIPK3, presented with altered bone micro-architecture and increased osteoclast number, highlighting a new biological pathway relevant to osteoporosis.

In summary, we have generated a unique osteoclast eQTL dataset and have used this to identify a number of potential effector genes for osteoporosis risk variants. We found that a significant proportion of the osteoclast eQTL associations identified appear to be specific to this cell type, and that high-confidence osteoclast eQTL are strongly enriched for osteoporosis risk variants. The results from this project will help focus functional studies in this area.

Keywords: osteoclast, osteoporosis, GWAS, eQTL, BMD.

Acknowledgements: This work was supported by the Australian National Health and Medical Research Council (Project Grants 1010494, 1048216, 1087407, 1107828, 1127156, 1163933), the Sir Charles Gairdner Osborne Park Health Care Group (SCGOPHCG) Research Advisory Committee (Grant 2016-17/017) and the iVEC/Pawsey Supercomputing Centre (Project Grants: Pawsey0260 (S.G.W.), Director2025 (S.G.W.)). The salary of B.H.M. was supported by a Raine Medical Research Foundation Priming Grant.
NEAT1 Long Noncoding RNA Isoform-switching Via Antisense Oligonucleotides Regulates Paraspeckle Formation In High-Risk Neuroblastoma

Ms Alina Naveed¹, Dr Ruohan Li¹, Mr Jack Cooper¹, Professor Sue Fletcher², Professor Steve Wilton², Associate Professor Archa Fox¹

¹University of Western Australia, Perth, Australia, ²Murdoch University, Perth, Australia

Paraspeckles are subnuclear organelles involved in the regulation of gene expression. Nuclear Enriched Abundant Transcript 1 (NEAT1) is a long non-coding RNA (IncRNA) required for paraspeckle formation that exists in two overlapping isoforms of sizes 3.7 kb and 23 kb, which are termed NEAT1_1 and NEAT1_2 respectively. NEAT1_2 is found to be the IncRNA back bone around which paraspeckles form, whereas NEAT1_1 can form paraspeckle-independent foci called microspeckles.

A clinical setting in which paraspeckle abundance may be important is the childhood brain cancer neuroblastoma, where changes in NEAT1 levels are correlated with patient outcome. Neuroblastoma is the most common infant cancer, ranging from low-risk, differentiated cancer cells to high-risk cells that maintain an undifferentiated, self-proliferative state. In this study, we show that paraspeckles may be implicated in the regulation of differentiation of high-risk neuroblastic cells, potentially reducing their self-proliferative capacity. We have developed antisense oligonucleotides that bind specific regions of NEAT1 RNA and induce the isoform switching of NEAT1_1 to NEAT1_2, thereby increasing paraspeckles in high-risk neuroblastoma cell lines. Hence, these oligonucleotides have been dubbed Boost paraspeckle (BoostPS) oligonucleotides. High-risk cells transfected with the BoostPS oligonucleotides displayed reduced cell viability and reduced cell confluence, indicating that reducing NEAT1_1 and increasing paraspeckle numbers may suppress proliferation. Accordingly, when we overexpressed NEAT1_1, cells had increased viability, suggesting microspeckles may have an oncogenic role in opposition to the tumour-suppressive paraspeckles. RNA-seq experiments with high-risk cells treated with BoostPS oligonucleotides show upregulation of differentiation pathways, which were further assessed studying RNA and protein levels. In summary, we have developed a method to transiently alter the formation of paraspeckles and have demonstrated that paraspeckles are associated with differentiation regulation. The BoostPS oligonucleotides may be useful in the clinical setting of high-risk neuroblastoma and hold therapeutic potential.

Keywords: NEAT1, neuroblastoma, antisense oligonucleotide, differentiation, paraspeckles
Characterization of the Pathogenetic Role of POLD1 Gene in Mandibular Hypoplasia, Deafness, Progeroid Features and Lipodystrophy (MDPL) Syndrome

Paola Spitalieri1, Giuseppe Novelli1, Michela Murdocca1, Claudia De Masi1, Rosaria D’Apice1, Monica D’Adamo2, Ion Udruiu3, Jessica Marinaccio3, Antonella Sgura3, Paolo Sbraccia2, Federica Sangiulio1.

1 Dept of Biomedicine and Prevention, Tor Vergata University of Rome, Italy.  
2 Dept of Systems Medicine, Tor Vergata University of Rome, Italy.  
3 Dept of Science, Roma Tre University, Rome, Italy.

Mandibular hypoplasia, Deafness, Progeroid features and Lipodystrophy define a multisystem disorder named MDPL Syndrome (OMIM #615381). This disease has been associated to mutations in POLD1 gene, which encodes the active site of DNA polymerase δ, involved in DNA replication and repair mechanisms.

After the identification of an in-frame deletion (p.Ser605del), segregating for the first time as an autosomal dominant mutation, we have derived dermal fibroblasts (HDFs) from patient’s skin biopsy in order to investigate the pathogenetic role of POLD1 gene in clinical manifestations of MDPL syndrome. Cells harboring the mutation revealed abnormalities of nuclear envelope morphology, presence of micronuclei, and significant accumulation of prelamin A, associated with altered cellular proliferation and senescence markers, strongly linked to genomic instability. Moreover, DNA damage-induced treatment in MDPL- HDFs revealed a poor capacity of DNA repair, the increase of micronuclei, an arrest in the phase G0/G1 transition, and a lower number of cells in the phase S. At the protein level, WB analyses exhibited a nuclear reduction of POLD1 in MDPL-HDFs compared to WT in basal condition and a statistically significant increase after induction of cisplatin/1-Gy X-ray treatment remaining drastically elevated compared to WT condition.

Analysis of telomere length revealed an increased rate of telomere shortening in vitro (accompanied by an accumulation of unrepaired telomeric DNA damage). This was paired with an ever-slow proliferation rate, which ultimately led to growth stasis. Thus, being these cells DNA-damage-checkpoint proficient, the shortened telomeres led to the onset of senescence. In fact also the ultrastructural analysis of MPDL-HDFs highlights the presence of a large number of autophagosomes, already reported by immunofluorescence and WB assays. Finally, we generated human induced pluripotent stem cells (hiPSCs) from patient’s fibroblasts showing peculiar presence of micronuclei, although they show all stemness markers and differentiation abilities.

Knowing the mechanistic basis for the association of DNA damage and DNA repair with aging would give insight into contravening age-related diseases and promoting a healthy life span.

Keywords: POLD1, mandibular hypoplasia, aging, DNA repair.
Non-Invasive Prenatal Testing (NIPT) in Maternal Blood Using Cell Free Fetal DNA (cffDNA): Experience on 12088 Pregnancies

Alessandra Tacconelli², Rosario Ruta², Marta Grispo², Andrea Pezzullo², Maria De Salvo², Lorenzo Cucina², Monika Faron², Alessandra Zagnoli³, Armando Castiello⁴, Giuseppe Mucci⁵, Giuseppe Novelli*¹
¹ Dept of Biomedicine and Prevention, Tor Vergata University of Rome, Italy.
² Bioscience Genomics, Rome, Italy.
³ Bioscience Institute SpA, San Marino.
⁴ Bioscience Institute SpA, Milano, Italy.
⁵ Bioscience Institute Group, San Marino.

In the last few years, non-invasive prenatal testing (NIPT) through the analysis of cffDNA has revolutionized prenatal screening to determine fetal risk for genetic disorders. The use of Next generation sequencing (NGS) technologies in NIPT leads to rapid and reliable results. The aim of this study is to report the clinical application of NIPT in detecting chromosome aneuploidies in 12088 pregnancies. The study set included both singleton and twins pregnancies. Maternal blood sample were analyzed through massive parallel sequencing (MPS) by the Ion Proton™ System (Life Technologies). The bioinformatic analysis was performed by using a standardized algorithm. Women enrolled in the study, after written informed consent, showed a mean gestational age of 12.24 ± 1.93 (range 10-32), a mean age of 35 years old (range 17-56) and a mean weight of 62.49 kg ± 11.65 (range 38-140). Fetal fraction was detected for each case, mean value of 8% (range 3.5-31.9%). We found 82 cases at high risk for chromosomal abnormalities among the samples (1.7%). Statistical analysis of results showed that there is a positive correlation between maternal age and high risk for chromosomal abnormalities, as expected. We also found a statistically significant correlation between fetal fraction and gestational age, while we did not find correlation between fetal fraction and maternal age. According to the literature, the fetal fraction was inversely correlated to maternal weight and it increased in high-risk cases of Trisomy 21.
Our study showed high sensitivity and specificity for common aneuploidies (sensitivity 99.17%, 98.24% and 100% respectively for T21, T18, T13 and specificity 99.95%, 99.95% and 99.96%). This confirms that the NIPT screening has higher detection rate respect other non-invasive screening tests. It is currently the most valid test to detect the risk for fetal genetic disorders.

Keywords: NIPT, Prenatal diagnosis, Chromosome aneuploidies, Cell free fetal DNA
Secretome Benefits Of Human Mesenchymal Stem Cells And Targets For Wound Healing

H.T. Ong1,2, S.L. Redmond1,2, D.B. Vargas-Landin3, A. Forrest4, R.J. Dilley*1,2,5
1 Ear Science Institute Australia, 2 Ear Sciences Centre, University of Western Australia (UWA), 3 School of Molecular Sciences, UWA, 4 Harry Perkins Institute of Medical Research, 5 Centre for Cell Therapy and Regenerative Medicine, UWA

Our previous studies showed human adipose-derived mesenchymal stem cells (ADSCs) produce an activity that stimulated wound healing in eardrum keratinocytes (hTMk). By regulating ADSCs in hypoxia (<0.1% O2), this wound healing stimulus was increased. To understand the molecular mechanism behind this paracrine activity of ADSCs on wound healing, we used a bioinformatics approach to determine ADSCs and hTMk transcriptome for secreted ligands and receptors. Primary ADSCs were cultured in ambient oxygen conditions (21%) or hypoxia for 48h without serum. hTMk were established under normoxic conditions. Conditioned media (CM) were collected from ADSCs to assess paracrine activity on hTMk proliferation and migration, and to quantify specific protein secretion using ELISA. Transcriptomic analysis of ADSCs and hTMk were assessed using RNAseq and bioinformatics. Transcripts differentially expressed (adj-P<0.05) between each primary hypoxic and normoxic ADSCs were filtered through databases to identify secreted ligands, and similarly for hTMk transcriptome to identify receptors. Both subsets were then matched to a FANTOM5 curated ligand-receptor pair database to establish the final list of ligand-receptor repertoires. Results show VEGFA consistently upregulated 3-fold by hypoxia in ADSC, with corresponding receptors expressed at mRNA level on the hTMk. This could potentially represent a previously unknown function in wound healing.

Keywords: Hypoxia, Mesenchymal Stem Cells, Wound Healing, Transcriptomics, and Keratinocytes

Acknowledgements: HuanTing Ong was supported by Ear Science Institutes Australia through the University of Western Australia Sarich and Stokes Research Scholarship.
Evolution and Extinction: A Genetic Drift Based on Allele Inference.

S. Oluwafemi Oyamakin
Department of Statistics, University of Ibadan, Nigeria

In order to present a series of stochastic models from population dynamics capable of describing rudimentary aspects of genetic evolution, we studied two-allele Wright–Fisher and the Moran models for evolution of the relative frequencies of two alleles at a diploid locus under random genetic drift in a population of fixed size “simplest form, selection, and random mutation”. In general, alleles drift to fixation was significantly faster in smaller populations while Probability of fixation of an allele say A was approximately equivalent to the initial frequency of that allele. With the inclusion of selection in our model, probability of fixation of a favoured allele due to natural selection increased with increase in fitness advantage and population size. The time taken to reach fixation was much slower, in case of no selective advantage, than a fixation under mutation with selective advantage.

Keywords: Allele, Genetics, Genetic Drift, Moran model, Wright-Fisher model
Exosomal and Plasma MiRNA Ratio As a Putative Prognostic Marker for Melanoma

Ms Alisa Petkevich, Dr Aleksandr Abramov, Dr Vadim Pospelov, Dr Mikhail Kiselevskiy
1N. N. Blokhin Russian Cancer Research Center, Moscow, Russian Federation, 2RUDN (Peoples’ Friendship University of Russia), Moscow, Russian Federation

Introduction
Exosomes may reflect the genetic events happening in tumor site what is especially advantageous in case of inability to make biopsy and for monitoring of disease progression and treatment effectiveness. Changes in plasma and exosomal miRNA ratio may indicate different changes in disease course such as disease progression.

Materials and methods
20 plasma samples of melanoma patients of 3-4 grades and 20 plasma samples of melanoma patients of 2 grade. Expression levels of plasma and exosomal miRNA were determined by real-time PCR. Exosomes were isolated with ultracentrifugation method. Additionally, there was performed mRNA sequencing on a MinIon sequencer of 50 genes. Detected mutations were confirmed by real-time PCR with specific highly sensitive LNA probes.

Results
Among three opportunities: plasma miRNA, exosomal miRNA and plasma exosomal miRNA ratio the most significant correlation with the disease stage was for plasma exosomal miRNA ratio. There was a slight correlation between the severity of the disease and the expression levels of exosomal mRNA. Generally, 78 mutant sequences were found in the ratio of 0.05% to 45% (mutant fragments compared to wild-type sequences). Detected with MinIon mutations were confirmed with real-time PCR in 8 cases, the differences in the percentage of sequencing results were 30%.

Conclusion
Identified transcriptome profiles may have the prognostic value for melanoma and allow to identify mutation and epigenetic changes in tumor site.

Keywords: melanoma, prognostic, exosomal, miRNA, mRNA

Acknowledgements:
Polymorphic SVAs Are Associated With Parkinson’s Disease Progression Markers and Modulate Gene Expression In The PPMI Cohort

Abigail L. Pfaff\textsuperscript{1,2*}, Vivien J. Bubb\textsuperscript{3}, John P. Quinn\textsuperscript{3} and Sulev Koks\textsuperscript{1,2}

\textsuperscript{1}Perron Institute for Neurological and Translational Science, Perth, Western Australia, \textsuperscript{2}Murdoch University, Perth, Western Australia, \textsuperscript{3}University of Liverpool, Liverpool, UK

Body: Parkinson’s disease (PD) is a complex disorder caused by a combination of genetic and environmental factors. Recent progress utilising modern genetic technologies has unveiled the increasing contribution of genetics in the pathogenesis of PD, however, there is still a significant proportion of the genetic component to be identified. The majority of genetic studies focus on single nucleotide variants and we propose that part of this ‘missing heritability’ is due to other types of genetic variation, such as retrotransposons. SINE-VNTR-Alus (SVAs) are a hominid specific composite retrotransposon consisting of a CCCTCT repeat, an Alu-like domain, a GC-rich variable number tandem repeat (VNTR), a SINE region and a poly A-tail. There are nearly 3000 SVAs in the reference human genome and a small subset are polymorphic for their presence/absence. SVAs can affect gene expression, mRNA splicing and have been identified as the cause of 12 genetic diseases including X-linked dystonia parkinsonism. The aim of our study was to characterise polymorphic reference SVAs in whole genome sequencing data from the Parkinson’s Progression Markers Initiative (PPMI), a longitudinal PD cohort with extensive clinical and phenotypic data, to address their potential role in the predisposition to and progression of PD. We identified 83 polymorphic reference SVAs in 179 controls and 371 PD cases and using logistic regression did not identify SVAs associated with disease risk. However, analysis of longitudinal data identified 14 SVAs associated with clinical features and progression markers of PD, including a SVA in the intron of the CASP8 gene whose presence was significantly associated with a lower Movement Disorder Society – Unified Parkinson’s Disease Rating Scale total score (FDR corrected p=0.02) after 36 months follow up. RNA sequencing data from the same individuals showed the presence of selected SVAs significantly modulated gene expression at their genomic locus. This study has integrated whole genome sequencing, RNA-sequencing and clinical data to address the role of polymorphic SVAs in Parkinson’s Disease identifying novel functional genetic elements associated with progression markers.

Keywords: Parkinson’s Disease, retrotransposon, SVA

Acknowledgements: Data were obtained from the Parkinson’s Progression Markers Initiative (PPMI) database (www.ppmi-info.org/data). For up-to-date information on the study, visit www.ppmi-info.org.
Development of combinatorial epigenome editing technologies through a massively parallelized single cell RNA-seq screen

Dr. Christian Pflueger¹,², Tessa Swain¹,², Dawid Makosa¹,², Jessica Kretzmann¹,², Dr. Jahnvi Pflueger¹,², Dr. Giovanni Pietrogrande³,⁴, Prof. Ryan Lister¹,²

¹University of Western Australia, Crawley, Australia, ²Harry Perkins Institute of Medical Research, Nedlands, Australia, ³University of Queensland, Brisbane, Australia, ⁴Australian Institute for Bioengineering & Nanotechnology, Brisbane, Australia

Chemical tags on histones and DNA, known as epigenetic modifications, are vital in regulating transcription and cell identity. Recently, first-generation targeted epigenome editing systems based on nuclease-dead Cas9 (dCas9) have been developed to investigate defined changes in the epigenome and their impact on transcription. However, the induced epigenome changes are predominantly transient, and frequently ineffective, likely due to differing chromatin states. Consequently, we are implementing a high throughput single-cell resolution combinatorial epigenome modifier screen to discover the most potent combinations of transcriptional activators and repressors. We have implemented a new barcoding strategy to readout targeted epigenome modification machinery at the single-cell level. This enables massively parallel testing of thousands of epigenome modifier combinations in a single assay. Furthermore, we have developed a novel dCas9-based recruitment platform that will allow precise recruitment of multiple independent epigenome modifiers to desired genomic regions. This enables customized changes to the epigenome based on the chromatin context at the target locus in order to regulate transcription. This work will fundamentally advance our understanding of transcriptional regulation and will transform our ability to control the epigenetic regulatory layers of the genome, with extensive benefits for cell identity manipulation and disease modeling.
Characterizing the spatial heterogeneity of tumour tissues by identifying cancer and immune cell-types, cell-subtype distribution and transcriptional regulation

**Mr Duy Pham**, Mr Jun Xu, Dr Quan Nguyen

1Institute for Molecular Bioscience, University of Queensland, Brisbane, Australia

**Body:** Spatial Transcriptomic (ST) data enable an integrative analysis of genome-wide transcriptional profiles for thousands of cells with information on spatial locations and distances of these same cells in their native tissue context. Adding the new spatial dimension to the conventional gene expression data opens a vast potential for deciphering novel biology of cell types in morphological tissues. However, analytical tools for utilizing these new data types are lacking. We developed stLearn - a comprehensive software that offers a user-friendly and complete downstream analysis toolkit for ST data, with both conventional and novel analysis functionalities.

stLearn implements essential analysis types such as data preprocessing, customized visualization and differential expression analysis. Importantly, stLearn introduces novel functionalities, including microenvironment detection by factor analysis, spatial clustering, tissue image segmentation, cell-cell interaction analysis and pseudo-time-space (PTS). PTS can be used to spatially reconstruct local and global differentiation within and between cell subtypes in a tissue. At a regional tissue level, PTS is based on locally weighted smoothing, cluster centroids mapping and elastic principal graph technique. At the global tissue level, stLearn connects spatial cell subtypes by diffusion pseudo-time trajectory. stLearn provides intuitive visualisation options to describe cell progression and transcriptional regulation based on high-resolution spatial transcriptomics data. We provide the documentation and tutorials to use stLearn here: https://st-learn.github.io/

We applied stLearn for a range of real-life datasets, representing different organs such as brain and kidney, and different cancer tissues including breast and gastric cancer. With the additional spatial information, we identified cancer and immune cell subtypes that are spatially separated. We then delineated the in vivo development of cancer cell clones and detected cancer-immune cell interactions within tumour tissue. We make stLearn publically available and we believe that stLearn will be widely applied to study at the molecular level of the spatial heterogeneity of cell types. For cancer studies, stLearn is especially powerful to quantify spatial heterogeneity, which can suggest mechanisms for differential responses to treatment and cancer recurrence.

**Keywords:** spatial transcriptomics, spatial trajectory inference, spatial clustering, gastric cancer, breast cancer

**Acknowledgments:** We thank members in Nguyen's Biomedical Machine Learning Lab for helpful discussion. This work has been supported by the Australian Research Council (ARC DECRA DE190100116), the University of Queensland, and the Genome Innovation Hub.
Dissecting the Genotype-Phenotype Correlation of Transthyretin Amyloidosis: From Population Genetics to Transcriptomic and Epigenomic Regulation

Renato Polimanti1,*, Antonella De Lillo2, Gita A. Pathak1, Flavio De Angelis2, Frank R. Wendt1, Joel Gelernter1, Maria Fuciarelli2
1Department of Psychiatry, Yale University School of Medicine, West Haven CT, United States
2Department of Biology, University of Rome “Tor Vergata, Rome, Italy

Body: Transthyretin (TTR) amyloidosis is a rare life-threatening disease. Coding mutations in the TTR gene cause misfolding of the protein and the formation of amyloid fibrils that deposit in different tissues, resulting in a heterogeneous set of disease symptoms. While different symptoms may be present in patients with different TTR mutations, the same point mutation can be associated with different phenotype combinations even within the same family. To dissect the molecular basis of the genotype-phenotype correlation of TTR amyloidosis, we applied a wide range of different approaches. We investigated the origin of the two most common TTR mutations, Val30Met (rs28933979) and Val122Ile (rs76992529). Val30Met is the most common TTR mutation in Europe; we observed three independent mutational events with respect to Sweden, Portugal, and Italy, in agreement with the different clinical presentations of the disease in these three geographic regions. Val122Ile is the most common TTR mutation in African-descent carriers and we identified two independent Val122Ile haplotypes for West and East Africa populations. Unfortunately, limited data are available regarding TTR amyloidosis in Africa. However, we determined that genetic differences between African and non-African populations in binding sites of transcription factors lead to a substantial difference between African and non-African populations of genetically-determined TTR gene expression in heart tissues in line with the fact that cardiomyopathy is the most common symptom in Val122Ile carriers. Among Italian patients, we identified carriers with tissue-specific expression patterns associated with certain disease presentations, supporting the role of transcriptomic regulation in the genotype-phenotype correlation. In a case-control epigenome-wide analysis conducted in Italian individuals, we identified a significant methylation change in the BACE2 gene, encoding a protein responsible for the proteolytic processing of the amyloid precursor protein, a critical step in the etiology of Alzheimer’s disease. Although there is ongoing debate regarding the analogies between the amyloidogenic processes of the two diseases, no previous study reported BACE2 as a possible molecular link between TTR amyloidosis and Alzheimer’s disease. In the analysis conducted in the African-American Val122Ile carriers, we identified methylation changes associated with a history of heart disease in the GLS gene, encoding the mitochondrial glutaminase that, due to its role in glutamine metabolism, has been previously proposed as a potential therapeutic target for cardiovascular diseases. In summary, our findings confirm the molecular complexity of TTR amyloidosis, providing novel insights that could lead to novel treatments more effective across patients with a diverse ancestral background.

Keywords: Amyloid, Coding Mutations, Heterogeneity, Genomic Regulation, Modifier Genes

Acknowledgements: The investigators were supported by a Global ASPIRE TTR Amyloidosis Competitive Grant from Pfizer Inc. (Yale University School of Medicine), a research grant from the Amyloidosis Foundation (Yale University School of Medicine), and an Investigator-Initiated Research from Pfizer Inc (University of Rome “Tor Vergata”).
Antisense Oligomer-induced Exon Skipping to Restore Dysferlin Function

Bal Hari Poudel1,2,3, Dr Loren Flynn1,3, Prof Sue Fletcher1,3, Prof Rohit Pokhrel4, Prof Norman Palmer1,3 and Prof Steve Wilton1,3

1 Center for Molecular Medicine and Innovative Therapeutics, Murdoch University, WA 2 Central Department of Biotechnology, Tribhuvan University, Nepal 3 Perron Institute for Neurological and Translational Science, WA, 4 Institute of Medicine, Tribhuvan University, Teaching Hospital Nepal.

Dysferlin is a calcium-dependent membrane-associated protein involved in membrane repair, vesicle trafficking, and T-tubule function. Mutations in DYSF, encoding the dysferlin protein, cause primary dysferlinopathies; a group of autosomal recessive diseases that includes Limb-Girdle Muscular Dystrophy type 2B (LGMD 2B), Miyoshi Myopathy and Distal Myopathy. More than 300 pathogenic mutations in DYSF has been reported within exons or near an intron-exon boundary. AAV-mediated gene transfer is unlikely to succeed as the large size of the DYSF mRNA protein coding sequence exceeds the capacity of a single AAV vector. We hypothesize that antisense-mediated exon skipping strategies could be applied to particular dysferlin mutations and restore dysferlin function. Antisense oligomers (2’O-methyl) modified bases on a phosphorothioate backbone were designed to skip exons 2, 3, 4, 25, 30, 32, 34, 35, 36, 37, 51 and 52, and subsequently transfected into healthy human myoblasts. The 2’O-methyl PS chemistry showed significant levels of exon skipping for exons 25, 34, 35, 36, 37, 51 and 52. These results warranted further testing with other chemistries and analysis of the truncated dysferlin function using western blotting and immunohistochemistry. The antisense oligomers targeting exons 30 and 32 induced almost 100% skipping at a transfection concentration of 400 nM. The most effective sequence targeting exon 32 was synthesized as a phosphorodiamidate morpholino oligomer, evaluated for exon 32 skipping in a LGMD 2B patient (compound heterozygous; exon 32 nonsense mutation and exon 52 nonsense mutation) myogenic cells and shown 100% exon 32 skipping at the concentration of 100 μM by RT PCR analysis, and verified by sequencing. Protein analysis by western blotting and protein localization by immunoassay supported the functional integrity of the protein after exon 32 skipping. The Loss of DYSF exon 32 had been associated with a milder phenotype of LGMD 2B. We will explore and evaluate the redundancy of other exons through antisense mediated skipping, however to date exon skipping appears to be a viable strategy to overcome DYSF mutations affecting exon 32.
Association Of A Poly-T Structural Variant Within The SCAF4 Gene And Amyotrophic Lateral Sclerosis

Julia Pytte1,2, Loren Flynn2,3, Frances Theunissen1,2, Ryan Anderton2,4, Ian James3, Ann Saunders5, Richard Bedlack5, Teepu Siddique6, Michael Lutz5, Allen Roses5, Anthony Akkari2,3*
1University of Western Australia, Perth, Australia, 2Perron Institute for Neurological and Transnational Science, Perth, Australia, 3Murdoch University, Perth, Australia, 4University of Notre Dame, Fremantle, Australia, 5Duke University, NC, USA, 6Northwestern University Feinberg School of Medicine, Chicago, IL, USA.

There are currently over 200 reported fALS associated genetic variants in the SOD1 gene (1). The functional significance of these associated variants remains largely undetermined. Structural variants (SVs) are highly polymorphic markers that have been implicated in altered gene function and can potentially explain significant phenotypic variability observed in complex disorders, such as ALS (2). Consequently, we have examined SVs within and surrounding the SOD1 gene loci to assess their relevance to ALS, and to examine if such SVs are associated with disease and phenotypic variation in ALS cohorts. To do this, a SV evaluation algorithm was used to identify and score candidate variants according to variability, proximity to regulatory elements, trait association, signal for transcription factor binding sites, conservation and intron size (3). One SV (termed SV1) was selected for further analysis due to its high potential to impact gene regulation, close proximity to SOD1 and high conservation score. SV1 was systematically assessed through polymerase chain reaction, capillary separation and Sanger sequencing. In the present study SV1, a 15-18 Poly T repeat, was identified in SCAF4, a gene downstream of SOD1. In a North American cohort of familial (n=180) and sporadic (n=29) ALS patients and age-matched healthy controls (n=555), we subsequently showed that carrying an 18T SV1 allele is associated with ALS (P=0.001). Furthermore, carriage of an 18T allele was strongly associated with a shorter survival by an average of 33 months (P=0.001). The potential association between SV1 length, SOD1 variants and fALS was examined in this study. From a biomarker perspective, common SOD1 ALS associated variants are not incredibly informative for disease. In this study we present a highly informative biomarker marker for ALS risk and survival. This is the first report of such an SV in the SCAF4 gene and highlights the importance and implications of further investigation into SVs that may provide new targets for cohort stratification and therapeutic development.

References:

Keywords: Structural variants, ALS, SOD1

Acknowledgements: We would like to thank patients for taking part in the study. Funding for this study was provided through a Perron Institute internal grant.
Functional Genomics Approaches for Characterising Human Epilepsy Genes and Developing Novel Therapies

Ms Natasha Radcliffe1, Dr Zeeshan Shaukat1, Dr Ciao Xin Lim1, Mr Akzam Saidin1, Dr Michael Ricos1, Prof Leanne Dibbens1

1University of South Australia, Adelaide, Australia

Utilising our assembled Biobank of 10,000 familial and sporadic patients affected with epilepsy, we continue to identify new genes and genomic variants which cause epilepsy and its comorbidities, including autistic features, intellectual disability and psychiatric features. These genes include DEPDC5 involved in mTOR signaling, PCDH19 involved in cell adhesion and signaling, and the ion channel KCNT1.

We are interested in identifying what changes in biological pathways and mechanisms are caused by the genetic variants and how these contribute to the epilepsy phenotype. To address this, we have employed a functional genomics approach for biological investigation through model organism studies. We have utilised Drosophila as model organism in which to recreate our disease conditions with the vast toolbox of genetic manipulation resources that this model offers.

We are able to use our Drosophila models of epilepsy to test putative novel therapeutic agents. In order to improve the potential hit rate and therapeutic index of our novel treatments we are investigating novel compounds, off label use - FDA approved drugs and nutritional and metabolic substances.

Keywords: epilepsy, functional genomics, Drosophila, model organism, dietary interventions

Acknowledgements: NHMRC Project Grant, NHMRC Senior Research Fellowship.
Genetic diagnosis of complex ataxia pedigrees using short-read sequencing data

Dr Haloom Rafehi1,2, Dr Mathew Wallis3,5, A/Prof Katja Lohmann6, Dr Angela Rosenbohm7, Dr Albert Ludolph7, Dr John Christodoulou8,9,10, Dr Michael Hildebrand3,8, Dr Natasha Brown8,9,10, Prof Samuel Berkovic3,4, Dr John Christodoulou8,9,10, Prof Martin Delatycki4,8,9,10, A/Prof Paul Lockhart8,10, Prof Melanie Bahlo1,2

1The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia, 2Department of Medical Biology, University of Melbourne, Melbourne, Australia, 3Epilepsy Research Centre, University of Melbourne, Austin Health, Melbourne, Australia, 4Austin Health, Melbourne, Australia, 5Tasmanian Clinical Genetics Service, Tasmanian Health Service, Hobart, Australia, 6Institute of Neurogenetics, University of Lübeck, Germany, 7Department of Neurology, University of Ulm, Germany, 8Murdoch Children’s Research Institute, Parkville, Australia, 9Victorian Clinical Genetics Service, Parkville, Australia, 10Department of Paediatrics, The University of Melbourne, Parkville, Australia

Body: Aims: Spinocerebellar ataxias (SCAs) are a group of rare, progressive neurological disorders. SCAs are often caused by expansions of short tandem repeats (STRs), which are difficult to diagnose. Recent methodological advances have made detection through whole genome sequencing (WGS) more feasible. Methods: Two large, multigenerational pedigrees with undiagnosed ataxia had genome wide screening for repeat expansions (REs) in WGS data using established tools exSTRa and ExpansionHunter. Results: Analysis of WGS from three patients from the first pedigree provided a rapid diagnosis (<5 days) of SCA36, a rare SCA caused by an intronic GGCCTG RE in the gene NOP56. The second pedigree had linkage at chr1:56,000,000-62,000,000, which overlaps with the SCA37 gene DAB1. SCA37 is caused by a complex intronic RE, with an ATTTC motif inserted into an expanded ATTTT reference motif, making diagnosis with WGS difficult. Initial screening of the proband WGS data identified possible expansion of the ATTTT motif, but no evidence of the ATTTC insertion. Manual filtering of the bam file for reads containing the ATTTC motif provided evidence of enrichment of this motif, suggesting a likely SCA37 diagnosis (validation ongoing). Conclusions: RE screening in WGS is highly feasible for the diagnosis of simple REs such as SCA36. Complex REs, such as SCA37, still require additional information, such as linkage analysis.

Keywords: ataxia, diagnosis, repeat expansion, short tandem repeats, bioinformatics.

Acknowledgements: NA.
Antisense Oligonucleotides targeting Required for Meiotic Nuclear Division 1 Homolog (RMND1) towards Tackling Solid Cancers

Ms Prithi Raguraman¹², Dr Stacey Edwards³, Dr Rakesh Veedu¹²
¹Centre for Molecular Medicine and Innovative Therapeutics, Murdoch University, Murdoch, Australia, ²Perron Institute for Neurological and Translational Science, Nedlands, Perth, Australia, ³QIMR Berghofer Medical Research Institute, Brisbane, Australia

Modulation of RNA splicing using synthetic antisense oligonucleotide (AO) has been established as a viable therapeutic strategy for tackling diseases (1). RNA targeting therapy using AOs have become an effective treatment strategy with eight new drugs approved for the treatment of cytomegaloviral retinitis, familial hypercholesterolemia, Duchene muscular dystrophy, spinal muscular atrophy, hereditary transthyretin amyloidosis, familial chylomicronemia syndrome and Batten disease. In this study, we have explored the potential of exon skipping mechanism to inhibit the expression of a gene, required for meiotic nuclear division 1 homolog (RMND1). RMND1 is a mitochondrial protein encoded by the mitochondrial DNA. Variations in the expression level of RMND1 gene are associated with different pathologies, and listed as one of the pan-cancer molecular (methylation) signatures. RMND1 gene is also present in the breast cancer susceptibility locus 6q25.1 and has an increased expression in breast cancer. Our study suggests that RMND1 is not only highly expressed in breast cancer but also in liver cancer and glioblastoma cells. We hypothesised that removal of one or more exons will inhibit the expression of RMND1. In this direction, we have designed several AOs targeting various exons of the RMND1 gene transcript and evaluated their potency. Our results showed that the AOs efficiently skipped exon-3 and also induced cytotoxicity in the breast cancer and hepatocellular carcinoma cells in vitro. Further validations, including protein analysis by Western blot, are currently underway.

Keywords: RMND1, antisense oligonucleotides, cancer.

Acknowledgements: P.R thanks the MIPS funding scheme of Murdoch University. R.N.V acknowledges the funding provided by McCusker Charitable Foundation and the Perron Institute for Neurological and Translational Science. We greatly appreciate and thank Dr. Bao Tri Le and Madhuri Chakravarthy for their technical assistance.

2D in-vivo Structures and Interactions of Inc-RNAs

Dr Jordan A. Ramilowski¹, Mrs Hiromi Hiromi Nishiyori-Sueki¹, Dr Kayoko Yasuzawa¹, Dr Youtaro Shibayama¹, Mr Jenkin Tsui¹,², Ms Jessica Severin¹, Dr Jay W. Shin, Dr Piero Carninci¹, Dr Michiel de Hoon¹
¹RIKEN Center for Integrative Medical Sciences, Yokohama, Japan, ²University of Toronto, Toronto, Canada

Body: Structure of biomolecules is a key component in understanding their function. Yet structural annotations, especially of long non-coding RNAs (IncRNAs; RNA transcripts longer than 200nt and not coding for a protein), lag behind those of protein-coding genes. Here, we modify Psoralen Analysis of RNA Interactions and Structures (PARIS) protocol to interrogate secondary structures and intermolecular interactions of RNAs in-vivo and then combine it with Sequencing of Psoralen crosslinked, Ligated, and Selected Hybrids (SPLASH) protocol. As a part of the FANTOM 6 project, we aim to make a comprehensive atlas of secondary structures and interactions of RNAs in the cytoplasm and in the nucleus of multiple cell types, compare and contrast them and interrogate their relationship with genomic properties of the RNA transcripts (ex., gene class, sequence conservation, SNPs, splicing, stability).

Keywords: 2D in-vivo structures, interactions, IncRNAs, cell compartments
Acknowledgements: Research Grant from the Japanese Ministry of Education, Culture, Sports, Science, and Technology (MEXT) to the RIKEN Center for Integrative Medical Sciences.
SQSTM1/p62 Expression Induces TDP-43 Pathology and Neuronal Death

Ms Adriana Foster¹,²,³, Dr Carmel Cluning², Dr Sarah Rea¹,²,³,⁴
¹Murdoch University, Murdoch, Australia, ²Department of Endocrinology and Diabetes, Sir Charles Gardner Hospital, Nedlands, Australia, ³Harry Perkins Institute of Medical Research, University of Western Australia, Nedlands, Australia, ⁴Perron Institute for neurological and translational science, Nedlands, Australia

Body:  Background: Amyotrophic lateral sclerosis (ALS) is a progressive and ultimately fatal neurodegenerative disease. ALS exists on a disease spectrum with Frontotemporal lobar degeneration (FTD). A hallmark of pathology is the deposition of aggregated cytoplasmic TDP-43, which is found in 97% of ALS cases and approximately 60% of FTD cases. Cytoplasmic mislocalisation and cleavage of TDP-43 to smaller fragments (35kDa or 25kDa) is associated with pathology. Disease associated protein aggregates also contain the autophagy regulator SQSTM1/p62 and ubiquitin. The cause of cytoplasmic aggregation of TDP-43 in ALS is currently unknown.

Objectives: To determine the effects of SQSTM1/p62 expression on TDP-43 pathology in motor neuron-like cells.

Methods: NSC34 cells were transfected with TDP-43-tomato and EGFP or EGFP-SQSTM1/p62 (wild type or various SQSTM1/p62 domain deletion constructs). For confocal experiments cells were fixed and stained with Hoechst to enable Pearson’s coefficient co-localisation analysis with the nucleus, which was calculated with ImageJ software. For aggregation and cleavage experiments cells were fractionated into soluble (RIPA buffer) and insoluble (1% SDS) fractions. Viability of cells expressing GFP-p62 was determined using fluorescence assisted cell sorting following staining with Sytox Red. Statistics were performed using ANOVA and post-hoc testing with significance set to <0.05 in SPSS.

Results: We observed that SQSTM1/p62 expression leads to TDP-43 nuclear depletion and cytoplasmic mislocalisation and reduces the soluble TDP-43 in cells such that it is shifted into insoluble aggregates. Additionally, expression of SQSTM1/p62 leads to TDP-43 cleavage to a 35-kDa fragment. TDP-43 cleavage and insolubility were dependent on expression of the self-dimerization (PB1) and ubiquitin-associated (UBA) domains as well as the nuclear export sequence (NES) of SQSTM1/p62. Expression of SQSTM1/62 induced significant neuronal death both when expressed alone and in combination with TDP-43.

Discussion and conclusions: Our study shows that increased SQSTM1/p62 expression, which occurs in response to proteasomal stress, oxidative stress or heavy metal exposure, all of which have been associated with ALS, leads to hallmarks of ALS and FTD pathology. This includes TDP-43 mislocalisation, aggregation and cleavage to a 35-kDa fragment, which has previously been observed in diseased tissue, but was not found in control tissues. We also find that expression of SQSTM1/p62 causes neuronal cells to die. Importantly, we have identified the regions of SQSTM1/p62 required to induce TDP-43 pathology. Future studies will determine whether antisense oligonucleotides to the PB1 domain can prevent the TDP-43 and SQSTM1/p62 interaction and thereby present a potential therapeutic strategy in ALS and FTD.

Keywords: SQSTM1/p62, TDP-43, amyotrophic lateral sclerosis, frontotemporal lobar degeneration, neurons

Acknowledgements: This work was supported by a National Health and Medical Research Council-Australian Research Council Dementia Fellowship (APP1102977) and a Motor Neuron Disease Research Institute of Australia, Multiple Sclerosis WA funded Innovator Grant (IG 1949).
Development and Evaluation Of A Diagnostic Algorithm For Babies Born With Ambiguous Genitalia

Alejandra Reyes¹,², Nayla León¹, and Vincent Harley¹.
¹Centre for Endocrinology and Metabolism, Hudson Institute of Medical Research, Melbourne, Victoria, Australia. ²Genetics Department, Hospital Infantil de México Federico Gómez, Mexico City, Mexico.

Is it a boy or a girl? Differences of sex development (DSDs) are complex genetically and clinically. Patients can present at birth with ambiguous genitalia. Potentially, there are a hundred different causes of ambiguous genitalia, and a precise diagnosis is essential for management of endocrine, surgical, reproductive, and psychosocial issues. While more than 60 genes have been associated with DSDs, most cases do not receive a definitive genetic diagnosis.

There is a need to improve the diagnostic approach for clinicians and researchers so that the decision process integrates traditional clinical practices (such as physical examination, biochemical and hormonal analysis, imaging evaluation, biopsy/laparoscopy) with data from DSDs gene panels, as well as, emerging genome-wide technologies such as DNA structural mapping and sequencing. We have developed an algorithm¹ based on a comprehensive evaluation of previous approaches. The algorithm will help specialists involved in multidisciplinary teams navigate through the process of making a diagnosis when presented with a newborn with ambiguous genitalia. The algorithm can differentiate as many as 48 different molecular aetiologies of ambiguous genitalia. We advocate where affordable, that genome technologies be employed as part of front-line screening.

Current clinical guidelines for patients with ambiguous genitalia are based mainly on expert opinion rather than clinical research. Here, we aim to evaluate the effectiveness of the algorithm in different populations worldwide. Our expected outcome is to increase the genetic diagnostic yield of patients born with DSD. The primary outcome measure is the number of diagnoses made using the algorithm compared to the previous assessment. The secondary outcomes are the uptake by the physicians, cost, reproducibility of the analysis, and feasibility of the algorithm based on the time taken to obtain a definitive diagnosis and the inter-rater reliability.

Keywords: disorders/differences of sex development, ambiguous genitalia, algorithm

References:
Investigating New Genetic Causes of Familial Epilepsies in a Large Biobank

Mr Akzam Saidin¹, Mrs Natasha Radcliffe¹, Dr Zeeshan Shaukat¹, Dr Michael Ricos¹, Prof Leanne Dibbens¹
¹University of South Australia, Adelaide, Australia

We have assembled and curated a Biobank from 10,000 familial and sporadic patients affected with a range of different types of epilepsy. Over a twenty year period we have identified a number of different genetic causes of epilepsy. With the current efficacy and availability of next generation sequencing we are exome sequencing this collection to discover novel genes and genomic variants that cause epilepsy.

We have undertaken exome sequencing of affected and unaffected family members in our larger families with epilepsy in whom we have not previously identified a genetic cause. We have also used this opportunity to improve our genomic data analysis pipelines for variant calling and variant analysis. Here we report on findings from the sequence analysis of over 400 individuals from 42 different families with epilepsy.

Keywords: genomics, epilepsy, gene discovery, exome sequencing, neurogenetics
Acknowledgements: NHMRC Project grant and NHMRC Senior Research Fellowship
A 10 Years Study of DMD/BMD Patients And Carriers In A Heterogeneous Malaysian Population

Sasi D. Saminathan1, Ahmad Akmal Azman1, David J. Bunyan3, Khoo Teik Beng2, Roziana Ariffin1
Molecular Genetics Laboratory, Women and Children Hospital Kuala Lumpur1, Institute of Paediatrics, Women and Children Hospital2, Wessex Regional Genetics Laboratory3

Body:
Duchene muscular dystrophy (DMD) and the milder Becker muscular dystrophy (BMD) are the two most common form of neuromuscular disease in children caused by a mutation in the DMD gene located on Xp21.2. DMD/BMD has a X-linked recessive inheritance pattern and causes irreversible weakness of muscles affecting both skeletal and cardiac muscles. This comprehensive study involved DMD/BMD patients and their close female relatives (carrier status) who were referred to the National Molecular Genetics Referral Laboratory in Malaysia in the past 10 years. The heterogeneous Malaysian population is a goldmine for studying population specific mutations that could be added to the DMD/BMD database and used as a reference in understanding the types of DMD/BMD causative mutation in an Asian population. This is the first time that information on carrier status of DMD is presented from a South East Asian population. We combined multiplex ligation-dependent probe amplification (MLPA) with Sanger sequencing to detect mutations in the Dystrophin gene. A total of 571 patients and carriers were studied and 236 (41.3%) of them carried a mutation for DMD or BMD either large deletions or duplications, single exon deletions or duplication and point mutations that mostly fell within the two hotspots of DMD/BMD mutation, 5’proximal region and mid-distal region. There were however some single exon deletions that were more commonly appearing in the Malaysian population. The highest number of mutations for single exon deletions were found for exon 45 (16 cases), exon 44 (14 cases), exon 51 (10 cases) while the highest single exon duplications were found in exon 3 (3 cases), exon 18 (4 cases) and exon 17 (2 cases). The results from this study that involves a big cohort of patients and carriers not only provides DMD/BMD mutation information for the three main ethnic groups within the Malaysian population which is very useful from a diagnostic perspective, but will also serve as a basis to a Genome Wide Association study for DMD/BMD in the Malaysian population.

Acknowledgements: All the Molecular Genetics Laboratory staff and referring Paediatric Neurologist, WCHKL
Keywords: Duchene Muscular Dystrophy, Becker Muscular Dystrophy, DMD, BMD, DMD/BMD Malaysia, MLPA, Sanger Sequencing
Exploring the Genetic and Phenotypic Heterogeneity of RFC1 Expansions in an Australasian Neurological Disease Cohort

Miss Carolin Scriba1,2, Dr Sarah Beecroft1,2, Dr Joshua Clayton1,2, Roisin Sullivan3, Dr Andrea Cortese3, Dr Henry Houlden3, Mary Reilly3, Dr Mark Davis4, Dr David Chandler5, Dr Richard Roxburgh6,7, Dr Phillipa Lamont8, Professor Nigel Laing1,2, Dr Gianina Ravenscroft1,2

1Harry Perkins Institute of Medical Research, QEII Medical Centre, Nedlands, Australia, 2Neurogenetic Diseases Group, Centre for Medical Research, QEII Medical Centre, University of Western Australia, Nedlands, Australia, 3Department of Neuromuscular Disease, UCL Queen Square Institute of Neurology and The National Hospital for Neurology and Neurosurgery, London, UK, 4Neurogenetics Laboratory, Department of Diagnostic Genomics, PP Block, QEII Medical Centre, Nedlands, Australia, 5Australian Genome Research Facility, Harry Perkins, Institute of Medical Research, QEII Medical Centre, Nedlands, Australia, 6Neurology Department, Auckland City Hospital, Private Bag 92024, Auckland, New Zealand, 7Centre for Brain Research Neurogenetics Research Clinic, University of Auckland, Auckland, New Zealand, 8Neurogenetic Unit, Royal Perth Hospital, Perth, Australia

**Body:** Cerebellar ataxia, neuropathy and vestibular areflexia syndrome (CANVAS) is a progressive late-onset, neurological disease. Prior to the discovery of a genetic basis, the clinical heterogeneity of CANVAS made definitive diagnosis challenging. Recently, a pentanucleotide expansion in intron 2 of the RFC1 gene was identified as the genetic cause of CANVAS. This pathogenic expansion, (AAGGG)exp differs from the reference (AAAAG)11 in both size and configuration. Biallelic pathogenic expansions underlie a substantial percentage (22%) of late-onset ataxias in the first published European cohort. We therefore suspected that the RFC1 expansion may underlie a number of unsolved Australasian neurogenetic disease cases. By screening three clinical cohorts, we showed that in the Australasian population the pathogenic RFC1 expansion underlies 17% of suspected CANVAS cases (n = 6), 10% of unsolved ataxia cases (n = 50), and 12% of unsolved neuropathies (n = 25). We also identified a novel likely pathogenic allele at the RFC1 locus in a subset of patients. This novel allele shares the same core haplotype as the previously reported pathogenic (AAGGG)exp allele, suggesting a single origin of this disease. These results highlight the genetic and phenotypic diversity of biallelic repeat expansions in RFC1, and provide important insight into the genetic cause of CANVAS. Diagnostic implementation of RFC1 expansion detection will provide accurate genetic diagnosis for many patients and families with late-onset neuropathies and ataxias.

**Key words:** CANVAS, repeat expansion, RFC1, ataxia, sensory neuropathy
Epigenetic Modifications Underlying The Neuroprotective Properties Induced By A Single Ultra-Low Dose Of THC

Mr. Guy Shapira¹, Prof. Yosef Sarne¹, Prof. Noam Shomron¹
¹Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv-yafa, Israel

The Endocannabinoid system (ECS) is an endogenous neuromodulatory system involved in the pathogenesis of neurodegenerative disease. Age-related dysfunction of the ECS is currently suggested to be partially responsible for the late onset of many neurodegenerative disorders, with some of its vital functions, such as attenuation of neuroinflammation and influence over neuronal cell survival, diminishing with age. Cannabinoid drugs became increasingly studied as a potential treatment for neurological disorders, through their complex interaction with the ECS.

Our research found that a single, ultra-low dose (3-4 orders of magnitude lower than the standard dose, pharmacologically distinct and non-psychoactive) of the plant derived cannabinoid, Delta-9-Tetrahydrocannabinol (THC) restored cognitive function in old-aged mice and resulted in long lasting morphological and biochemical alterations of the brain. In an effort to elucidate the mechanisms underlying these effects, we explored significant alterations in hippocampal gene expression, lasting over a month after the THC treatment. Many of the differentially expressed genes were heavily implicated in neuroprotection and some are prominent therapeutic targets for neurodegeneration. We currently focus our research on genes that are potentially driving the observed beneficial effect of our treatment, such as neurodegeneration and cognitive improvements, and explore their potential exploitation as a part of a novel, disease-modifying treatment for neurodegenerative disorders.

Our hypothesis is that acute, ultra-low dose THC treatment induce a long-term neuroprotective effect, with substantial therapeutic potential for a large variety of neurodegenerative disorders, in addition to it's cognitive benefits for aging.

Keywords: neurodegenerative disease, Endocannabinoid system, neuroprotection, Gene expression, neurogenesis
Analysis of Differentially Expressed Genes from Whole-transcriptome Sequencing of Patients With Esophageal Squamous Cell Carcinoma From Kazakhstan

A. Sharip¹, S. Rakhimova¹, A. Molkenov¹, U. Kozhamkulov¹, Y. Zhukov², M. Omarov², A. Akilzhanova¹, U. Kairov*¹

Introduction: Esophageal cancer is the eighth most common cancer worldwide and sixth in Kazakhstan. Esophageal squamous cell carcinoma (ESCC) is the predominant histological subtype of esophageal cancer and diagnosed at late stage. The aim of the project was to identify genetic basis of ESCC by analysing differentially expressed genes (DEGs) from whole-transcriptome sequencing of Kazakhstani patients.

Materials and Methods: Tissue samples were obtained from 25 ESCC-affected individuals immediately after Ivor-Lewis esophagectomy from Oncology Center in Nur-Sultan. Whole transcriptome sequencing was performed following the TruSeq RNA Protocol. STAR software and DESeq2 package have been used for mapping and defining differentially expressed genes. Functional analysis of DEGs was performed using various R packages.

Results: The study sized 14 men and 11 women, average age of patient 65.5±7.7 years. 88% of the patients were diagnosed with advanced stages T3-T4. Analysis of tumor and normal esophageal tissues identified 1197 DEGs, comprising 883 upregulated and 314 downregulated genes (with adjusted p-value <0.05). We found significant 4 up-regulated and 6 down-regulated KEGG pathways (p-value<0.05). Among up-regulated pathways oxidative phosphorylation (p-value=0.002), hematopoietic cell lineage (p-value=0.005), cardiac muscle contraction (p-value=0.01) and complement and coagulation cascades (p-value=0.03) have been identified. Whereas the most significant down-regulated pathways are peroxisome (p-value = 0.001), ubiquitin mediated proteolysis (p-value=0.001) and phosphatidylinositol signalling system (p-value=0.007). Top 300 DEGs were mapped to PPI network and three modules consisting from closely connected nodes (genes) were identified. Functional enrichment analysis of these modules showed that “module_1” is significantly associated with histone functions, “module_2” is linked with oxidative phosphorylation and electron transport chain, “module_3” is principally associated with degradation of extracellular matrix (ECM).

Conclusion: ESCC with moderate dysplasia is the most common histologic subtype of esophageal cancer in our patients and is characterized by a poor prognosis. High-throughput sequencing approach allows identifying molecular pathways involved in esophageal carcinogenesis that could improve diagnosis and treatment strategies.

Keywords: Esophageal squamous cell carcinoma, Kazakh population, transcriptomics

Acknowledgements: The work was supported by grants of the Ministry of education and science #AP05134722, #AP05135430 and #AP05136106.
The rise of rapid implementation science: A worked example of solving an existing problem with a new method by combining concept analysis with a systematic integrative review

James Smith, 1 Frances Rapport, 1 Tracey A. O’Brien, 2,3 Stephanie Smith, 4,5 Vanessa J. Tyrrell, 6 Emily V.A Mould, 6 Janet C. Long, 1 Hossai Gul, 1 Jeremy Cullis 7 and Jeffrey Braithwaite 1

1 Centre for Healthcare Resilience and Implementation Science, Australian Institute for Health Innovation, Macquarie University, Australia
2 School of Women’s and Children’s Health, Faculty of Medicine, University of New South Wales, Australia
3 Kids Cancer Centre, Sydney Children’s Hospital, Randwick, Sydney
4 School of Nursing and Midwifery, Edith Cowan University, Australia
5 Nuffield Department of Orthopaedics, Rheumatology & Musculoskeletal Sciences, Botnar Research Centre, University of Oxford, UK
6 Children’s Cancer Institute, Lowy Cancer Research Centre, University of New South Wales, Australia
7 Macquarie University Library, Macquarie University, Australia

Body: Abstract

Background: The concept of rapid implementation has emerged in the literature recently, but without a precise definition. Further exploration is required to distinguish the concept’s unique meanings and significance in the field of implementation science. The study clarifies the concept of rapid implementation and identifies its attributes, antecedents, and consequences. We present a theoretical definition of rapid implementation to clarify its unique meaning and characteristics as well as present a model case on precision medicine and next generation sequencing.

Methods: Rodgers evolutionary concept analysis method, combined with a systematic integrative review, were used to clarify the concept of rapid implementation. A comprehensive search of four databases, including EMBASE, MEDLINE, SCOPUS, and WEB OF SCIENCE was conducted, as well as relevant journals and reference lists of retrieved studies. After searching databases, 2,442 papers were identified from 1963 to 2019; 24 articles were found to fit inclusion criteria to capture data on rapid implementation from across healthcare settings in four countries. Data analysis was carried out using descriptive thematic analysis.

Results: The results present the introduction of rapid implementation as part of implementation science. Rapidly evolving areas being viewed across the biomedical enterprise are addressed, such as molecular immunohaematology (molecular oncology), molecular profiling (oncology), molecular tumour boards (precision oncology), and genotyping (biostatistics) that require rapid deployment of actionable data. Guidance for further conceptualisation to bridge the gap between research and practice and redefine rigour, adapting methods used (current approaches, procedures and frameworks), and challenging clinical trial design (efficacy-effectiveness-implementation pipeline) is provided.

Conclusions: It is possible that we are on the cusp of a paradigm shift within implementation science brought about by the need for faster results into practice and policy. Researchers can benefit from a deeper understanding of the rapid implementation concept to guide future implementation of rapid actionable results in clinical practice.

Keywords: Concept analysis; precision medicine; healthcare; rapid implementation; systematic integrative review

Acknowledgements: N/A
Signals of Positive Selection in Human Populations of Northern Russia

Vadim A. Stepanov*, Vladimir Kharkov, Kseniya Vagaitseva, Irina Khitrinskaya
Institute for Medical Genetics, Tomsk National Medical Research Center, Tomsk, Russian Federation,

**Body:** Human adaptation to extreme climatic and geographic conditions mediated by natural selection was of the major factors for formation of genetic structure in North Eurasian populations. Using data on genome-wide set of single nucleotide polymorphisms (SNPs) we have searched for the signals of positive selection in 5 populations of Siberia and Russian European North. From 113 to 185 genomic regions with extended homozygous haplotypes blocks, containing altogether 771 genes, were found in each of the populations. Cross-population search of the selection targets resulted in about 150 genomic regions, 57 of which overlap with the results of haplotype analysis in individual populations. Genomic loci with the most profound signals of positive selection in northern populations include regions of SLC30A9, CACNA1C, KCNQ5, ABCA1, ALDH1A2, CSMD1, RBFOX1, WWOX as well as some other genes. Bioinformatic analysis has demonstrated that major biological processes where selection targets are implicated, are such processes as response to external stimuli, including proteins, nutrients and glucose; and defense reactions, including inflammatory immune response. The network of protein-protein interactions of genes under positive selection forms distinct clusters related to some of the biological processes indicated above. Results of the study indicate that non-neutral microevolution mechanisms may play a substantial role in the genetic structuring of the human populations during long-term adaptation to unfavorable environmental conditions.

**Keywords:** human evolution, population genomics, positive selection, North Eurasia

**Acknowledgements:** This work is supported by RFBR project #18-29-13045
Genetic heterogeneity of polymicrogyria: study of 124 patients using deep sequencing

Dr Chloe Stutterd1,2,3,4,5, Dr Stefanie Brock6,7, Dr Katrien Stouffs6,8, Dr Miriam Fanjul-Fernandez4, Prof Paul Lockhart1,2, Ms Kate Pope1, Dr George McGillivray4, A/Prof Simone Mandelstam1,2,3, A/Prof Anna Jansen6,9, A/Prof Richard Leventer1,2,3
1Murdoch Children’s Research Institute, Melbourne, Australia, 2University of Melbourne Department of Paediatrics, Melbourne, Australia, 3Royal Children's Hospital, Melbourne, Australia, 4Victorian Clinical Genetics Service, Melbourne, Australia, 5Austin Hospital, Heidelberg, Australia, 6Neurogenetics Research group, Vrije Universiteit, Brussel, Belgium, 7Department of Pathology, UZ Brussel, Brussel, Belgium, 8Centre for Medical Genetics, UZ Brussel, Brussel, Belgium, 9Pediatric Neurology Unit, UZ Brussel, Brussel, Belgium

Body: Polymicrogyria is a malformation of cortical development (MCD) characterized by overfolding and abnormal lamination of the cerebral cortex. Manifestations include epilepsy, speech disturbance and motor and cognitive disability. Causes include acquired prenatal insults and inherited and de novo genetic variants. The proportion of patients with polymicrogyria and a causative germline or mosaic variant is not known. The aim of this study was to identify the genetic causes of polymicrogyria in a heterogeneous cohort of patients reflective of a clinical service. Patients with polymicrogyria were recruited from two research centers in Australia and Belgium. Patients with evidence of congenital infection or causative chromosomal copy number variants were excluded. 124 patients were tested with a deep sequencing gene panel including known and candidate genes for malformations of cortical development. Causative and potentially causative variants were identified in known MCD-associated genes and correlated with phenotypic features. Pathogenic or likely pathogenic variants were identified in 25/124 (20%) patients. A variant of uncertain significance (VUS) with potential clinical significance was identified in one patient for whom parental segregation studies were not available and required to confirm mode of inheritance and 'likely pathogenic' classification. Incorporating this candidate variant, the total number of genetic causes identified was 26/124 giving a diagnostic yield of 21%. Of the 22 dominant variants identified, seven appeared to be mosaic (AF <0.45) and the lowest allele fraction was 9%. The most commonly implicated genes were TUBA1A and PIK3R2. The other eleven genes implicated were PIK3CA, NEDD4L, COL4A1, COL4A2, GPSM2, GRIN2B, WDR62, TUBB3, TUBB2B, ACTG1 and FH. A genetic cause was more likely to be identified in the presence of an abnormal head size or additional brain malformations suggestive of a tubulinopathy, such as dysmorphic basal ganglia. Clinical assessment for head size and additional brain malformations can therefore assist in identifying patients with a likely genetic cause. A gene panel test provides greater sequencing depth and sensitivity for mosaic variants but is limited to the genes included, potentially missing variants in newly-discovered genes. The diagnostic yield of 21% indicates that polymicrogyria may be associated with genes not yet known to be associated with brain malformation, non-genetic factors or brain specific somatic mutations.

Keywords: Brain, Cerebral cortex, Genetic testing, High-Throughput Nucleotide Sequencing, Mosaicism

Acknowledgements: We thank the patients, their families and their referring clinicians for their participation in this study. CA Stutterd was supported by the Flora Suttie Neurogenetics Fellowship funded by the Suttie family and their supporters, the Thyne Reid Foundation and the Macquarie Foundation. The research at the MCRI was supported by the Campbell Edwards Trust, the Genet Foundation and the MCRI Translation Research Fund. A Jansen was supported by an Senior Clinical Investigator Fellowship from the Research Foundation - Flanders (FWO).
Genome-wide Variably Methylated Tumour DNA Regions and Association with Overall Survival in Invasive Lobular Breast Cancer

Ms Medha Suman1, Dr. Pierre-Antoine Dugué2,3,4, Dr. JiHoon Eric Joo1, Dr. Ee Ming Wong1,2, Prof. Catriona McLean5, Dr. Tu Nguyen-Dumont1,2, Prof. John Hopper4, Prof. Graham Giles3,4,6, Prof. Roger Milne2,3,4, Prof. Melissa Southey1,2,3

1Department of Clinical Pathology, The University of Melbourne, Melbourne, Australia, 2Precision Medicine, School of Clinical Sciences at Monash Health, Monash University, Melbourne, Australia, 3Cancer Epidemiology Division, Cancer Council Victoria, Melbourne, Australia, 4Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Melbourne, Australia, 5Department of Anatomical Pathology, Alfred Hospital, Melbourne, Melbourne, Australia, 6School of Public Health and Preventive Medicine, Monash University, Melbourne, Australia

Background: Invasive lobular breast cancer (ILBC) is the second most common histological subtype of breast cancer, accounting for 10-15% of all cases. Tumour DNA methylation profiling has shown potential to refine current breast cancer subtyping and several studies have shown the utility of tumour DNA methylation as a diagnostic and prognostic biomarker. However, there is very limited data on DNA methylation alterations specific to ILBC.

Aims: (1) To identify the most variably methylated regions (VMRs) across ILBC tumour genome. (2) To test the possible association between tumour methylation levels at the most significant VMRs and overall survival in women with ILBC.

Methods: Cases of ILBC (n=130), were identified in participants in the Melbourne Collaborative Cohort Study (MCCS) and tumour-enriched DNA was prepared by macrodissecting formalin-fixed paraffin embedded (FFPE) tumour tissue. Genome-wide DNA methylation was measured using the HumanMethylation 450K (HM450K) BeadChip array. VMRs (contiguous CpGs less than 1000 bp apart) were identified across the genome using the DMRCate package in R. Cox proportional hazards regression models were used to assess the association between average methylation at the 10 most significant VMRs and overall survival. Replication of the VMR and survival analysis findings was examined using data for ILBC cases (n = 168) retrieved from The Cancer Genome Atlas (TCGA).

Results: We identified 2,771 VMRs (P < 10^-9) within ILBC. The most variable cluster was located in the promoter region of APC (16 CpGs). Other notable VMRs were in ISM1 (29 CpGs), TMEM101 (16 CpGs), ASCL2 (41 CpGs) and NKX6 (39 CpGs). Many regions including APC, ISM1, HIST3H2A/HIST3H2BB and HCG4/P3/HLA-J appeared strongly variably methylated in both the MCCS and TCGA data. Associations between tumour methylation and reduced overall survival were observed for the VMR in APC (hazard ratio (HR) per 10% methylation increase =1.23, 95% CI:1.06-1.42) and in four other genes: TMEM101 (HR=1.20, 95% CI:1.01-1.43), HIST3H2A/HIST3H2BB (HR= 1.23, 95% CI:1.04-1.62), NKX6 (HR= 1.24, 95% CI:1.01-1.54) and CELF2 (HR= 1.26, 95% CI: 1.08 - 1.48). The VMR in CELF2 was also associated with reduced overall survival in TCGA data (HR= 1.42, 95% CI: 1.07 - 1.89).

Conclusion: Substantial variability of methylation was identified across the ILBC genome. The promoter region of APC was identified as the most variably methylated cluster. Higher methylation at APC, TMEM101, HIST3H2A/HIST3H2BB, NKX6 and CELF2 showed an association with reduced overall survival in the discovery set. Associations observed for APC, TMEM101 and CELF2 were replicated using TCGA data.

Keywords: Genome-wide tumour DNA methylation, variably methylated regions, survival biomarker, epigenetics, breast cancer

Acknowledgements: Melbourne Collaborative Cohort Study (MCCS) cohort recruitment was funded by VicHealth and Cancer Council Victoria. The MCCS was further augmented by Australian National Health and Medical Research Council grants 209057, 396414 and 1074383 and by infrastructure provided by Cancer Council Victoria. Cases and their vital status were ascertained through the Victorian Cancer Registry and the Australian Institute of Health and Welfare, including the National Death Index and the Australian Cancer Database.
Integrate Single Cell RNA Sequencing Data to Reconstruct Heart Development at Single Cell Resolution

**Miss Yuliangzi Sun¹, Dr Quan Nguyen¹, Dr Nathan Palpant¹**

**Body:** Cardiovascular diseases are the leading cause of death globally. Research on cardiac development is the key to uncover approaches that can help heart rebuild itself since heart cannot repair itself in cardiovascular diseases. Single-cell RNA sequencing (scRNA-seq) as an emerging technology can assess cells directly, and it offers a whole-transcriptome expression profile of individual cells. In addition, scRNA-seq could uncover valuable discoveries relative to rare cell populations, cell type identification, and gene network across cells. However, current published *in vivo* cardiac scRNA-seq data only covered partial time points due to sample preparation and cost. With implemented advanced bioinformatic approaches, which enables integration of different scRNA-seq data sets, we are able to build a roadmap on cardiomyocyte maturation which provide new insights for heart development from embryonic blastula stage to the postnatal heart. In this project, we applied different integration methodology, such as canonical correlation analysis (CCA), linear regression and Euclidean distance. Integration results were visualized by Uniform Manifold Approximation Projection (UMAP) dimensionality reduction. Furthermore, integration performance was validated by gene ontology enrichment analysis. We demonstrated meaningful integration extend our understanding of heart development in a complete time scale. This project reveals a potential direction on integration of *in vivo* mouse heart data and *in vitro* human induced stem cell-derived cardiac cells at single-cell resolution to computationally map stem cell differentiation to *in vivo* heart development.

**Keywords:** Single cell genomics
The Clinical Utility Of Chromosomal Microarray Studies In the Evaluation Of Patients with Intellectual Disability And Global Developmental Delay: A Malaysian Tertiary Centre Experience

Tae S.K1, Mazlan R.A2, Thong M.K1
1Genetic and Metabolism Unit, Department of Paediatrics, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia
2Medical Genetics Unit, University Malaya Medical Center, Kuala Lumpur, Malaysia

Introduction: Intellectual disability (ID)/global developmental delay (GDD) is a diverse group of disorder that may occur with or without associated co-morbidities. Chromosomal microarray (CMA) is widely used as a first-tier clinical diagnostic test in evaluation of unexplained ID and GDD in developed countries. Our study aim is to assess the usefulness of CMA in a developing country and study the phenotype-genotype correlation.

Methods: A retrospective collection and review of data, for all patients with ID/GDD attending the genetic clinic in University Malaya Medical Centre (UMMC), who were investigated with CMA from the year 2015-2019. These patients were further grouped under their main clinical co-morbidities.

Result: A cohort of 80 patients with ID/GDD was reviewed and all of them had a prior conventional karyotyping done. A total of 26 patients (32%) were identified to have pathogenic copy number variants (CNV). Majority of these pathogenic CNVs were copy number losses. The most common associated phenotype was dysmorphism 43 (53%), follow by failure to thrive 40 (50%) and short stature 32 (40%). Other associated co-morbidities included microcephaly, skeletal abnormalities and congenital heart disease. Out of the 26 patients, 9 (11.2%) had an abnormal karyotype.

Discussion: Establishing an aetiology diagnosis in ID/GDD patient is crucial for genetic counselling and management. CMA is a useful diagnostic tool in the evaluation of ID/GDD patient. Our centre showed a higher diagnostic yield compared to other centres worldwide most probably due to the patient selection. Certain phenotype or co-morbidities are more commonly associated with pathogenic CNVs. Thus, the presence of co-morbid conditions is one of the factors to consider when considering CMA in resource-limited country.

Conclusion: CMA is an important diagnostic tool when used in combination with careful assessment of the patient’s phenotype as this will increase the diagnostic yield in evaluation of ID/GDD patients.

Keywords: Chromosomal microarray, Global developmental delay, Intellectual disability, developing country

Acknowledgements: We thank all the families of patients who participated in this review. We thank the staff and University of Malaya Medical Centre for their assistance in this study.
Development of a population-scale genetic variant data management and analytical infrastructure

Tan Joanna HJ1, Maxime Hebrard1, Ow Jack Ling1, Ang Shimin1, Chen Jieqi Pauline1, Justin Jeyakani1, Ng Boon Hsi Sarah1, Shih Chih Chuan1, SG10K Health Consortium, Liu Jian Jun1, Tan Boon Ooi Patrick1, Shyam Prabhakar1, and Nicolas Bertin1,*
1Genome Institute of Singapore, A*STAR, Singapore

Body:
To date, the vast majority of studies linking genetic variations to diseases predisposition, aetiology, and eventually informing therapeutic options are heavily skewed toward populations of Caucasian ancestry. The Asian genomes in current public databases are underrepresented, which makes meaningful disease comparisons severely compromised. Being composed of Chinese, Indian, and Malay descendants, the population of Singapore offers, an unique opportunity to capture a very large fraction of South Asian genetic diversity to develop a clinical-genetic dataset of Asian normality.

The SG10K Health project is a multi-institutions project aiming to sequence the genomes of ten thousand healthy Singaporeans. It represents the first stepping stone towards the development of the computational infrastructure and institutional policies necessary for a comprehensive characterization of the genetic variations.

We have deployed infrastructure-agnostic (on-cloud and on-premise High Performance Computing) scalable analytics pipelines based on international best-practices (GATK, GA4GH) as well as a variety of tools for ensuring data quality at scale and enabling privacy preserving data aggregation utilization (imputation web-service) and exploration (variant browser) by researchers and clinicians.

Through the SG10K Health project’s genetic variant catalogue, it is possible to develop a clinical-genetic database of South Asian normality, a key step towards data driven medicine, improving patient outcomes and mitigating rising healthcare costs.

Keywords: Clinical-genetic database, Data driven medicine, South Asian genetic diversity, Scalable analytics pipelines, Privacy preserving data aggregation

Acknowledgements: This project is funded by Singapore’s Agency for Science, Technology and Research (A*STAR) Industry Alignment Fund (Pre-Positioning) IAFPP: H1701a0007.
Deep Learning Model for Automated Cancer Classification based on Image data and Molecular Markers

Mr Xiao Tan¹, Mr Andrew Su¹, Dr. Hanlee Ji², Dr. HoJoon Lee², Dr. Quan Nguyen¹
¹Institute for Molecular Bioscience, The University of Queensland, St Lucia, Australia, ²Stanford Centre for Clinical Research, Stanford University, Stanford, United States

Body: The development of automated cancer classification systems has been dependent on pathologist annotated tissue images, however, these qualitative annotations are variable between pathologists, time consuming and often not at the pixel-level. Whereas these annotations rely on prior knowledge to link diseases with tissue morphological features, the combination of molecular information with tissue images has been a major focus, with data sharing from the The Cancer Genome Atlas (TCGA) and The Cancer Imaging Archive (TCIA).

To overcome current pathological classification limitations and to make use of recent advances in genomics technologies as well as the availability of public cancer genomics and imaging data, we have developed a deep learning model that uses molecular markers to train H&E imaging data. The model, instead of using pathologist annotations, can automatically and accurately classifies cancer from standard Haematoxylin & Eosin (H&E) stained histology images. Our model uses a convolutional neural network to learn morphological features corresponding to cancer cells as identified by positive staining for TP53, a molecular marker of cancer. We trained our model on paired TP53 stained and H&E stained tissue sections from adjacent slices within the same tissue block. Each TP53 image was used to label the corresponding H&E image. We implemented an image tiling strategy to increase the size of the sample. To achieve this, we developed automated approaches to segment the TP53 stain and register the TP53 images to the H&E images, correcting for differences in tissue alignment as well as tissue artefacts.

We evaluated this model on tissue from 25 colon cancer and 36 gastric cancer patients. We show that our model performs better than whole slide level labelling methods. We further show that our approach can be used for any molecular marker, including drug targets, and can use annotations based on genetic marker like that generated from spatial transcriptomic technologies. We expect that the model can also be generalised to other cancer types, such as by utilising tissue images from public datasets like TCGA and TCIA.

Keywords: Cancer Genomics, Deep Learning
Prevalence of ESR1 gene mutations and their Clinical significance in newly diagnosed metastatic and endocrine-Therapy treated breast cancer patients

Dr Faisel Mohammad AbuDuhier, Dr Rashid Mir
1Prince Fahd Bin Sultan Research chair, Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, University of Tabuk, Saudi Arabia., 2Prince Fahd Bin Sultan Research chair, Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, University of Tabuk, Saudi Arabia.,

Purpose
Estrogen receptor alpha (ERα), encoded by the estrogen receptor1 (ESR1) gene, is expressed in approximately 70% of all breast cancers, and Tamoxifen-based hormone therapy represents a major treatment modality in all stages of ER positive breast cancers. ESR1 mutations have attracted attention as a potentially important marker and treatment target in endocrine therapy-resistant breast cancer patients. Emerging mutations in the ESR1 gene result in resistance to different endocrine therapies leading to disease progression or recurrence. Several studies reported 10–50% ESR1 gene mutations in metastatic, endocrine therapy-resistant Breast cancer patients and most of them were associated with a shorter progression-free survival. Therefore the goal of the present study was to determine the incidence, clinical significance of ESR1 gene mutations in newly diagnostic and metastatic Breast cancer patients treated with endocrine therapies in Tabuk region-Saudi Arabia.

Methodology:
The study was conducted on 100 clinically confirmed cases of breast cancer. Most of the cases were treated with endocrine therapy (Tamoxifen). The estrogen receptor alpha (ERα) gene mutations were correlated with metastasis, staging and other clinicopathological features.

RESULTS:
All 100 cases of breast cancer patients were screened for ERα gene mutations including L536Q, Y537N, S463P, ESR1 Y537C, ESR1 Y537S by mutation specific PCR. Twelve of 100 were carrying ESR1 S463P mutation, 45/100 were positive for ESR1 Y537N mutation and no one were positive for (0/100) ESR1 L536Q. Higher incidence of ERα mutations S463P, Y537N mutations were found in metastatic cancer cases and might be responsible for inducing resistance for Tamoxifen in our patients. Also the higher frequency of ERα S463P mutation was reported in advanced stage breast cancer. The incidence of ESR1 Y537N mutation was higher in our breast cancer metastases cases than the non metastatic cases treated with endocrine therapies.

CONCLUSION
It was concluded that clinically relevant ESR1 mutations are prevalent in newly diagnosed, metastatic endocrine-treated breast cancer patients. ESR1 mutation screening status could improve the therapeutic strategies in controlling ER signaling before the occurrence of widespread disease metastasis. Therefore, detection of ERα gene mutations in newly diagnostic and metastatic Breast cancer patients is clinically useful in the selection of appropriate treatment strategies to prevent disease progression.

Keywords: Breast Cancer, ESR1 gene mutations, Tamoxifen resistance, The Amplification Refractory Mutation System (ARMS), An allele-specific oligonucleotide (ASO).
Structural Variant in STMN2 is Associated with sALS Disease Risk, Age of Onset and Clinical Presentation

Miss Frances Theunissen1,4, Dr Ryan Anderton1,2,4, Prof Frank Mastaglia1,2, Miss Julia Pytte1,5, Dr Loren Flynn1,2, Prof Ian James7, Prof Rick Bedlack8, Miss Leanne Jiang1,6, A Prof Stuart Hodgetts1,5, Prof Sue Fletcher2,3, Prof Steve Wilton1,2,3, Prof Merrilee Needham7,9,10, Prof Anthony Akkari1,2,3

1The Perron Institute for Neurological and Translational Science, Nedlands, Australia, 2Centre for Neuromuscular and Neurological Disorders, University of Western Australia, Nedlands, Australia, 3Centre for Molecular Medicine and Innovative Therapeutics, Murdoch University, Perth, Australia, 4School of Health Sciences and Institute for Health Research, University of Notre Dame Australia, Fremantle, Australia, 5School of Human Sciences, University of Western Australia, Nedlands, Australia, 6School of Biological Sciences, University of Western Australia, Nedlands, Australia, 7Institute for Immunology and Infectious Disease, Murdoch University, Nedlands, Australia, 8School of Medicine, Department of Neurology, Duke University, Durham, America, 9Faculty of Medicine, Notre Dame University, Nedlands, Australia, 10Department of Medicine, Fiona Stanley Hospital, Murdoch, Australia

Body: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder characterized by the degeneration of upper and lower motor neurons, resulting in paralysis and respiratory failure. At present, there is a lack of specific genetic markers for the different ALS disease subtypes, indicators of disease trajectory and disease risk. Structural variants within the genome can play a significant role in neurodegenerative disease risk and may be a source of unidentified heritability in ALS. Stathmin 2 (STMN2) encodes a protein responsible for axonal outgrowth, microtubule stability and axonal transport. It is a strong candidate for investigation due to its close relationship to, and regulation by, the known ALS-associated protein TDP-43. STMN2 contains an unresolved structural variant, a CA repeat located in intron 3 that is highly polymorphic. The STMN2 CA repeat was investigated in a cohort of North American sALS patients (n = 321) and age matched controls (n = 332) and was associated with increased disease risk for those that had the L/L genotype (p = 0.042). In particular, the presence of at least one copy of the 24CA repeat, in those that had the L/L genotype, was highly significant in sALS cases (p = 0.0023). In addition, the presence of at least one long allele was associated with a 7.5 year earlier age-at-disease onset (p = 0.039). Preliminary data also suggest that the 24CA repeat impacts patient survival in a subset of 53 bulbar onset patients (p = 0.008). The effect on survival was also tested in a small local Perth follow up cohort of longitudinal patients (n = 69) with various sites of onset. Trends for reduced survival was seen in the L/L genotype (p = 0.2) as well as reduced ALSFRS score over time, compared to other genotypes (p = 0.1). This is the first report of a structural variation in STMN2 to be associated with sALS disease risk and age-at-disease onset, and may be a genetic marker with both prognostic and therapeutic potential that warrants further investigation.

Keywords: Amyotrophic lateral sclerosis1, structural variant2, genetic markers3, missing heritability4, clinical trial stratification5.

Acknowledgements: We would like to acknowledge the patients that contribute to our ongoing work, the MNDRIA Ice Bucket Challenge SALSA-SGC project for support in providing clinical records for the participants included in this study, Prof Naomi Wray, Prof Nigel Laing and Mandi McShane.
The Australian Inherited Retinal Disease Registry And DNA Bank

Jennifer A. Thompson¹, John N. De Roach¹,², Terri L. McLaren¹,², Ling Hoffmann¹, Isabella Urwin¹, Fred K. Chen¹,²,³,⁴,⁵, David A. Mackey¹,²,³ and Tina M. Lamey¹,²*

¹Australian Inherited Retinal Disease Registry & DNA Bank, Department of Medical Technology & Physics, Sir Charles Gairdner Hospital, Nedlands, Western Australia, ²Centre for Ophthalmology & Visual Science, The University of Western Australia, Nedlands, Western Australia, Australia, ³Lions Eye Institute, Nedlands, Western Australia, Australia, ⁴Department of Ophthalmology, Royal Perth Hospital, Perth, Western Australia, Australia. ⁵Department of Ophthalmology, Perth Children’s Hospital, Nedlands, Western Australia, Australia.

Body: The Australian Inherited Retinal Disease Registry and DNA Bank (AIRDR) was established in 1984 as a local inherited retinal disease (IRD) register, and evolved in 2001 to include the collection and storage of DNA samples. In 2009 the register expanded into a national IRD resource.

The focus of the AIRDR is to characterize the genetic spectrum of IRD in Australia to facilitate development of personalized therapies, encourage Australian-based clinical trials, facilitate recruitment of potential candidates for future trials and treatments, and expand our knowledge of IRDs. Our internal investigations into disease- or gene-specific cohorts are prioritized based on current research and clinical trial status. We also collaborate nationally and internationally, providing participant data or samples to support IRD research.

The AIRDR currently contains information on 4066 IRD-affected individuals and 5170 family members from 2936 unrelated families. Participants are enrolled into the registry either directly, by completing an expression of interest to participate, or indirectly, by referrals from ophthalmologists and eye care providers. Pertinent information encompassing participant demographics, clinical diagnosis, participant-reported symptoms, medical history and family relationships are sourced.

To-date, DNA samples have been collected and stored for 3052 IRD-affected individuals and 4137 family members, from 2127 unrelated families. Genetic analyses at various stages of assessment and interpretation have been undertaken on 2440 individuals from 934 families, encompassing 75 IRDs involving 209 genes. This has resulted in the provision of 976 genetic research reports, of which 75% were considered resolved. We provide genetic research reports where it is envisaged that they may inform patient management or when they are requested by a clinician. This information may also be provided to a clinician or genetic counsellor when requested by a participant, when sufficient resources are available. Sequence variants are assessed to accepted international standards (ACMG/AMP).

Taken together, the research activity and output of the AIRDR aims to expand the genetic knowledge of IRDs and subserves our core function, which is to facilitate the translation of research findings into clinical applications for the advancement of IRD research and development of novel therapies.

Keywords: Inherited retinal disease, Registry, retinal dystrophy, retina, genetics

Acknowledgements: The Australian Inherited Retinal Disease Registry and DNA Bank is supported by generous donations from Retina Australia. The authors gratefully acknowledge the support of the Department of Medical Technology & Physics, Sir Charles Gairdner Hospital, Telethon-Perth Children’s Hospital Research Grant, MD Foundation Australia Research Grant, the Australian National Health and Medical Research Council grants, collaborating ophthalmologists and clinical geneticists, and the participants of the Registry.
Long Non-Coding RNA NEAT1 Seeded Paraspeckles In Cancer Cells Show Mechanosensitive Responses To Matrix Stiffness And Microchannel Confinement

Ms Vanja Todorovski¹, Ms Annika Meid², Doctor Andrew Holle², Professor Joachim Spatz², Doctor Yu Suk Choi¹, Doctor Archa Fox¹,³
¹School of Human Sciences, The University of Western Australia, Crawley, Australia, ²Max Planck Institute of Medical Research, Stuttgart, Germany, ³School of Molecular Sciences, The University of Western Australia, Crawley, Australia

Body: Cancer progression is driven by the integrated biochemical and biomechanical changes that occur within the tumour microenvironment. During tumorigenesis, the extracellular matrix (ECM), which forms part of the tumour microenvironment, is remodelled, ultimately leading to the stiffening of the surrounding tissue. These changes in the ECM composition and stiffness are further implicated during metastasis, whereby cancer spreads to a distal site and accounts for over 90% of cancer related deaths. Cells are able to sense changes in their surrounding microenvironment and convert mechanical stimuli into biochemical signals through a process known as mechanotransduction. Despite this, our understanding of the mechanisms underpinning mechanotransduction in cancer cells and metastasis remains limited.

The long non-coding RNA Nuclear Paraspeckle Assembly Transcript 1 (NEAT1), which forms the backbone of subnuclear ‘paraspeckle’ bodies, has been identified as a key genetic regulator in numerous cancers. Here, we investigated whether paraspeckles in cancer cell lines are influenced by various mechanical stress, including two extreme stiffnesses (3 vs. 40 kPa) or microconstrictive channels of varying width (3 vs. 10 μm), mimicking microenvironmental changes in cancer. Using tuneable polyacrylamide hydrogels, we observed an inverse relationship between paraspeckles and matrix stiffness, with an increase in paraspeckles in cells cultured on soft (3 kPa) hydrogels compared to stiffer (40 kPa) hydrogels in several cancer cell lines. This response to soft substrate is erased when cells are first conditioned on stiff substrate, then transferred onto soft hydrogels, suggesting a mechanomemory effect. We also examined well-characterized mechanosensitive markers, but found that Lamin A expression, as well as YAP and MRTF-A nuclear translocation did not show consistent trends between stiffnesses, despite all cell types having increased migration, nuclear and cell area on stiffer hydrogels.

We next investigated the effect of microconstriction on paraspeckles in metastatic breast cancer cells migrating through Polydimethylsiloxane (PDMS) microchannels. Cells confined inside 10 μm channels showed increased paraspeckle formation compared to cells outside the constriction, however paraspeckles did not change when cells migrated through 3 μm channels. Furthermore, using an antisense oligonucleotide that influences NEAT1 RNA processing, to increase paraspeckle abundance, we observed that cells treated with the oligonucleotide migrated through 10 μm channels faster (0.68 μm/min) and had increased permeation (71%), compared to non-targeting (control) antisense transfected cells (0.39 μm/min and 55% permeation). We therefore propose that paraspeckles may act as novel markers of mechanotransduction and may prove useful in characterising the effects of mechanical stress in cancer and metastasis.

Keywords: Paraspeckles, NEAT1, mechanotransduction, cancer migration, metastasis
Acknowledgements: This was supported by an ARC Future Fellowship FT180100204 (to AF), the Cancer Council of Western Australia (to AF), National Health and Medical Research Council PG1098449 (to YSC), Heart Foundation Future Leader Fellowship 101173 (to YSC), Dr Roy D. Bloebaum and Lois D. Bloebaum 2019 Graduate Student Travel Award (to VT) and the Australian Government Research Training Program Scholarship (to VT).
Whole Genome Sequencing for Cold Medicine-Related Stevens-Johnson Syndrome

Yosuke Kawai¹, Yuki Hitomi², Mayumi Ueta³, Seik-Soon Khor¹, Chie Sotozono³, Shigeru Kinoshita³, Masao Nagasaki⁴, Katsushi Tokunaga*¹
¹Genome Medical Science Project, National Center for Global Health and Medicine, Tokyo, Japan
²Department of Microbiology, Hoshi University School of Pharmacy and Pharmaceutical Sciences, Tokyo, Japan
³Departments of Ophthalmology and Frontier Medical Science and Technology for Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan
⁴Human Biosciences Unit for the Top Global Course Center for the Promotion of Interdisciplinary Education and Research, Kyoto University, Kyoto, Japan

Stevens-Johnson syndrome (SJS) and its severe condition with extensive skin detachment and a poor prognosis, toxic epidermal necrolysis (TEN), are immunologically mediated severe cutaneous reactions of the skin and mucous membranes such as the ocular surface. We have identified susceptibility variants on the HLA-A and other autosomal genes to cold medicine-related SJS/TEN with severe ocular complications (CM-SJS/TEN with SOC) by means of Single nucleotide polymorphism (SNP)-based genome-wide association studies. In this study, using a whole-genome sequencing (WGS) approach, we explored other susceptible variants to CM-SJS/TEN with SOC, especially among rare variants and structural variants (SVs). WGS was performed on samples from 133 patients with CM-SJS/TEN with SOC and 418 healthy controls to obtain SNPs and SVs. Genome-wide association tests with these variants reproduced the associations of the common variants of HLA-A and loci on chromosome 16q12.1. We also identified novel associations of SVs on these loci and an aggregation of rare coding variants on the TPRMB gene. In silico gene expression analysis on the HLA-A locus revealed that the SNP (rs12202296), which was significantly associated with susceptibility to CM-SJS/TEN with SOC, was correlated to higher expression level of HLA-A in the whole blood (P = 2.9 × 10⁻¹⁷), based on the GTEx database.

Keywords: Stevens-Johnson syndrome, whole-genome sequencing, genome-wide association study, HLA, structural variation
Zebrafish regulatory interaction clustering as a novel approach to elucidate regulatory architectures

Mr Lucas van Duin¹, Dr Sarah Rennie¹, Dr. Robin Andersson¹
¹The Bioinformatics Center, Dept. of Biology, University of Copenhagen, Denmark

Body: Interactions between regulatory elements in the genome, such as enhancers and promoters, are crucial for the regulation of gene expression and can play highly specific roles in tissue-specific or developmental programmes. Here, we draw upon a wealth of data sets in Zebrafish to identify genomic modules from inferred activity-associated interactions, highlighting distinct signatures of features related to accessibility (ATAC-seq), histone modifications (ChIP-seq) and gene expression (RNA-seq).

These interactions were inferred in an unbiased way, by defining potential regulatory elements based on accessibility and subsequently scoring their interaction potential using accessibility, activity and contact, based on an activity-by-contact model (Fulco et al, 2019). We then called sub-clusters in this genome-wide interaction network, resulting in distinct TAD-like domains, but unlike TADs, not only based on Hi-C signal. These clusters were subsequently annotated with chromatin assays from the DANIO-CODE database.

From this analysis, it can be concluded that housekeeping-containing clusters are on average larger, have higher chromatin accessibility, higher average expression, and have a higher promoter to enhancer ratio.

Further work will focus on identifying how the properties of these clusters changes over developmental time, looking into genes that have significant expression and at the same time little to no interactions, and relating the types of regulatory interactions present at genes to their expression levels.

Keywords: Regulatory Interaction, Zebrafish, Housekeeping, Epigenetics, Chromatin Conformation
Expression levels and Genetic Polymorphism of SRB1 and CETP as Biomarkers of Type 2 Diabetes Mellitus

Mohd Wamique¹, Wahid Ali¹, Dandu Himanshu²
¹PG Department of Pathology, King George’s Medical University, Lucknow, Uttar Pradesh 22603, India, ²Department of Medicine, King George’s Medical University, Lucknow, Uttar Pradesh 26003, India

Body: The aim of the present study was to determine whether the expression levels of scavenger receptor class B member I (SRB1) and cholesteryl ester transfer protein (CETP) may be used as biological markers in type 2 diabetes mellitus (T2DM). A total of 600 individuals, including 300 T2DM and 300 healthy individuals, were enrolled into the study. Blood samples were collected from each T2DM and healthy individual. Total proteins were determined using western blot analysis. In addition, restriction fragment length polymorphism (RFLP) analysis was achieved to detect the incidence of genetic polymorphisms. Furthermore, the western blot analysis results revealed that the protein expression levels of SRB1 was significantly decrease in the T2DM. The genotype distribution and the allelic frequencies for the SRB1 polymorphism were significantly different between T2DM and controls. CC genotype of the SRB1 polymorphism showed potential association with incidence of T2DM (OR =1.19, 95% CI 0.63 - 2.25, P=0.577). Furthermore, after adjusting confounding factors multivariate analysis showed significant association of SRB1 C allele with type T2DM. No significant association was observed in CETP rs3764261 polymorphism. The protein expression levels of CETP were not significantly different in T2DM when compared with the levels in the healthy controls. In conclusion, the expression levels and genetic polymorphisms of SRB1 rs5888 may be potential biomarkers for the occurrence of T2DM.

Keywords: Type 2 diabetes mellitus, SRB1, CETP
Acknowledgements: We are thankful to Vice-Chancellor of King George’s Medical University, Lucknow for providing research support and facilities
Vitamin D receptor mutations influence on course of Parkinson’s disease in patients treated with L-Dopa

Phd Barbara Zapała², Md Agnieszka Spychałowicz², Phd Monika Piwowar³, Phd Urszula Ciałowicz², Miss Adrianna Wasińska¹, Miss Martyna Kościuszko¹, Mr Olaf Chmura¹

¹Jagiellonian University Medical College, Kraków, Poland, ²Department of Clinical Biochemistry, Jagiellonian University Medical College, Kraków, Polska, ³Department of Rehabilitation, The Center of Movement Organ Rehabilitation “KRZESZOWICE” SP ZOZ, Poland, Kraków, Polska, ⁴Department of Bioinformatics and Telemedicine, Jagiellonian University Medical College, Kraków, Polska

Body:

BACKGROUND-AIM

Parkinson’s disease (PD) is second most often occurring neurodegenerative disease after Alzheimer’s disease. Age is being considered the most important factor for PD risk. Vitamin D(VD) is steroid hormone crucial for calcium homeostasis and bone metabolism. Contrary to other vitamins, VD is being produced in human organism in presence of sunlight. VD metabolism is multi-factorial process which involves specific enzymes of liver and kidneys with 1,25-D3 being active product. Latest research indicated that VD modulates over 1000 genes involved in cellular growth, protein synthesis and immunological processes. Several animal studies showed potential protective attributes of VD in dopamine cells. The aim of the study was to search for the connections between VDR gene mutations and course of PD development.

METHODS

Sequential analysis of VDR gene was performed on genomic DNA isolated from peripheral blood leukocytes of 100 patients with diagnosed Parkinson’s Disease treated with Levodopa. Sequencing was performed in 3130xl Genetic Analyzer(Applied Biosystems) and statistical analysis was conducted using AB DNA Sequencing Analysis Software v. 5.2.(Applied Biosystems).

RESULTS

From analyzed VDR gene fragments splicing region of exon 1 turned out to be the most interesting one. Mutation of “start”(ATG) codon was detected in most cases. In examined patients C/C genotype was present 32 times, C/T 53 times and T/T 23 times. Patients in research group had statistically significant prevalence of SNP. We found that dominant C/C alleles showed statistically earlier average age of diagnosis. In addition, the presence of each subsequent T allele significantly delayed the onset of the disease (p = 0.014). The T/T genotype could have also extended the time from diagnosis to the implementation of l-dopa treatment, but data did not reach statistical significance (p = 0.07) We have also connected C/T genotype of rs2228570 variant with higher chance of levodopa-induced dyskinetias. We will also present results of Vitamin D metabolome assessment and vitamin D levels which are currently under statistical processing.

CONCLUSION

We conclude that due to connections between VDR Gene mutations and clinical consequences gene sequencing may in the future be a viable way to predict future Parkinson Disease course

Keywords: Vitamin D receptors 1, genetic variability 2, Parkinson’s disease 3,

Acknowledgements: The study was financially supported by the Ministry of Science and Higher Education (grant No: K/DSC/003558).
‘A Spitting Image’ of Human Cytomegalovirus Micro RNA Molecules in Clinical Samples

Ms Shelley Waters1,2, Dr Silvia Lee3, Dr Kylie Munyard1, Dr Ashley Irish4, Associate Professor Patricia Price1, Associate Professor Bing Wang2

1School of Pharmacy & Biomedical Science, Curtin Health Innovation Research Institute, Curtin University, Bentley, Australia, 2Baker Heart and Diabetes Research Institute, Melbourne, Australia, 3Department of Microbiology, Pathwest Laboratory Medicine, Perth,

Body: Human cytomegalovirus (HCMV) is a common-cause of serious morbidity following organ transplant, and renal transplant recipients (RTR) maintain higher levels of HCMV antibodies than healthy controls for many years. MicroRNAs (miRNA) are small non-coding RNA molecules which can regulate cellular gene expression by interacting with mRNA. Herpesviruses, including HCMV, encode miRNAs that target human mRNA. Functions of the 28 known HCMV-encoded miRNA have been studied in cell culture and in silico, with limited data available from clinical samples.

We sought HCMV miRNAs miR-UL112, miR-US5-1, miR-US5-2, miR-UL36, miRUS25-2 and miR-UL22A in blood and saliva samples from RTR and healthy controls. Data were linked with measurements of the burden of HCMV, including antibody and T-cell responses. miRNAs were detected using commercially available quantitative PCR (qPCR) assays. Sensitivity was determined by diluting a positive control template (CMV strain AD169) via 10-fold serial dilution. Samples that amplified at a CT higher than that of the lowest AD169 dilution were defined as negative. Positive samples displayed two clear qPCR curves with CT lower than that of the lowest AD169 dilution.

Optimized experimental procedures revealed that miRNAs are more readily detected in saliva samples compared to plasmas and buffy coats, so the optimized assays were applied to saliva samples from RTR (n=35) and healthy controls (n=15). HCMV miR-US5-2-3p was more frequently detected in saliva from RTR compared with any other miRNA (p=0.0001-0.0044). Saliva samples from RTR with any detectable HCMV miRNA had increased levels of HCMV immediate-early 1 (IE-1) specific T-cells (p=0.01) and displayed a trend of increased HCMV lysate reactive antibody levels (p=0.08). When the presence of HCMV miRNA in saliva from RTR was assessed alone, positive samples had marginally increased IE-1 specific T-cell responses (p=0.0507). HCMV miR-US5-2 is involved in inhibiting two human mRNAs that encode proteins involved in the secretory pathway (SNAP23 and CDC42), so inhibition of these gene transcripts may influence the secretion of cytokines. Future studies will assess cytokines and HCMV-reactive antibodies in saliva from RTR with and without critical miRNAs.

Keywords: Micro RNA, Human Cytomegalovirus, Renal Transplant, Saliva, Secretory Pathway

Acknowledgements: The authors thank all individuals who participated in this study and acknowledge the Baker Heart and Diabetes Research Institute and Curtin Health Innovation Research Institute for provision of laboratory space and technology platforms. The authors also acknowledge the support of the Australian Government Research Training Program Scholarship and Graduate Women Western Australia Open Scholarship 2018.
**TGG-Viewer: A Web-based Interactive Genome-wide Visualization Tool for Splice Junctions and CNVs**

**Mr Ben Weisburd**
1Broad Institute, Cambridge, United States

**Body:** Visualization and spot-checking are important parts of sequencing data analysis. A number of tools provide visualizations for core data types, including desktop applications like IGV [Thorvaldsdóttir 2013] and IGB [Nicol 2009] as well as web-based tools like LocusZoom and IGV.js. We surveyed existing tools for their utility in visualizing RNA-seq splice junctions and CNV normalized coverage tracks in the context of rare disease analysis, and then developed a new web-based tool that removes several common limitations.

For RNA-seq analysis, sashimi plots are widely used to visualize splice junctions. Multiple packages can generate these plots as static images at particular loci - including Miso [Katz 2013], and ggsashimi [Garrido-Martín 2018]. While the images are informative and visually appealing, these tools require computational expertise and constrain exploration to pre-defined loci. Desktop IGV provides an interactive genome-wide sashimi track which solves these issues. However, both the IGV track and the other plotting tools are based on alignment files and so can typically only render regions smaller than ~100kb from no more than ~10 samples. While this constraint allows the tools to avoid processing an overwhelming amount of read data, it also makes it impossible to visually compare splice junctions in case samples vs large collections of reference data such as all muscle samples in GTEx.

Similarly, for copy number variation, multiple tools can generate static images at specific loci - including SVPV [Munro 2017] and CNView [Collins 2016], while IGV can show interactive tracks with copy number variant calls. However, to our knowledge, there is no tool that can display the widely used normalized coverage plots in a way that allows interactive genome wide exploration and comparison across many samples.

To improve on this, we developed the TGG Viewer - a web-based visualization tool based on IGV.js with improved support for RNA-seq splice junctions and CNV normalized coverage plots. By using .bed files as the underlying data-type for both the splice junctions and the normalized coverage tracks, our tool allows for significantly smaller data files and much more efficient visualization. As a result, we are able to provide an interactive web-based interface that can render splice junctions and normalized coverage at scales from 100’s of bases to an entire chromosome, and from single samples to aggregated tracks of 1000 samples. This tool is free and open-source and allows anyone to load their own data directly from Google buckets. It is available @ http://tgg-viewer.broadinstitute.org.

**Keywords:** Visualization, Bioinformatics, RNA-seq, CNVs, Rare Disease
Correlative And Causal Relationships Between Complex Traits And Brain Volumes Are Masked By Education And Socioeconomic Status

Dr. Frank Wendt\textsuperscript{1,2}, Dr. Gita Pathak\textsuperscript{1,2}, Dr. Todd Lencz\textsuperscript{3,4,5}, Dr. Joel Gelernter\textsuperscript{1,2,6}, Dr. Renato Polimanti\textsuperscript{1,2}

\textsuperscript{1}Department of Psychiatry, Yale School of Medicine, New Haven, United States, \textsuperscript{2}United States Department of Veteran Affairs, West Haven, United States, \textsuperscript{3}Division of Psychiatry Research, The Zucker Hillside Hospital, Glen Oaks, United States, \textsuperscript{4}Department of Psychiatry, Zucker School of Medicine at Hofstra/Northwell, Hempstead, United States, \textsuperscript{5}Institute for Behavioral Sciences, Feinstein Institute for Medical Research, Manhasset, United States, \textsuperscript{6}Departments of Genetics and Neuroscience, Yale School of Medicine, New Haven, United States

**Body (word count: 371/400):** Education (EDU) and socioeconomic status (SES) are phenotypically and genetically associated with psychiatric disorders and human behavior. The extent of their genetic influence on genetic risk discovery and genetically predicted biology of mental health traits remains unclear. Using information from >1 million individuals, we removed the effects of EDU (cognitive performance, highest math class completed, self-rated math ability, and educational attainment) and SES (Townsend deprivation index and median household income) from the genetic liability to psychiatric disorders, personality traits, brain imaging phenotypes, and externalizing behaviors and test for (1) shared latent factors with genomic Structural Equation Modeling (SEM) and (2) causality using Latent Causal Variable (LCV) analyses. After removing the effects of EDU/SES, we uncover two correlated latent factors (0.36 ≤ correlation ≤ 0.44) largely described by (a) mood disorders and related symptomology and (b) anxiousness and neuroticism substantiating recent findings about the distinct genetic architectures of these phenotype groups. LCV uncovered putative causal relationships between (a) extraversion\textsuperscript{left subcallosal cortex volume} (genetic causality proportion (gĉp) = 0.188 ± 0.107, 1.23x10^{-13} ≤ p-values ≤ 1.83x10^{-6}) and (b) left subcallosal cortex volume\textsuperscript{subjective well-being} (gĉp = 0.745 ± 0.009, 1.45x10^{-9} ≤ p-values ≤ 1.16x10^{-6}) which appear to precede associations between alcohol dependence and mood disorders (schizophrenia, bipolar disorder, and major depressive disorder) and (c) openness\textsuperscript{left insular cortex volume} (gĉp = 0.296 ± 0.050, 2.54x10^{-23} ≤ p-values ≤ 3.63x10^{-8}) which appear to precede associations between depressive symptoms and autism spectrum disorder. Removing the effects of EDU/SES revealed cell types putatively mediating the latent causal relationships linking left subcallosal cortex volume and mood disorders: (a) inhibitory and lateral geniculate nucleus GABAergic neurons related to major depressive disorder and (b) gestational week (GW) 10 stem cells and GW26 GABAergic neurons related to schizophrenia and bipolar disorder. By conditioning mental health outcomes for the shared genetic etiology with EDU/SES phenotypes, this study elucidates (1) novel biology of behavioral traits related to specific brain cells and (2) correlative and putative causal relationships among these phenotypes. These could not have been detected without assessing the effects of EDU and SES, because, as indicated by our findings, the pervasive effects of EDU/SES may mask underlying biology of mental health outcomes in support of multi-trait analyses of GWAS to enable trait-specific discoveries.

**Keywords:** causal inference, psychiatry, brain volume, latent causal variable, genetic correlation

**Acknowledgements:** We would like to thank the research participants and employees of 23andMe, Inc for making this work possible. This study was supported by the Simons Foundation Autism Research Initiative (SFARI Explorer Award: 534858 (RP)), the American Foundation for Suicide Prevention (YIG-1-109-16 (RP)), the National Institutes of Health (R21 DC018098 (RP), R21 DA047527 (RP), and R01 MH117846 (TL)), and the National Center for PTSD of the U.S. Department of Veterans Affairs.
EWAS of Thyroid Function Markers Identifies Novel Associations with Differentially Methylated Positions and Connections to Metabolic Pathways


1Department of Endocrinology & Diabetes, Sir Charles Gairdner Hospital, Nedlands, WA, Australia; 2Department of Twin Research & Genetic Epidemiology, King’s College London, London, UK; 3School of Biomedical Sciences, University of Western Australia, Crawley, WA, Australia; 4Pathwest Laboratory Medicine, Nedlands, WA, Australia; 5Telethon Kids Institute, University of Western Australia, Nedlands, WA, Australia; 6Centre for Genetic Origins of Health and Disease, School of Biomedical Sciences, University of Western Australia, Crawley, WA, Australia; 7School of Pharmacy and Biomedical Sciences, Curtin University, Perth, WA, Australia; 8Department of Health Sciences, University of Leicester, Leicester, UK; 9Queensland Brain Institute, University of Queensland, Brisbane, QLD, Australia; 10Centre for Advanced Imaging, University of Queensland, Brisbane, QLD, Australia; 11QIMR Berghofer Medical Research Institute, Brisbane, QLD, Australia; 12Medical School, University of Western Australia, Crawley, WA, Australia.

Acting on almost every tissue in the body, thyroid hormones affect basal metabolic rate, protein synthesis, fat and carbohydrate metabolism, and cellular response to catecholamines. They are essential to development and cellular differentiation in a range of tissues, as demonstrated by the severe mental and physical clinical consequences of untreated congenital hypothyroidism. Clinically, pituitary-thyroid axis function is evaluated by measuring circulating concentrations of thyrotropin (TSH), free thyroxine (fT4) and free triiodothyronine (fT3). Circulating concentrations of TSH and fT4 have a strong heritable component, but previously identified loci from large GWAS meta analyses only explain 33% and 21% of their estimated genetic variance, respectively. However, recent research by Brix and Hegedüs (2012) has linked DNA methylation to thyroid-related phenotypes.

To find novel associations between thyroid function and differentially methylated positions (DMPs) in the genome, we performed epigenome-wide association studies (EWAS) using DNA from blood assessed with Illumina 450K methylation array. Study subjects were euthyroid and of European ancestry. We studied two population-based cohorts, the Brisbane Longitudinal Twin Study (age 16 years; n=590) and the Raine Study (age 20 years; n=678), and meta-analysed the EWAS data from the two cohorts. Thyroid hormone levels were measured by immunoassay (Abbott). Analyses employed a likelihood ratio test in a generalised linear mixed model; smoking, blood cell composition, sex, age, family and analytical batch were included as covariates.

The analyses revealed 5 novel epigenome-wide significant DMPs with thyroid traits. Among the novel DMPs associated with thyroid traits were DMPs at chromosomal locations 1p22.2, 7q11.23, 9q21.12, 17q25.3, 19p13.3. The most significant pathways highlighted were related to regulation of behavioural, cardiovascular and homeostatic traits. The results of this EWAS highlight the close connection of thyroid function and metabolic phenotypes. The data provide a basis for follow-up functional studies and assessment of the causality of associations between thyroid function and DNA methylation. Together, these data will facilitate an improved understanding of the systemic effects of thyroid hormones.

**Keywords:** epigenetics, DNA methylation, EWAS, thyroid hormones, TSH

**Acknowledgements:** The plasma samples were collected in the context of the Brisbane Longitudinal Twin Study 1992–2016, supported by grants from Australian National Health and Medical Research Council and Australian Research. We thank Anjali Henders, Lisa Bowdler, Tabatha Goncales for biobank collection and Kerrie McAloney for collating samples for this study. We would like to acknowledge the Raine Study participants and their families for their participation in the study, and the Raine Study Team for cohort co-ordination and data collection. We also thank Abbott for donating the immunoassay reagents. This work was also supported by the NHMRC and the Sir Charles Gairdner Osborne Park Health Care Group (SCGOPHCG) Research Advisory Committee.
Morpholino Oligomer-Induced Dystrophin Isoforms: Mapping the Functional Domains in the Distal Third of Dystrophin Protein

Dunhui Li, Abbie Adams, Russell Johnsen, Sue Fletcher, Steve Wilton*
Centre for Molecular Medicine and Innovative Therapeutics, Murdoch University; and Perron Institute for Neurological and Translational Science, Perth, WA 6009, Australia

Dystrophin is the cytoskeleton protein that maintains the sarcolemma stability during muscle contractions, deficiency of which causes the progressive muscle wasting disorder, Duchenne muscular dystrophy. Antisense oligonucleotide-mediated manipulation of pre-messenger RNA splicing has been approved of safety and efficacy to overcome disease-causing mutations and restore the dystrophin expression level in patients. This strategy is inspired by the genotype-phenotype correlations as indicated by the allelic milder Becker muscular dystrophy, where in-frame deletions of some of the dystrophin exons especially in the central rod domain result in internally truncated but semi-functional dystrophin protein. However, the paucity of Becker-causing mutations in the last third of the dystrophin gene makes the amenability of mutations in this region to exon skipping strategies undetermined. In this study, dystrophin isoforms were induced in vivo by intraperitoneal injection of the peptide-conjugated phosphorodiampate morpholino oligomers in C57BL/10ScSn and C57BL/10ScSnmdx mice for the functionality evaluation to indicate any therapeutic potential. We demonstrate that the dystrophin expression level was decreased after the induction of dystrophin isoforms by skipping in-frame exon block exons 56+57, or 58+59, with severe dystrophic pathology being examined in the mouse muscle.

Keywords: Antisense oligonucleotide, Pre-mRNA splicing, Duchenne muscular dystrophy;
Acknowledgements: This work was supported by funding to S.D.W. and S.F. from the National Health and Medical Research Council (Grant # 1144791). D.L. receives a postgraduate scholarship from Muscular Dystrophy Western Australia. The authors thank Sarepta Therapeutics Inc. for generously providing the PPMOs. This work was conducted in Perth, Australia.
An Efficient, Tunable Solution For Pre-Capture Multiplexing In Targeted Sequencing Via Novel Chemistry And Semiconductor DNA Synthesis Technology

Edward Wong, Irene Song, Raymond Miller, Jiashi Wang, Jianpeng Wang, Long Fan, Lumeng Ye, Hong Li, Heidi Huang

Target enrichment of NGS libraries enables routine profiling of specific genomic regions within samples in a cost-effective manner and thus becomes a popular and well-accepted method in both research and clinical diagnostics fields. The pooling of NGS libraries ahead of target enrichment further improves the sequencing economy. This well-established workflow is now facing the challenge by the high-throughput demands generated by Novaseq, Illumina’s flagship platform that occupies the research and industry market rapidly in recent years. Novaseq creates terabytes of sequencing data per run, allowing an unprecedented level of sample multiplexing, especially for targeted NGS. The challenges scientists facing during high-throughput targeted sequencing include index hopping during library construction and target capture, the inconsistent performance of capture panel, and low capture efficiency.

To allow high-throughput pre-capture pooling without compromising the sequencing quality, GenScript team has developed library preparation to target enrichment reagents by leveraging 17+ years of DNA and protein production experience. To lower the index hopping while increasing the capacity per run during high-throughput sequencing, we have developed 384 unique adaptor pairs with customization options (Ex: UMIs), and an even larger index repertoire to explore high-throughput multiplex sequencing. With the combination of stringent control of cross-contamination during adapter production, the computationally designed custom index sequences, the refined library preparation and target enrichment protocols, we demonstrated that we could perform 384+ plex sequencing with lower index hopping rate than the traditional workflow. Also, to accommodate the various length of indices in adaptors and simplify the workflow, we develop TRUE universal blockers that bind to different indices unbiasedly regardless of length. Leveraging our semiconductor DNA synthesis technology, we can deliver a tunable and highly efficient hybrid capture solution in a fast and cost-effective manner — the precise control of semiconductor DNA synthesis technology also allows the potential evolution of capture panel via the test-learn cycle.

Keywords: NGS library, high-throughput, workflow, DNA synthesis, semiconductor
Comprehensive Detection of Germline and Somatic Structural Mutations In Cancer Genomes By Bionano Genomics Whole Genome Imaging

Yingying Wu¹, A.W.C. Pang¹, Yingying Wu¹, J. Lee¹, K. Hong¹, T. Anantharaman¹, E.T. Lam¹, A. Hastie¹, and M. Oldakowski*¹
¹Bionano Genomics, San Diego, California, US

Body: The ability to identify structural variants (SVs) is crucial in cancer genetics. Karyotype and cytogenetics are manually intensive. Microarrays and sequencing cannot detect calls in segmental duplications and repeats, miss balanced variants and low-frequency mutations. We describe the Bionano’s Saphyr system, a genome imaging platform for ultra-sensitive and ultra-specific genome-wide structural variation detection to identify SVs in cancer genomes. DNA >100 kbp is extracted, labeled at specific motifs, and linearized through NanoChannel arrays. Molecule images are digitized and de novo assembled, creating chromosomal-arm scale genome maps. Cancer mutations >500 bp are detected by aligning the molecules or the genome maps to the public reference. We ran the Bionano’s rare variant pipeline on multiple human cancer cell lines. While the number of SVs varies among samples, we typically observe > 3,500 calls per genome. In the SK-BR-3 breast cancer genome, we detected a cluster of amplifications, and translocations on chr8, impacting the gene MYC. In the CML genome K562, the BCRABL translocation was detected, while we also detected novel rearrangements, such as insertion and inversion interrupting the gene NAALADL2 in a prostate cancer cell line LNCaP. In conclusion, with one platform, the Bionano genome imaging technology can discover a broad range of traditionally refractory but relevant SVs, and improves our understanding of cancer.

Keywords: Whole genome mapping, structural variants, cancer, cytogenetics, karyotype
Comprehensive Detection of Germline and Somatic Structural Mutations In Cancer Genomes By Bionano Genomics Whole Genome Imaging

Yingying Wu¹, A.W.C. Pang¹, J. Lee¹, K. Hong¹, T. Anantharaman¹, E.T. Lam¹, A. Hastie¹, and M. Oldakowski¹*
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Keywords: Whole genome mapping, structural variants, cancer, cytogenetics, karyotype
High throughput assessment of tandem repeat contraction associated with Facioscapulohumeral Muscular Dystrophy (FSHD) by genome imaging

Grace Xie, Jian Wang¹, Ernest T Lam², Andy WC Pang³, Tom Wang⁴, Dong Zhang⁵, Henry B Sadowski⁶, Alex R. Hastie⁷, Mark Oldakowski* Bionano Genomics, Inc;

Abstract: Tandem repeats play important roles in gene regulation and chromosome structures, and are associated with various diseases. PCR and sequencing can characterize short repeats, but are ineffective when repeat size exceeds the PCR amplicon or sequence read. For large repeats, gel electrophoresis plus Southern blot analysis and fluorescent in situ hybridization (FISH) are used. While relatively effective, these procedures are laborious and highly specialized to each disease. We introduced a new workflow based on the Bionano Genomics Saphyr genome imaging platform to assay for repeat-instability disorders. As an example, we examined samples with Facioscapulohumeral muscular dystrophy (FSHD). It is the third most common genetic diseases of skeletal muscle. FSHD can be diagnosed by looking for a contraction of the D4Z4 repeats at the chromosome region of 4q35 with the permissive haplotype, commonly referred to as the 4qA haplotype. We showed that in 12 samples with known FSHD phenotypes we can correctly detect the D4Z4 repeat contractions and assign the correct haplotype in the disease allele. We further show that in 58 control samples without known FSHD, we size the repeat and determine the haplotypes correctly and detect no FSHD-type contraction in these samples. Reproducibility experiments on a subset of these samples show that we can consistently obtain equivalent results in all tested cases. Bionano offers sample preparation, DNA imaging and genomic data analysis technologies combined into one streamlined workflow that enables high-throughput analysis of tandem repeat regions of interest.

Key words: FSHD, Bionano, Saphyr, DNA imaging, Tandem repeat;

Acknowledgements:
FANTOM6 pilot study: Systematic perturbation of long non-coding RNA in induced pluripotent stem cells reveal functional roles in pluripotency

Dr Chi Wai Yip, Dr Chung Chau Hon, Dr Divya M. Sivaraman, Dr Kayoko Yasuzawa, Dr. Saumya Agrawal, Dr Joachim Luginbühl, Dr Youtaro Shibayama, Dr Piero Carninci, Dr Jay W. Shin

RIKEN Center for Integrative Medical Sciences, Yokohama, Japan

**Body:** The recent effort on characterizing long non-coding RNA (lncRNA) in stem cells have suggested their indispensable roles in pluripotency and differentiation. However, a systematic approach to address the involvement of lncRNA remains unavailable. This study aims to perturb selected lncRNAs in human induced Pluripotent Stem cell (iPSC) to identify functional lncRNAs and to characterize their roles in pluripotency and differentiation.

We perturbed 390 IncRNAs expressed in iPSC by a high-throughput screening method adopting LNA GapmeR anti-sense oligonucleotides (ASOs) lipofection and real-time cell growth assay. When quantified by qPCR assay, 166 (43%) IncRNAs showed successful knockdown (KD), while cell growth assay further revealed 31 (18%) of them with suppressed cell growth. Next, we selected 311 transfected samples plus controls for transcriptomic analysis by low-quantity Cap Analysis Gene Expression (LQ-CAGE). The transcriptomic profiles supported the observed growth phenotype according to Gene Set Enrichment Analysis (GSEA). Among 122 IncRNA targets, 15 (12%) of them showed concordant differential expression by at least two independent ASOs. Cell type enrichment assay identified 2 novel lncRNAs which regulate cell pluripotency, while KD of 10 other lncRNAs drive the cells to different cell type. Comparison of 59 IncRNA between human dermal fibroblast (HDF) primary cells and iPSC revealed 2 of them exerted similar cellular phenotype while 4 of them with similar transcriptomic changes. We further perform Hi-C and RADICL-seq on our iPSC to evaluate functional mechanism of the IncRNA targets. Five IncRNAs which showed significant chromatin contacts were identified with supportive KD profiles. Overall, combination of cellular and molecular functional genomics screening elucidates novel lncRNAs with functional impacts in stem cells.

**Keywords:** long non-coding RNA, induced pluripotent stem cell, pluripotency, high-throughput screening, low-quantity Cap Analysis Gene Expression sequencing

**Acknowledgements:** This study is supported by the MEXT funding.
A large-scale web-based survey on various symptoms related to computer vision syndrome and the genetic understanding based on multi-trait genome-wide association study

Mr Keito Yoshimura1, Dr Yuji Morita2, Dr Kenji Konomi3, Dr Daisuke Fujiwara2, Mr Keisuke Kobayashi1, Dr Masami Tanaka1
1DeNA Life Science, Inc., Tokyo, Japan, 2Kirin Holdings Company, Limited, Tokyo, Japan, 3Keio University Hospital, Tokyo, Japan

Body:
With the growth of digital devices, there are many complaints nowadays about a variety of eye-related symptoms such as eye strain, blurred vision, and double vision, and it is collectively referred to as computer vision syndrome (CVS). CVS is not directly measurable, and the latent phenotype of interest is assessed as an approximation based on multivariate symptom data from medical examinations by interview. This situation often occurs in psychological, psychiatric, and behavioral research as well, and the multi-trait genome-wide association study (GWAS) has found increasing use in these data to systematically provide knowledge about genetic overlaps between phenotypes. This study conducts multi-trait GWAS towards CVS-related survey data and will give a clue to approach biological mechanisms behind CVS.

Among customers of a direct-to-consumer genetic testing service (MYCODE), 1,998 Japanese volunteers (male to female ratio: 50.5%, average age: 49.3 ± 10.0 years) were recruited, and approximately 600,000 quality-controlled single nucleotide polymorphisms (SNPs) were obtained from each customer’s genotyping data. A drop in mind and body function known as CVS-related symptoms were evaluated using a total of web-based 14 questionnaires, such as eye strain, headache, annoyance, etc. In addition to that, based on the dry eye-related quality-of-life score QOL (DEQS) questionnaires, participants who were likely positive for dry eye syndrome were screened at a cutoff of >15 DEQS. Multi-trait GWAS was performed using a multivariate statistical analysis technique, structural equation modeling (SEM), and SNPs with a significant relationship to CVS were explored modeling a score for all observations of a latent variable, that may capture the CVS itself.

As a result, four loci were identified at a genome-wide suggestive level of p-value, and they were not hit by single-trait GWAS in each questionnaire item. With referring to publicly-available eQTL datasets, colocalization analysis between GWAS and eQTL signals derived a posterior probability where both are associated and share a single causal variant, and a locus on 3p22.1 was co-localized with eQTL signals of ENTPD3 and ZNF621 genes in retina with above 0.7 of the probability, while not in other tissues. As optionally available knowledge, the factor loadings in SEM can tell what sort of symptoms can be more frequently observed from CVS, and that were head-related and asthenopic symptoms. From these results, this study shows the possibility that the use of multivariate questionnaire data and multi-trait GWAS approach can enhance our genetic understanding of complex relationships among symptoms related to CVS.

Keywords: computer vision syndrome, statistical genetics, genome-wide association study, multivariate analysis, structural equation modeling
Acknowledgements: None. No funding to declare.
Mutation Characterisation in Patients with Usher Syndrome type 2A and Application of Therapeutic Alternative Splicing

Khine Zaw1,2,3, May Thandar Aung-Htut1,2, Fred K. Chen4, Sue Fletcher1,2, Chalermchai Mitrpant3, Steve Wilton1,2

1 Centre for Molecular Medicine and Innovative Therapeutics, Murdoch University, Western Australia.
2 Centre for Neuromuscular & Neurological Disorders, The University of Western Australia and Perron Institute for Neurological and Translational Science, Western Australia.
3 Department of Biochemistry, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.
4 Centre for Ophthalmology and Visual Science (incorporating Lions Eye Institute), The University of Western Australia, Perth, Western Australia.

Mutations in the USH2A gene are responsible for Usher syndrome type 2A, a combined deaf blindness disease. USH2A encodes usherin, a basement membrane protein highly expressed in photoreceptors of the retina and hair cells of the cochlea. On screening in different cell types, the early part of the USH2A transcript was amplified from Huh7 cells, the ARPE-19 cell line and primary human myotubes, but not from healthy or patient fibroblasts. Therefore, mutations in the early and later parts of the USH2A transcripts were analysed in induced myogenic cells from fibroblast and primary fibroblast cultures, respectively. We found that an intronic mutation in intron 46 of USH2A weakened the natural splice donor site and activated a cryptic splice site, resulting in deletion of the last 153 nucleotides of exon 46 from the mature transcript. Using patient fibroblast-derived induced myogenic cells, we also confirmed a previous report that the synonymous mutation, c.949C>A, caused the deletion of the last 93 nucleotides of exon 6. In future studies, patient fibroblasts will be reprogrammed to induced pluripotent stem cells and differentiated into retinal organoids that will be treated with antisense oligonucleotides to induce alternative splicing as a molecular therapy for Usher syndrome type 2A.
Association Of Gene Polymorphisms In Patients With Implanted Mechanical Circulatory Support Device

Mrs Madina Zhalbinova1,2, Mrs Saule Rakhimova1, Mrs Makhbubat Bekbosynova3, Mrs Saltanat Andosova3, Mrs Bagdat Abdirova3, Ms Ainur Akilzhanova1,2

1National Laboratory Astana, Nazarbayev University, Nur-Sultan, Kazakhstan, 2N. Gumilyov Eurasian National University, Nur-Sultan, Kazakhstan, 3JSC “National Research Cardiac Surgery Center”, Nur-Sultan, Kazakhstan

Introduction: Heart transplantation (HT) is the main treatment of the chronic heart failure in Kazakhstan. Nowadays, alternative way of HT is implantation of the mechanical circulatory support devices - left ventricular assist device (LVAD). Thrombosis and bleeding are side effects which are very common in patients with implanted LVADs. Heart failure patients with LVADs are normally prescribed with antithrombotic therapy for the prevention of the side effects. The aim of this study is to evaluate patients for gene polymorphisms: CYP2C19(*3) rs4986893, CYP2C19 (*2) rs4244285, ITGB3 rs5918

Methods: The venous blood samples were recruited from patients with implanted LVAD devices such as HW, HM2 and HM3 at the National Research Cardiac Surgery Center since 2011. Study included 2 groups: Case study (n=98) and Control group (n=95). Patients were prescribed with the dose of the warfarin according to the clinical protocol of the Ministry of Healthcare of the Republic of Kazakhstan. Genomic DNA samples were extracted by using the PureLinkTM Genomic DNA Mini Kit (Invitrogen, UK). Therefore, samples were genotyped by real-time polymerase chain reaction (PCR) with TaqMan probes. Genotyping was done for two groups for gene polymorphisms: CYP2C19(*3) rs4986893, CYP2C19 (*2) rs4244285, ITGB3 rs5918

Results: Statistical analysis was done on IBM SPSS Statistics 23. Genotyping results of ITGB3 polymorphism showed that mutant alleles (C/C) are common in LVAD patients (Case study). Only one patient from control group had mutant allele C/C. Statistical analysis of gene polymorphism ITGB3 reflected that mutant genotype C/C is strongly associated (P = 0.000) to the risk of myocardial infarction and aspirin resistance which can be common in heart failure patients. Genotyping results of CYP2C19(*3) polymorphism didn’t show mutant A/A genotype in both groups. Results are not statistically significant (P = 0.266). On the other hand, polymorphism CYP2C19 (*2) is not statistically significant (P = 0.888) too.

Conclusions: After statistical test results our research need to study other gene polymorphisms and amount of samples (patients) need to be increased. If patients number will be increased mutations might be identified in LVAD patients.

Keywords: heart failure, antithrombotic therapy, ITGB3, genotyping

Acknowledgements:
Setting Global Standards for Germline Genome Editing

Mr Yujia Zhu¹, Dr Owen Schaefer¹, Mr Markus Labude¹, Dr Vicki Xafis¹
¹SHAPES Initiative, Centre for Biomedical Ethics, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

Body: Several suggestions have been presented on how the experimental clinical application of germline genome editing (GGE) is to be regulated. Some have argued that existing mechanisms such as national laws are sufficient; that states impose on themselves a temporary moratorium on the clinical applications of GGE; or that binding international law against clinical applications of GGE be used. However, all three proposals risk leading to a permanent ban even in the face of subsequent significant changes to scientific and societal conditions. The third proposal would be especially difficult to achieve. However, international harmonization should be vigorously pursued, in part to protect individuals from being harmed by “rogue” GGE services offered by providers in places with less stringent regulations. Legal scholars recognize that formal international law-making is stagnating and that new international norms tend to be non-binding and negotiated with increased participation of a broader range of stakeholders.

We therefore propose that international harmonization could be achieved via a body resembling how some standard setting organizations (SSO) operate. In line with calls for broad participation, the proposed body would involve diverse stakeholders such as individuals affiliated with universities, hospitals, biotechnology and pharmaceutical companies, government agencies, including drug regulators, and patient and disability advocate groups, tasked to set standards relating to the suitability of experimental clinical applications of GGE.

Drawing on how the SSO Internet Engineering Task Force works, the proposed body would make decisions on the basis of “rough consensus”. Underlying the concept of rough consensus is the consideration of all issues raised in discussions and agreement reached by the vast majority of stakeholders who actively participate in the decision making, i.e. “those who care”. This process would foster more in-depth discussion, increase stakeholder understanding of others’ perspectives, and promote agreement. Drawing on the lessons from the failed attempts to negotiate an international convention to ban human reproductive cloning, increased stakeholder buy-in is especially important in the resolution of conceptual debates, such as those in GGE. The body’s output would not be enforceable but stakeholders would abide by the standards due to their perceived legitimacy. Not abiding by standards set would result in reputational damage. Criticisms of the proposal are also addressed in the paper.

Keywords: Germline genome editing, standards setting organizations, rough consensus

Acknowledgements: The following authors of this paper (YZ, OS, ML, and VX) were funded by the Singapore National Medical Research Council Research, Innovation and Enterprise 2020 Grant.