⁴*South Western Sydney Clinical School, University of New South Wales, Sydney, NSW, Australia*

PLK1 promotes cell cycle and is involved in recovery from DNA damage. PLK1 overexpression is present in CRC, and it is associated with poor patient prognosis. The upstream proteins are affecting the expression of PLK1. However, the contribution of the intrinsic factors to the causes of deregulation remains uncertain. This study investigates mutation and CpG island methylation of PLK1 in CRC and their associations with PLK1 overexpression. PLK1 gene mutations in HCT116, SW48, Colo320DM and T84 cells were assessed by Sanger sequencing. The methylation statuses were analysed by mass spectrometry. Subsequently the methylation study was expanded to a cohort CRC patients using pyrosequencing. The *in vitro* studies show only single deletions were found in the silencer region upstream of promoter region of PLK1 gene in SW48 and a subpopulation of HCT116 cells. Furthermore, no specific trend of methylation status was observed in the majority of the CG sites of the irradiated and control cells. In the patient cohort, low methylation was detected in CRC tissues independent of the expression of PLK1 and normal mucosa tissues. In conjunction with the findings on the literature, overexpression of PLK1 appears to be due to the effects of upstream proteins rather than intrinsic factors.

A COMPARISON OF BENCHTOP HIGH-THROUGHPUT SEQUENCING PLATFORMS IN THE DIAGNOSTIC LABORATORY SETTING

Sarah L. Nickerson, Debra O. Prosser, Stella W.S. Lai, Donald R. Love
Diagnostic Genetics, LabPLUS, Auckland City Hospital, Auckland, New Zealand

The advent of massively parallel sequencing (MPS) technologies has profoundly altered clinical genetic testing. Whilst MPS platforms vastly increase the number of tests that can be offered in the diagnostic setting, it is imperative that data quality, reliability and reproducibility are maintained. We evaluated two benchtop high-throughput sequencing instruments; the MiSeq (Illumina) and Ion Torrent PGM (Life Technologies). We designed a custom panel, comprising 24 genes predominantly implicated in hereditary cancer and metabolic diseases. The performance of the two instruments was compared by sequencing 16 samples which had previously tested positive for a pathogenic variant by Sanger-based sequencing in one of the genes included on the panel. We assessed the sensitivity and concordance of each platform, with particular focus given to homopolymeric regions and regions of high GC content. Base-call quality scores, read depth and uniformity of read coverage were also assessed, and an indication provided of the financial cost involved both in the initial set-up and the running cost necessary to meet our quality control requirements.

The results and discussion have not been included here as these are not yet available but will be complete prior to Pathology Update 2016.

A WORKFLOW FOR THE DETECTION AND CLASSIFICATION OF VARIANTS IN THE *BRCA1* AND *BRCA2* GENES

Sarah L. Nickerson, Stella W.S. Lai, Rongying Tang, Debra O. Prosser, Donald R. Love
Diagnostic Genetics, LabPLUS, Auckland City Hospital, Auckland, New Zealand

Massively parallel sequencing (MPS) technologies facilitate unprecedented levels of sequencing throughput; however, the analysis and interpretation of the large volumes of data generated present a challenge. The American College of Medical Genetics and Genomics (ACMG) have recently produced standards and guidelines for the interpretation of sequence variants, with the aim of addressing the potential for inconsistent variant classification among diagnostic laboratories (Richards *et al.*, Genet Med 2015). We describe our pipeline for analysis of *BRCA1/2* MPS data using JSI SeqPilot software, and report on our adoption of the ACMG guidelines. We illustrate how we have automated a large part of this process through the design of a web-based program, 'SOLVA', capable of extracting and integrating information from multiple population databases, *in silico* protein-prediction and splice-site prediction programmes. We also look at variants that we have re-evaluated since our implementation of the ACMG guidelines and highlight any cases where our new protocol has led to a change in a variant's previously reported pathogenicity.

SNP ANALYSIS AND WHOLE EXOME SEQUENCING: THEIR APPLICATION IN THE ANALYSIS OF A CONSANGUINEOUS PEDIGREE SEGREGATING ATAXIA

Sarah L. Nickerson¹, Renate Marquis-Nicholson², Karen Claxton¹, Fern Ashton¹, Ivone U.S. Leong¹, Debra O. Prosser¹, Jennifer M. Love¹, Alice M. George¹, Graham Taylor², Callum Wilson³, R.J. McKinlay Gardner⁴, Donald R. Love¹
¹*Diagnostic Genetics, LabPLUS, Auckland City Hospital, Auckland, New Zealand;* ²*Centre for Translational Pathology, University of Melbourne, Parkville, Vic, Australia;* ³*Paediatric Metabolic Service, Starship Children's Hospital, Auckland, and* ⁴*Clinical Genetics Group, Dunedin School of Medicine, University of Otago, Dunedin, New Zealand*

Autosomal recessive cerebellar ataxia encompasses a large and heterogeneous group of neurodegenerative disorders. We employed single nucleotide polymorphism (SNP) analysis and whole exome sequencing to investigate a consanguineous Maori pedigree segregating ataxia. We identified a novel mutation in exon 10 of the *SACS* gene: c.7962T>G p.(Tyr2654*), establishing the diagnosis of autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS). This is the first report of ARSACS in the Maori population. Our findings expand both the genetic and phenotypic spectrum of this rare disorder, and highlight the value of high-density SNP analysis and whole exome sequencing as powerful and cost-effective tools in the

diagnosis of genetically heterogeneous disorders such as the hereditary ataxias.

IN PRAISE OF ANELOVIRUSES, SAVIOURS OF THE NUCLEOTIDE ECONOMY: A CASE BASED ON EVIDENCE FROM SYNTHETIC BIOLOGY AND GENETICS

Tim Shaw¹, Andrew Peel²

¹Victorian Infectious Diseases Reference Laboratory, The Doherty Institute, and ²Department of Computing and Information Systems, The University of Melbourne, Melbourne, Vic, Australia

Synthetic biology aims to mimic complex biological networks using electronic equivalents and *vice versa*, an idea inspired by the recognition of striking similarities between evolved (genetic) and engineered (computational) control systems. In this context, viral infection is analogous to re-programming a control system by adding a regulatory module ('modulon') to change the performance of the entire network.

The recently recognised anellovirus family comprises four genera of small single-stranded DNA viruses typified by genetic heterogeneity, broad tissue tropism and exceptionally high global distribution. Human torquovirus (TTV), the founding member, was discovered in 1997 and subsequently associated with a wide variety of pathological conditions but without convincing evidence for a causal role in disease aetiology. Widespread chronic anellovirus infection has now been detected in all families of ectothermic vertebrates (mammals and birds) that have been tested. Infection is generally asymptomatic, suggesting that it might confer a survival advantage.

A simple electronic circuit model was designed to mimic anellovirus infection. Input data was obtained from recent publications. The model predicts that during physiological stress, TTV replication provides a temporary nucleotide bank, saving energy by rescuing nucleotides from catabolism. TTV could therefore be regarded as a viral commensal.

THE IMPORTANCE OF REPEATING A BONE MARROW EXAMINATION – A FATAL CASE OF EPSTEIN-BARR VIRUS (EBV) DRIVEN HAEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS (HLH) IN A 1 YEAR OLD

Rebecca Barton, Anthea Greenway, Janine Campbell
Royal Children's Hospital, Department of Haematology,
Melbourne, Vic, Australia

Aim: To describe progressive bone marrow changes in a case of EBV associated HLH in a 1 year old.

Methods: Retrospective case review

Results: This case review describes a previously well, immunised 1-year-old girl, who presented with a 5 day history of fever with no focus. On examination she was febrile, tachycardic with hepatomegaly. Initial investigations demonstrated severe pancytopenia, coagulopathy, positive EBV serology and deranged liver function tests. The patient was commenced on high dose broad spectrum antibiotics with additional investigations revealing a metabolic acidosis and elevated ferritin. A bone marrow aspirate (BMA) showed a myeloid left shift with

minimal haemophagocytosis. Despite inconclusive bone marrow findings a probable diagnosis of EBV driven HLH was made and Alemtuzumab was commenced. A repeat BMA 24 hours later showed rapid progression with florid haemophagocytosis. Further results demonstrated an elevated soluble CD25. Clinical course was complicated by rapid disease progression with multi-organ failure. Despite maximal treatment there was no improvement in disease burden and care was withdrawn.

Conclusion: Infection associated HLH can have a rapidly progressive course. Morphological changes in the BMA may lag behind clinical deterioration and confirmatory results such as CD25 may not be immediately available. Repeat BMA if clinical suspicion is high has an important role in confirming the diagnosis.

References

1. Gupta A, Tyrell P, Valani R, *et al.* The role of the initial bone marrow aspirate in the diagnosis of haemophagocytic lymphohistiocytosis. *Pediatric Blood Cancer* 2008; 51: 402–4.
2. Jordan MB, Allen CE, Weitzman S, *et al.* How I treat haemophagocytic lymphohistiocytosis. *Blood* 2011; 118: 4041–52.

ORAL ANTICOAGULANT THERAPY INTERRUPTION IN CHILDREN: A SINGLE CENTRE EXPERIENCE

Sally Campbell, Paul Monagle, Fiona Newell
Clinical Haematology – Paediatric Thrombosis and Haemostasis, Royal Children's Hospital, Melbourne, Vic, Australia

Aim and background: The use of anticoagulant therapy in paediatrics is common and there is a weak evidence base behind many of the recommendations for anticoagulant therapy in paediatric patients. To date institutional protocols for bridging anticoagulation are based on adult guidelines. This study seeks to review the clinical outcomes associated with current bridging practices employed at a tertiary metropolitan children's hospital.

Methods: The patient population was selected from the warfarin management registry of a Clinical Haematology service. Data relating to demographics, anticoagulant therapy interruption plan, and clinical outcomes were recorded.

Results: A total of 61 admissions for children aged between 1 year and 17 years and 11 months were analysed for this study. Congenital heart disease (CHD) was the primary underlying disease for which long-term oral anticoagulation with warfarin was indicated. There were no instances of major bleeding ($n=0$) or thrombotic events ($n=0$).

Conclusion: This study describes the current practices and outcomes associated with anticoagulant therapy interruption at one paediatric institution representing the largest and most representative cohort to date. This project is a stepping stone from which future studies of safety and efficacy of paediatric anticoagulation interruption management can be developed.

EVALUATION OF SNP MICROARRAY BASED CHROMOSOME TESTING FOR DETECTION OF PROGNOSTICALLY RELEVANT GENOMIC ABNORMALITIES IN CHRONIC LYMPHOBLASTIC LEUKAEMIA

Melody Caramins¹, Nicole Chia², Stephen Mulligan³

¹Specialist Diagnostic Services (SDS Pathology), University of New South Wales, NSW, ²QML Pathology, Qld, and ³University of Sydney, NSW, Australia