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Poster Presentations

BIOCHEMICAL GENETICS AND METABOLIC AND DIET DISEASE MANAGEMENT

1. DIHYDROOROTATE DETECTED IN MILLER SYNDROME CONFIRMS FUNCTIONAL DIHYDROOROTATE DEHYDROGENASE DEFICIENCY

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Introduction: Dihydroorotate dehydrogenase (DHODH) is a flavin (FMN)-linked enzyme located on the inner mitochondrial membrane, with cytochrome-C and oxygen as final electron acceptors. It converts dihydroorotate (DHO) to orotic acid (OA) during pyrimidine de novo synthesis. Gene mutations in *DHODH* cause Miller (Genee-Wiedemann) syndrome (OMIM:263750) syndrome, typified by post-axial acrofacial dystosis. However, despite the description of *DHODH* mutations predicted to cause in vivo loss of activity, DHO has not been found to accumulate in patients; on the contrary, urinary OA is raised, representing a metabolic paradox that confounds diagnosis. *Patient and Results:* We report here the metabolic results for a 3-year-old male patient with Miller Syndrome who presented with cleft palate, congenital heart disease, oligodactyly and dysmorphia. He was not taking orotate supplements. HPLC-tandem mass spectrometry detected DHO in his plasma (3.3 micromol/L; normal range: <1) and urine (140 mmol/mol creatinine; <0.5), the first confirmation of functional DHODH deficiency. Importantly, orotic acid was undetectable in plasma (<0.1 micromol/L; <1) but raised in urine (27 mmol/mol; <5). Urinary uridine and uracil were normal. The patient had heterozygous frameshift and missense *DHODH* mutations. *Conclusions:* Miller patients previously reported in the literature had elevated urinary OA only. We demonstrated loss of DHODH activity leads to plasma DHO accumulation, placing the focus back on to DHODH and the mitochondrion as causative of the clinical presentation. DHO is degraded to OA during urinary excretion. We found DHO does not degrade to OA spontaneously in vitro, suggesting in vivo degradation occurs via another enzymatic mechanism.

2. DEVELOPMENT AND EVALUATION OF STANDARDIZED SICK DAY REGIMES FOR PATIENTS WITH MAPLE SYRUP URINE DISEASE (MSUD)

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Background: Management of Maple Syrup Urine Disease (MSUD) requires strict adherence to dietary therapy, particularly during illness, to prevent metabolic decompensation. The diagnosis of four children with MSUD in Victoria in 3 years prompted the development of a standardized sick-day protocol to streamline, safely and effectively manage acute illness both at home and in hospital. *Method:* A literature review guided recommendations. From these standardized feeds were designed to meet requirements when fed at maintenance fluid requirements for age groups : <4 months, 4–12 months and 1–4 years. A protocol for storage of frozen feeds was developed for out-of-hours admissions. For home management, written instructions provide feed recipes and monitoring requirements for implementation after phone consultation. *Results:* Since November 2011, the MSUD sick-day protocol has been initiated for >42 hospital admissions and >32 home events. The protocol includes education using 2–4 Dinitrophenylhydrazine to measure keto-acids at home and training for naso-gastric tube insertion for home feeding as required. The nutritional recommendations consider total energy and synthetic protein intakes (up to 4gm/kg/day), include isoleucine (30mg/kg/day) and valine supplementation (50mg/kg/day) and the reintroduction of natural protein (leucine) after temporary cessation. Standardized regimes have prevented feed commencement delays and due to their familiarity have minimized miscommunication between metabolic and ward staff and parents thereby reducing the risk of error. *Conclusions:* Standardized MSUD feeding regimes support parents in the management and monitoring of their child during illness and improves communication among care-givers. Ongoing development will allow nutritional needs to be met as these children age.

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3. A-*N*-ACETYLGALACTOSAMINIDASE DEFICIENCY: A NEW ADULT ONSET CASE WITH NO ANGIOKERATOMA CORPORIS DIFFUSUM

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Background: α-*N*-acetylgalactosaminidase (α-NAGA) deficiency (Schindler/Kanzaki disease) is a rare autosomal recessive disorder with a broad spectrum of disease manifestation. Up to now only 13 patients with α-NAGA deficiency have been described; a newly identified case of adult onset Schindler disease (Kanzaki disease) is described here. *Case details:* The patient is a 66 year old female with a clinical manifestation that includes acroparathesis, hypohydrosis and the suggestion of diminished cognitive abilities, with no angiokeratomas. *Results:* Enzyme analysis was performed on plasma and leucocytes, showing a markedly reduced α-NAGA level with plasma levels decreased to 4% of the mean normal activity. Urine oligosaccharide excretion was abnormal and showed the characteristic oligosaccharides for α-NAGA deficiency. Sequence analysis was performed on the 9 exons constituting the *NAGA* (α-NAGA) gene. The patient was found to homozygous for the p.E325K (c.973G>A) mutation in exon 8. *Discussion:* These findings reveal an atypical presentation of Kanzaki disease. This case is the first described Kanzaki disease patient to be homozygous for the p.E325K mutation, where this mutation was previously thought to be associated with the earlier onset and more severe phenotype of Schindler disease. Second, the absence of angiokeratomas from this patient is unique, as disseminated angiokeratomas is described as being one of the clinical features of Kanzaki disease. The patient's unusual presentation, together with other reported cases of clinical heterogeneity within the same family suggests that α-NAGA deficiency is not always a monogenetic disorder, but that other factors or genes are involved.

4. LCMS/MS ANALYSIS OF PLASMA ADENOSINE AND DEOXYADENOSINE FOR THE DIAGNOSIS AND MANAGEMENT OF ADENOSINE DEAMINASE DEFICIENCY

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Adenosine deaminase (ADA) deficiency is a rare disorder of purine metabolism characterized by the accumulation of deoxyadenosine and adenosine leading to severe combined immunodeficiency (SCID). Without early intervention this condition is fatal. Recently our laboratory developed and validated a LCMS/MS assay for the quantitation of plasma deoxyadenosine and adenosine to aid management of a patient with ADA deficiency. After the addition of $^{13}C_5$-adenosine, plasma samples were ultrafiltrated and precipitated with acetonitrile/acetone. Supernatants were evaporated, and reconstituted. Chromatographic separation was achieved in 15 minutes by a RP-C_{18} column. For deoxyadenosine, MRM transitions 252.1/136.1, and 252.1/119.1 were used. For adenosine 268.1/136.1 and 268.1/119.0 were used. The method was validated for linearity, precision, recovery, LOQ, LOD, matrix effect and stability. BSA (4%) in 0.9% saline and EHNA-HCL treated plasma were used as the matrix during the validation process. A Shimadzu Prominence HPLC and ABSciex QTRAP® 4000 were used. Both analytes were linear to 4000 nmol/L. Within-run precision was less than 8% and recoveries were 77–108%. The LOD was 14 nmol/L (deoxyadenosine) and 9 nmol/L (adenosine) and LOQ was 50 nmol/L (deoxyadenosine) and 30 nmol/L (adenosine). Matrix effects were minimal. Autosampler stability (8°C) was found to be at least 72 hours. Plasma samples left at room temperature were not stable and samples tested at 4°C and -20°C had questionable stability. The described method is a useful tool in the diagnosis and management of ADA deficient patients.

5. DIAGNOSIS OF FRUCTOSE 1,6-BISPHOSPHATASE (FBPASE) DEFICIENCY BY URINE TANDEM MASS SPECTROMETRY SCREENING

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Fructose 1,6-bisphosphatase (FBPase) deficiency is a disorder of gluconeogenesis due to mutations in the FBP1 gene. Patients present with recurrent episodes of hypoglycaemia, ketosis and lactic acidosis. Glycerol and glycerol phosphate have been described as markers for this disorder. We discuss the use of electrospray ionization tandem mass spectrometry in negative-ion mode to detect glycerol phosphate in urine samples. Data from 13,173 samples was reviewed. Glycerol phosphate levels >500 μmol/mmol creatinine had a positive predictive value of 50% for FBPase deficiency in three affected patients. Elevated glycerol, detected by organic acid profiling, can be multifactorial and is not a definitive metabolite to diagnose FBPase deficiency. Glycerol phosphate would be a preferred metabolite for the diagnosis. Both metabolites were only present in samples collected in the acute period and rapidly normalized during recovery. This disorder had not been diagnosed in Victoria prior to the addition of glycerol phosphate to the urine metabolic screen. It is proposed that the analysis of glycerol phosphate using tandem mass spectrometry is important in the first tier diagnosis of FBPase deficiency.

6. CLASSIFYING PKU ON THE BASIS OF A BH4 LOAD - CAN WE DO IT?

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Tetrahydrobiopterin (BH4) is a co-factor for phenylalanine hydroxylase (PAH), an enzyme used in the breakdown of phenylalanine to tyrosine. BH4 loads are conducted on all babies in whose newborn screening sample shows an elevated level of phenylalanine. The primary purpose of a BH4 load is to detect defects in BH4 metabolism where the treatment is different from PAH deficiency. BH4 loads were attended on 78 babies through The Children's Hospital at Westmead from 1998–2013, whose phenylalanine level was >380umol/L immediately prior to the test. Of these 78 babies, 7 were detected to have a metabolic disorder in the BH4 pathway. The BH4 load test is also used to predict whether or not a baby is likely to have BH4 responsive PKU. Traditionally, the clinic has used a 30% drop in phenylalanine over 24 hrs after giving BH4 as the marker for suggesting the baby may be BH4 responsive, with the numbers expected to be about one-third of all babies with PKU. Of the 71 babies with a PAH deficiency, 29 (41%) had a >30% drop in phenylalanine level over the load study period. A comparison was made between those predicted to be BH4 responsive on BH4 load and those whose mutations in the gene had been identified and predicted to be BH4 responsive. There seems little correlation between the two methods of predicting BH4 responsiveness with significant overlap of the two methods. We concluded the BH4 load alone is not reliable in classifying PKU.

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7. AN ELECTRONIC UNWELL REGIMEN (EUR) TOOL FOR USE IN PATIENTS WITH METABOLIC DISORDERS

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Metabolic decompensation occurs in patients with disorders of intermediary metabolism during times of increased metabolic demand associated with illnesses. In common clinical practice, unwell regimens are usually initiated at the first sign of metabolic stress to prevent or reverse catabolism and hence accumulation of potentially toxic metabolites. It is the metabolic dietitian's role to regularly update the unwell regimens, which is a time-consuming and complex task. This study aimed to produce an electronic pre-programmed tool to assist in calculating individualized unwell regimens for metabolic patients to minimise calculation errors and clinician time requirements. To develop the components of the Electronic Unwell Regimen (EUR) tool consultation was undertaken with the metabolic team at the Royal Children's Hospital, Brisbane. Programming was undertaken through assistance of hospital information services. The tool was able to automatically calculate the unwell regimens once a patient's details including weight, energy and protein requirements, energy and protein composition of current formulas and dietary intake were entered into the pre-programmed tool. The tool was piloted over a one year period on 50 metabolic patients. The pilot testing found the EUR tool produced not only a reduction in the time spent in formulating unwell regimens, but also ensured safe, accurate data by reducing the risk of calculation errors. The EUR tool offers the clinician a simple electronic alternative for the calculation of unwell regimens for metabolic patient.

8. PGM1-CDG: A CHALLENGING DIAGNOSIS IN AN INFANT WITH HYPOGLYCAEMIA

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A four month old male infant with Pierre Robin sequence and a bifid uvula was referred for review. Development was normal. At 5 months the infant represented with a hypoglycaemic seizure with a diarrhoea and vomiting illness. At presentation glucose was <1.2 mmol/L and insulin 13 mlU/L with a suppressed beta hydroxybutyrate of 0.5 mmol/L, consistent with a diagnosis of hyperinsulinism. The coagulation profile was deranged. Further episodes of hypoglycaemia did not appear to be insulin related. Fasting studies reached 7 hours with an insulin of 0.3 ml/L, ketones of 3.4 mmol/L and a glucose of 2.3 mmol/L. At discharge he was commenced on diazoxide and hydrochlorothiazide, polyjoule and 3 hourly feeds. Glucose levels remained variable (2.4–13.0 mmol/L). PEG feeding was commenced and no further hypoglycaemic episodes occurred. Genetic studies for hyperinsulinism including *ABCC8*, *KCNJII*, *KIR6.2*, *GCK* and *GLUD1* genes were all normal. SNP microarray was normal. Transferrin isoforms showed a markedly abnormal pattern with decreased amounts of penta- and tetra-sialotransferrin and markedly increased asialo-, monosialo- and disialo-transferrin. The apolipoprotein CIII (ApoCIII) showed a mildly elevated ApoC-III zero level with normal mono- and disialo-ApoCIII. This pattern is not diagnostic of a disorder of O-glycosylation. Subsequent analysis by QTOF mass spectrometry of the intact transferrin protein revealed severe loss of complete glycans that lack galactose. This profile and the clinical symptoms are consistent with a diagnosis of phosphoglucomutase deficiency (PGM1-CDG). This patient is the youngest presentation to date and with the most severe glycosylation detects. Studies are being performed on the pathogenic mutations.

9. VARIATION IN RESPONSE TO INITIAL DIETARY THERAPY IN NEWLY DIAGNOSED PKU

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Background: Best practice for infants diagnosed with Phenylketonuria (PKU) requires a rapid reduction in Phenylalanine (Phe) to within the therapeutic range. *Aim:* To describe potential relationships between initial Phe levels, dietary intake and rate of Phe reduction to <400 μmol/L in infants treated at the Melbourne Metabolic Genetics Unit. *Method:* A review of dietary records of infants diagnosed with PKU during 2010-2013 assessed Phe levels, dietary protein, and time until Phe level was <400μmol/L. BH_4 responsive patients were excluded. *Results:* Twenty infants were identified with complete data available for only 12/20 due to an organizational transition from paper to electronic medical records. Median age at Phe-free formula commencement was 11 days (range 9–17). Median time to Phe levels <400 μmol/L was 4.75 days (range 3–40). In 6/12 patients Phe reached <400 μmol/L in <4 days (2–4 days Phe-free formula only) despite no difference in NBS Phe levels, weight or breastfeeding status. Mean reduction in Phe was 500μmol/L/24hrs (median 510μmol/L, range 225–815). 4/5 patients with initial Phe level 2100–2500μmol/L, took >20 days for Phe <400 μmol/L due to poor feeding or slower than expected responsiveness. Initial results do not suggest an association between synthetic Phe-free amino acids/kg/day and rate of Phe reduction. *Conclusion:* A planned prospective study to detail total and synthetic protein and energy intake, and Phe levels over time will allow analysis of relationships between NBS Phe, dietary prescription and rate of Phe reduction. We will then develop evidenced based guidelines for newly diagnosed PKU in our service.

10. WHAT WOMEN WITH PHENYLKETONURIA (PKU) FIND STRESSFUL DURING PREGNANCY, AND THE SUPPORTS THEY FIND HELPFUL

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The purpose of this study is to explore the pregnancy-related stresses anticipated and experienced by women with phenylketonuria (PKU) and the coping strategies and supports utilized or anticipated to be beneficial during pregnancy. Thematic analysis of interview data from eight women with PKU was used in this cross-sectional, qualitative study. Five of the participants had never had a pregnancy but were planning to in the future, two participants had children, and one participant was pregnant. The central concern regarding pregnancy was achieving and maintaining the essential low Phe levels, in context of the devastating effects of high levels. The Transactional Model of Stress and Coping was utilized to understand the coping strategies and supports utilized or anticipated to be beneficial during pregnancy. Similarities and differences between the women who had experienced pregnancy, and those who were planning a pregnancy in the future were evident in key coping strategies, with

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knowledge seeking, positive reappraisal, and reassurance seeking reported. Support from health professionals and other mothers with PKU was key for all women. Psychological support was identified as a resource perceived to be beneficial to promote psychological well-being during pregnancy but not yet provided. These results suggest that pregnancy is associated with significant stresses for women with PKU, with clinical implications of the findings including provision of psychological support.

11. SCREENING FOR FABRY DISEASE USING DRIED BLOOD SPOTS

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Background: Fabry disease (FD) is an X-linked recessive lysosomal storage disorder resulting from a deficiency in the enzyme a-galactosidase A (a-gal). Deposition of glycosphingolipids occurs throughout the body, particularly in the kidney, nerves, heart, and brain. Female FD carriers can have clinical presentations ranging from asymptomatic to severely affected and can often display leucocyte a-gal activities within the normal range, making diagnosis difficult. The aim of this study was to determine whether analysis of a-gal in dried blood spot (DBS) samples offers a more definitive means of diagnosis, particularly for FD carriers. *Method:* a-gal activity in DBS was determined using a 4-methylumbelliferyl-conjugated substrate. *Results:* Hemizygous males were shown to have markedly reduced levels of a-gal activity (0.18 ± 0.13 nmol/h/mL, $n = 21$, NR = 1.7-11.9, mean $\pm$ *SD*). Females who were known carriers of a FD mutation also displayed reduced levels of a-gal activity (1.1 ± 0.52 nmol/h/mL, $n = 43$), however ~16% of those tested overlapped with the bottom of the normal range, up to 2.0 nmol/h/mL. *Conclusion:* Measurement of a-gal activity in DBS is a fast, cheap and robust method for screening for FD. Caution must be exercised when interpreting results from females with a-gal activities at the bottom of the normal range and repeat samples should be requested.

12. DIETARY MANAGEMENT OF 26-MONTH-OLD MALE RECENTLY DIAGNOSED WITH HYPERAMMONEMIA-HYPERORNITHINEMIA-HOMOCITRULLINURIA (HHH) SYNDROME IN SOUTH AUSTRALIA

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Aim: To describe the dietary management of a child diagnosed with HHH syndrome. HHH syndrome is a defect of the mitochondrial ornithine transporter causing increased plasma levels of ornithine and ammonia, elevated liver enzymes, and increased urinary excretion of homocitrulline, polyamines and orotic acid. Clinical presentation may include acute encephalopathy, developmental delay and poor growth. Treatment includes citrulline supplementation, sodium benzoate and dietary protein restriction. Dietary management aims to prevent ammonia toxicity and optimise nutritional status. *Case report:* 15-month-old Aboriginal boy diagnosed with HHH syndrome after presentation with developmental delay and poor growth. He commenced L-citrulline, sodium benzoate and protein restriction of 1g/kg. Oral skills and growth improved, and ammonia normalized. Protein was increased to 1.5g/kg. 6 months later despite good biochemical control his eating regressed and weight gain slowed. He was admitted and received allied health support. Protein intake was 1.7g/kg and calorie intake 71Cal/kg. Due to persisting poor weight gain a nasogastric tube (NGT) was inserted and a high energy protein free formula was given to support oral intake. Calorie was increased to 105Cal/kg. With adequate energy intake, dietary protein was increased to 2g/kg with no adverse effect to plasma ammonia levels. A trial off feeds was attempted with no success and he was discharged with a NGT. His current management is 2g/kg of protein, high energy protein free formula given via NGT if refused orally, fortnightly plasma amino acids, ammonia assays and weight. Literature indicates that clinical outcomes are variable even with good metabolic control.

13. TOWARDS BH4 THERAPY IN PHENYLKETONURIA: A SURVEY OF BASELINE CHARACTERISTICS, NUTRITION, FOOD VARIETY AND IMPACT OF MANAGEMENT

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Sapropterin (BH4) therapy for phenylketonuria (PKU) is not yet routinely available in Australia. This study determined nutritional intake, food variety, clinical measures and psychosocial impacts of dietary management of hyperphenylalaninaemia/PKU in 28 of 40 children, likely to be responsive to BH4 but not currently receiving BH4 therapy. Four were classified as classical PKU, 24 with milder variants. Clinical records were retrospectively reviewed for diet prescription, anthropometry and blood phenylalanine (phe). Dietary intake and food variety was determined using three non-consecutive 24-hour recall phone interviews and a qualitative food frequency questionnaire. A modified version of the PKU Worry Checklist rated concern about current management and expectations of BH4 therapy. Growth was in the normal range and median blood phe in previous year was 295 μmol/l. Reported protein intake ranged from 0.7–2.9 g protein/kg/day of which a median of 68% was from phe free protein supplement (prescribed for 27 patients). Moderate to high protein foods or low protein food products were not eaten regularly and half the group did not eat vegetables daily. Mothers expressed a range of concerns about PKU management but most commonly were a little or not worried overall (12/22). There was high expectation of BH4 therapy.

14. MEETING THE CHALLENGE OF MAINTAINING ENERGY INTAKE, SATIETY AND VARIETY WHEN ADDING A PROTEIN RESTRICTION TO A MINIMUM FRUCTOSE DIET, A CASE STUDY

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We report the case of a 26-year-old man, referred to our service, with Hereditary Fructose Intolerance on a strict minimal fructose diet who developed renal failure due to Focal Segmented Glomerulosclerosis and was advised by his renal dietitian to reduce his protein intake from 300 g/day to 100 g/day. Dietary assessment revealed that the patient previously ate large serves of meat and other animal products with only small amounts of refined cereals, savoury snacks, potato and minimal vegetables. He had reduced his meat intake but was reluctant to increase lower protein carbohydrate foods due to concerns that this would increase his fructose intake too much. The patient was having problems adjusting to the smaller meat serves, was finding the diet boring, was constantly hungry and continuing to lose weight. He asked for help with translating the principles of the diet into daily meals that were more filling and varied. A flexible meal plan, providing 11,800 kJ, 100–110g protein and 41 mg fructose/kg body weight, was devised using protein exchange lists incorporating fructose exchanges, and an energy exchange list of fructose free, low protein foods providing 420 kJ/serve. The plan included eight suggested daily menus with accompanying recipes.

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At 2 months follow-up the patient had regained 7 kg and he reported he had found the menus useful, had tried some of the recipes, was managing the smaller meat serves and making use of low protein products. At 4 months he was able to decreased kilojoule intake to avoid further weight gain.

CANCER GENETICS

15. A CASE OF CHIMAERIC FAMILIAL MDS/AML OR SPORADIC AND DE NOVO AML IN FATHER/DAUGHTER?

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Familial and germline de novo mutations predisposing to MDS/AML may be suspected if the age of disease onset is in the teens or early adult years. Patients are screened for predisposing germline mutations in *RUNX1*, *CEBPA* and *GATA2*. We have identified a family in which the father was diagnosed with AML at 28 years of age and received an autologous bone marrow transplant 2 years later. He is currently alive 13 years post-transplant. His daughter subsequently presented with AML (age 17 years). Genetic screening of the father on pretransplant BM, blood, buccal epithelium or hair failed to identify mutations in *GATA2* or *RUNX1*. However, a pre-transplant BM sample does harbour somatic *CEBPA* N- and C-terminal mutations, which is a signature of disease progression in patients with germline *GATA2* mutations. Genetic screening of the daughter revealed a germline *GATA2* (p.Arg69Leufs∗115) mutation confirmed on blood, buccal epithelium and hair. Similar *GATA2* frameshift mutations have been reported in several cases of familial AML. The mutation was further confirmed using an AmpliSeq custom 29 gene MDS/AML panel on the Ion Proton; no other mutations were found. We are currently in the process of deep sequencing the father's AML and germline samples to determine whether he is a low grade germline and somatic mosaic carrier of this *GATA2* mutation or whether this is a very rare case of de novo germline GATA2 mutation with sporadic AML in a first degree family member.

16. A NOVEL COMPOUND IN-CIS GERMLINE *GATA2* MUTATION IN AN INDIVIDUAL PRESENTING WITH AML WITH CONCURRENT THROMBOCYTOPENIA

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GATA2 is a zinc finger transcription factor that is essential for hematopoiesis. We have previously identified germline GATA2 mutations that predispose individuals with these mutations to Myelodysplastic Syndrome (MDS) and Acute Myeloid Leukaemia (AML). In this study we have identified a novel compound in cis GATA2 mutant (T358N/L359V) in an individual from a family presenting with MDS/AML with concurrent thrombocytopenia. The individual tested negative for RUNX1 mutations normally associated with this phenotype. This is the first example of in-cis germline mutations on the same allele observed in GATA2. We have demonstrated that T358N is a LOF mutation, and that T358N is dominant over L359V, a known GOF somatic mutation that plays a role in progression from chronic phase to blast crisis in Chronic Myeloid Leukaemia. Molecular modelling of the T358N/L359V variant suggests the larger asparagine residue in the T358N transition inhibits protein/DNA contact, overriding the closer contact allowed by the L359V mutation. We have further shown the T358N/L359V mutation significantly reduces the ability of GATA2 to synergise with PU-1 to activate myeloid specific promoters.

17. GERMLINE AND SOMATIC MUTATION OF FANCONI ANEMIA RELATED GENES IN THE PATHOGENESIS OF ACUTE MYELOID LEUKAEMIA

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Acute myeloid leukaemia (AML), which is the most common acute leukaemia in adults, is a particularly devastating disease with a 5-year overall survival of only 24%. In addition, there are a number of familial diseases described that have as a feature an increased susceptibility to AML. One such disease, Fanconi Anemia (FA) is characterized by hypersensitivity to DNA cross-linking agents, progressive bone marrow failure and a greatly increased predisposition to AML. Interestingly, many genes associated with predisposition to hematological malignancy, such as CEBPA, RUNX1 and GATA2 have also been described as targets of somatic mutation in sporadic AML. The purpose of this study was to comprehensively assess the frequency and somatic status of mutations in FA related genes in a large cohort of sporadic diagnostic AML samples. We have performed whole exome capture and next generation sequencing (NGS) of a series of 96 diagnostic AML samples. Using this analysis we identified 28 mutations in the 16 FA associated genes. Studies comparing AML diagnostic material to matched non-tumour DNA (mesenchymal stromal cells) has shown that germline mutations in these 16 FA genes are frequent (including known FA causing mutations), However, we have also identified novel somatic mutations in FANCD2 and FANCM. Meta-analysis has also shown that as a group FA genes are more frequently mutated in the AML cohort compared to a non-AML control cohort ($n = 200$). Together this information suggests that mutation of FA genes is important for both AML predisposition and pathogenesis.

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18. ANALYSIS OF SOMATIC *GATA-1* MUTATIONS AS AN EARLY DETECTION METHOD FOR MYELOID LEUKAEMIA IN CHILDREN WITH DOWN SYNDROME

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Individuals with Down syndrome (including mosaic Down syndrome) have a significant risk of developing acute lymphoblastic and myeloid leukaemia in childhood. Between 5–10% of neonates with Down syndrome develop the pre-leukaemic condition transient myeloproliferative disorder (TMD). TMD is characterized by accumulation of megakaryoblasts (blasts) in peripheral blood, spontaneously resolving in the majority of cases by the age of 3 months. Furthermore, 25% of these children will develop acute megakaryoblastic leukaemia (AMKL) between 2 and 4 years of age, with reported event-free survival over 80%. TMD and AMKL are characterized by somatic mutations of transcription factor GATA-1. Studies show that the same GATA-1 mutation is present in TMD and AMKL blasts of the same individual, providing evidence of a clonal evolution of disease. The PreP 21 study is a prospective multi-centre study which aims to predict which children with Down syndrome will develop leukaemia by testing for GATA-1 mutations. Peripheral blood samples will be collected from children with Down syndrome at defined intervals over the first four years of life. Correlating the persistence of GATA-1 mutant clones with clinical outcome will help further our understanding of leukaemogenesis in this unique study population. This is hoped to predict which children with Down syndrome will develop AMKL, and identify possible molecular targets for therapeutic intervention in the future. Recent developments in the diagnosis of TMD, subsequent understanding of progression to AMKL in children with Down syndrome and the potential for the study to provide additional information will be presented.

19. QUALITY DRIVEN REVIEW IN CASCADE TESTING UNMASKS THE DOUBLE ENTENDRE OF BRCA2:c.7806-2_7806-1dupAG

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Errors in splicing that effect functional *BRCA2* haploinsufficiency are well demonstrated as contributing to the autosomal dominant nature of familial breast/ovarian cancer. Novel variants which are juxtaposed to the splice boundary are usually obvious and readily testable in vitro. We present here our encounter with the DNA sequence variant BRCA2 8034_8035insAG (as presented in BIC, or BRCA2:c.7806_7807insAG in kConFab). In both databases, this has been classified as an exonic frame-shift variant, that is, pathogenic. A recent review by our laboratory at SA Pathology as a prelude to predictive testing noted that the nomenclature in the databases masks an untested but plausible splicing effect. As described in BIC and kConFab, the insertion lies at the start of exon 17 and is therefore interpreted as encoding an AG dinucleotide frame-shift. Alternatively and perhaps more precisely however, this is an AG duplication of the conserved splice acceptor site of intron 16 (i.e., BRCA2:c.7806-2_7806-1dupAG), which would not necessarily be pathogenic as splicing and the reading frame could be maintained. Despite web based predictions to the contrary, in this report we demonstrate the first empirical evidence that BRCA2:c.7806-2_7806-1dupAG does indeed predominantly encode normal wild type splicing. Our experience is a reminder of the importance of considering DNA variants in the context of the surrounding sequence. It also highlights the importance of reviewing DNA sequence variants, either periodically or in response to a new request for testing, as part of an effectively applied quality laboratory management system.

20. SOMATIC HYPERMUTATION OF THE IGHV GENE IN CLL: THE SA PATHOLOGY EXPERIENCE

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Somatic Hypermutation (SHM) of the immunoglobulin heavy chain variable (IgHV) gene sequence is a strong indicator of prognosis in Chronic Lymphocytic Leukaemia (CLL) patients. When a single productive rearrangement is detected, analysis of mutation status and interpretation is simple. However, it has been reported that double productive rearrangements have been detected in up to 5% of CLL patients and one study showed that in approximately 1% of cases double rearrangements of discordant mutation status were detected making prognostication difficult. The detection and sequence analysis of multiple rearrangements present difficulties for the laboratory and interpretation is sometimes inconclusive. Patients with progressing CLL were assessed for IgHV clonality and SHM status. The IgHV gene of each patient was amplified using six Variable gene (V-gene) Framework-1 (FR-1) consensus primers and a single Junction gene (J-gene) consensus primer by standard PCR, Sanger sequencing, and the mutation frequency was determined using the IMGT/V-Quest database. Those samples in which more than one rearrangement was detected were also analyzed using the Invivoscribe IGH Somatic Hypermutation Assay v2.0 which employs two multiplex PCRs which target the sequence between the leader-joining regions and FR1-joining regions. The results of the IgHV SHM status for our cohort of patients is presented including the comparison of the two methods in the small proportion of samples showing multiple rearrangements. The complexities encountered in the analysis of these samples is also discussed.

21. SULFATION OF HEPARAN SULFATE MEDIATES THE INVASIVE POTENTIAL IN A HUMAN BREAST CANCER MODEL

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Breast cancer is a highly heterogeneous disease at both the molecular and clinical level. With survival rates greatly increased by early diagnosis, understanding the micro-environmental changes involved in tumour development and progression will likely provide improved limitation strategies and treatment success. The extracellular matrix (ECM), a central component of the cellular niche provides structure to support optimal conditions for cell proliferation and maintenance. Heparan sulfate proteoglycans (HSPGs) are key components of the ECM where they mediate proliferation, invasion and cell signalling. In this study we have examined the gene expression profile of a number of HSPG family members, including initiation and modification biosynthesis enzymes, as well as the two major families of core proteins, the syndecans (SDC1-4) and glypicans (GPC1-6). Gene expression in relation to cell proliferation was examined in the human breast cancer MCF-7 and MDA-MB-231 cell lines following treatment with the sulfation inhibitor sodium chlorate (chlorate). Predominantly, chlorate treatment resulted in reduced SDC and GPC gene expression indicating reduced cell signalling likely through both FGF and Wnt pathways. The observed changes in gene expression provide further evidence for the

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role of these complex proteins in these significant cellular events. In the case of cancer, these proteins are involved in the development and progression of disease and may provide new markers of intervention.

22. PHYTOCONSTITUENTS FROM BUTEA MONOSPERMA HAVE ANTIGENOTOXIC AND ANTICANCER ACTIVITY

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Chemoprevention is prevention of cancer by chemical compounds. India possesses many still not explored traditional medicinal plants that can be used to obtain chemopreventive agents from natural products. *Butea monosperma* is one of those plants. This study was aimed to exploit phytoconstituents and pharmacological potential of hexane fraction (B3) of its bark and methanol extracts (L1) of its leaves. Reverse Phase High Performance Liquid Chromatography (RP-HPLC) was employed for identification and quantification of phenolic compounds. Antigenotoxicity was conducted by SOS chromotest (without metabolic activation) and DNA nicking assays. Extracts were evaluated for cytotoxic activity against MCF-7 cancer cell line by MTT assay. HPLC analysis revealed that most abundant polyphenols present in B3 and L1 were kaemferol and umbelliferon respectively. Extracts showed statistically significant antioxidant activity ($p \leq .05$). Antioxidant activity of polyphenols is attributed to their hydrogen donating ability to free radicals as their reduction potential is lower than those of Reactive Oxygen Species. The IC50 of B3 and L1 against MCF-7 cells was found to be 199.47 and 96.42 μg/mL respectively. Extracts were effective in reducing SOS response induced by 4-NQO (directly acting genotoxin). L1 possessed stronger antioxidant, cytotoxic and antigenotoxic activities than B3. Based on present study, it is quite apparent that *B. Monosperma* has tremendous potential for prevention of ailments related to genotoxins and environmental carcinogens.

23. T-LYMPHOBLASTIC LYMPHOMA ASSOCIATED WITH t(8;22)(p11.2;q11.2)

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The t(8;22)(p11.2;q11.2) has been reported in fewer than 20 patients. This genomic lesion is postulated to occur in a pluripotent hemopoietic stem cell with multilineage potential and patients may present with a CML-like disease, which may rapidly evolve to acute leukaemia, or with overt acute leukaemia of either myeloid or lymphoid lineage. The molecular consequence of this translocation is formation of a BCR-FGFR1 fusion gene. The resulting chimeric protein has constitutively active tyrosine kinase activity and confers growth factor-independence. While FGFR1-rearranged cells are responsive to tyrosine kinase inhibitors in vitro, the in vivo results are unsatisfactory and allogeneic HSCT currently provides the only hope for achieving long term remission in these patients. We report the unusual presentation of a 66 year old man diagnosed with T-Lymphoblastic Lymphoma whose bone marrow cells revealed 46,XY,t(8;22)(p11.2;q11.2)[14]/46,XY[2]. Interphase FISH analysis confirmed the involvement of FGFR1 and BCR. Our patient exhibits relatively mild symptoms for what is usually considered to be a clinically aggressive disease.

24. FISHING FOR THE GOLD STANDARD IN RB1 MOSAICISM

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Retinoblastoma is a malignant neoplasm of the retina that occurs in children. Tumours develop in cells carrying mutations in both copies of the *RB1* gene. The disease may be multifocal or unifocal, and may present with bilateral or unilateral retinoblastoma. Patients carrying germline mutations are at increased risk of bilateral eye disease and extra-ocular tumours and require regular surveillance. Due to a high de novo mutation rate sporadic cases, with no family history, are also common. Gonadal mosaicism, typically during spermatogenesis, has been implicated in these cases, leading to germline mutation in the offspring. Mutation may also occur at a post-zygotic stage leading to varying degrees of tissue mosaicism, which is seen in about 6% of patients. The detection of such mosaicism can be challenging. Large deletions and duplications account for up to 16% of *RB1* mutations, and in our laboratory gene sequencing is followed by MLPA to detect these abnormalities. However, MLPA is optimized for the detection of a single or two copy deletion of the gene. We describe a case in which the detection of a non-integer copy loss in a peripheral blood sample prompted further investigation by FISH and CGH array. Initial FISH analysis performed on fixed cells after 72-hour culture gave a normal result. Subsequent analysis of uncultured cells confirmed mosaicism in our patient. We consider this finding has implications for the use of FISH in retinoblastoma and other disorders where mosaicism is a possibility.

CLINICAL GENETICS

25. CROUZON SYNDROME WITH A BEARE-STEVENSON-LIKE PRESENTATION

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Crouzon syndrome (OMIM 123500) and Beare-Stevenson syndrome (BSS, OMIM 123790) are both craniosynostosis syndromes caused by mutations in the fibroblast growth factor 2 (*FGFR2*) gene. Crouzon syndrome is more common (1 in 60,000 live births) than Beare-Stevenson syndrome (BSS), with fewer than 20 cases of BSS reported. The cardinal features of BSS are cutis gyrata, acanthosis nigricans, skin furrows, skin tags, craniosynostosis (Crouzon syndrome-like features in some cases and cloverleaf skull in others), anogenital anomalies and a prominent umbilical stump. Here we present an infant with a previously reported Crouzon syndrome *FGFR2* mutation with features of BSS, the first such reported infant. The infant presented with evidence of sagittal and bicoronal synostosis at birth. The clinical features with phenotypic overlap with BSS were the presence of marked redundant skin involving the scalp, deep furrows in both ears, linear rugations very similar to cutis gyrata involving the lips, deep palmar and solar creases and a prominent umbilical stump. Successful fronto-orbital advance surgery was done at 6 months of age and early developmental progress is reassuring. Sequencing of *FGFR2* revealed a mutation in exon 8 (c.799T>C

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p.Ser267Pro) previously identified in Crouzon syndrome. No mutations in exon 11 of *FGFR2*, where previously reported BSS mutations have been located, were identified. This case expands the phenotypic spectrum of Crouzon syndrome and highlights the overlap between conditions caused by mutations in *FGFR2*.

26. CLINICAL AND GENETIC FEATURES OF AUSTRALIAN FAMILIES WITH LONG QT SYNDROME (LQTS)

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Familial long QT syndrome is a primary arrhythmogenic disorder caused by mutations in ion channel genes. Genetic testing plays a key role in clinical management. We performed a cross-sectional study to evaluate cohort features. Individuals were recruited from the Australian Genetic Heart Disease Registry and Genetic Heart Disease Clinic, RPA Hospital Sydney with a definite or possible clinical and/or genetic diagnosis. Clinical and genetic information was obtained through review of registry data, direct from the patient and medical records. Ninety nine individuals from 72 families were recruited; 22 (22%) had sudden cardiac death (SCD) events (including SCD, defibrillator therapy or resuscitated cardiac arrest). Cardiac arrest was the presenting symptom in 18 (18%). Patients with a SCD event were more likely to have non-sustained ventricular tachycardia (NSVT) (27% vs. 9%, $p = .01$). 38/71 (54%) probands underwent genetic testing with a pathogenic mutation identified in 21 (55%). There was no significant difference between the mean QTc, defibrillator frequency, NSVT, age of diagnosis, or gender between probands with or without results. Probands with at least one relative affected (54%) were more likely to have a mutation identified (76% versus 24%, $p = .001$). Following a positive proband result, a median of 2 relatives underwent predictive testing (range 0–10). Of the 35 individuals with a positive predictive test 12 (34%) did not meet diagnostic criteria but 8 (67%) were placed on beta -blockers. This is the first exploratory study comparing features of Australian LQTS families. This study provides insight into factors which impact on management and outcomes.

27. DILATED CARDIOMYOPATHY: THREE BROTHERS AND A *BAG3* MUTATION

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Three brothers have been diagnosed with dilated cardiomyopathy (DCM). Their father was diagnosed with DCM, but died awaiting a heart transplant at 55 years of age. A paternal aunt died suddenly at 21 years of age, cause unknown. Brother 1 has advanced DCM requiring a heart transplant. Echocardiogram prior to transplant reported a moderately dilated left ventricle (LVEDD 5.7cm, LVEDD Index 3.1cm/m^2). Ejection fraction was 10% by M-mode method. Brother 2 has a severely dilated left ventricle (LVEDD 6.4cm, LVEDV 220mL) with mild/moderate global impairment of systolic function. Ejection fraction is 44% by Simpson's biplane method. Brother 3 has a mildly dilated left ventricle, (LVEDD 5.4cm, LVEDV 172mL) with low normal systolic function. The ejection fraction is 54% by Simpson's biplane method. Brother 1's genetic testing identified a *BAG3* mutation, c.925C>T, p.R309X and a *MYH6* variant of uncertain significance, c.166G>A, p.G56R. Brother 2 was positive for the *BAG3* mutation, but negative for the *MYH6* variant. Brother 3 has yet to undergo testing. The familial cause of the DCM is likely to be the *BAG3* mutation. It could be hypothesized that Brother 1's severe presentation may be due to a confounding effect by the *BAG3* mutation and *MYH6* variant. Additional family studies may assist in this identifying this effect.

28. OUTFOXED BY *RBFOX1*: A CAUTION ABOUT ASCERTAINMENT BIAS

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We were challenged to interpret *RBFOX1* CNVs in three families with strong family histories of autism and intellectual impairment and initially hypothesized that *RBFOX1* was another susceptibility locus for neurodevelopmental disorders. We obtained aCGH data from 2,446 individuals with autism spectrum and compared this with aCGH data from 13585 controls. This data suggests that *RBFOX1* CNVs are not pathogenic and contradicts previous reports that examined smaller numbers of patients and controls.

29. SKIN MANIFESTATIONS AS A NOVEL CLINICAL PRESENTATION ASSOCIATED WITH *SUFU* MUTATIONS

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Medulloblastoma tumours may arise sporadically or as part of an inherited syndrome. A subset of children with medulloblastoma carry germline mutations in the SUFU tumour suppressor gene. Medulloblastoma is the main feature associated with SUFU mutations described to date; penetrance is currently estimated to be around 30%. We propose that skin manifestations and macrocephaly may be part of the spectrum and describe two cases that are consistent with this notion. The first is a 55-year-old woman referred for investigation of skin lesions and a family history of two children from different unions with medulloblastoma. Examination revealed facial papules (classified as benign folliculosebaceous harmartomatous lesions) and dysmorphology (macrocephaly, hypertelorism and prognathism). Her father and son are reported to share the same dermatological features. Cowden and Gorlin syndromes were excluded before a SUFU splice-site mutation predicted to lead to exon skipping was identified. The second case is an 18-month-old child born to non-consanguinous parents and diagnosed with nodular medulloblastoma at 7 months. Examination revealed macrocephaly and hypertelomerism with no other features of Gorlin or Cowden syndromes. The unaffected mother and maternal grandmother also have macrocephaly. SUFU mutation analysis identified a mutation in the proband; cascade testing confirmed the mutation was inherited from his mother, who is now considering pre-implantation genetic diagnosis. We suggest that the emerging SUFU phenotype should include hamartomatous skin lesions and macrocephaly. Presence of these features in combination with medulloblastoma may alert clinicians to the syndrome, affording the opportunity for genetic counseling of at-risk families.

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30. SOTOS SYNDROME: AN UNUSUAL PRESENTATION WITH INTRAUTERINE GROWTH RESTRICTION, GENERALIZED LYMPHEDEMA AND INTENTION TREMOR

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Background: Sotos Syndrome (SoS) is a childhood overgrowth syndrome characterized clinically by a distinctive facial gestalt, advanced bone age, excessive growth and non-progressive developmental delay; and genetically by haploinsufficiency of nuclear receptor binding SET Domain 1 (NSD1) gene, on chromosome 5q35. Generalized lymphedema has not previously been associated with SoS. It can be defined as the pathological accumulation of protein rich fluid because of dysfunction of the lymphatic system. Generalized lymphedema has been associated with mutations in numerous genes including FLT4, which is involved in the regulation of VEGFR3, a key governor of lymphatic-endothelial cell development and function. *Case:* We report on a 28-year-old Caucasian female with a novel NSD1 intragenic insertion mutation, 5848Ins(CCGCACAT), leading to a frameshift mutation. She had characteristic clinical features of SoS. Unusually, she had intrauterine growth retardation and post-pubertal onset of generalized lymphedema. She also has congenital hypothyroidism, generalized hair loss, intention tremor and small joint arthritis. *Conclusion:* To our knowledge, no link between SoS and generalized lymphedema has previously been described in the literature. We propose a mechanism by which disruptions in NSD1 gene may lead to generalized lymphedema. Aberrations of the mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK)-signalling pathway has been identified in both SoS and lymphedema. This finding extends the known phenotype of SoS through the inclusion of lymphedema. This case also indicates that presence of low birth weight does not exclude the possibility of Sotos syndrome.

31. CASE REPORT OF 8q13.1-q13.2 DELETION ASSOCIATED WITH INFERIOR VERMIAN HYPOPLASIA AND TALE-NAIL-LIKE DIGITAL REDUCTION ABNORMALITIES

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Cerebellar vermis hypoplasia has been associated with a large number of chromosomal abnormalities and metabolic disorders, with few candidate genes for isolated cerebellar vermis hypoplasia. We report on a 12-year-old boy diagnosed with inferior vermian hypoplasia associated with a novel de novo microdeletion. He presented with intellectual, speech and language impairment, unilateral facial nerve weakness, marked constipation, and bilateral hand and foot anomalies. His hand features were digital reductions similar to those seen in 4q34 deletion syndrome and a small number of other disorders. Cranial MRI demonstrated isolated inferior cerebellar vermis hypoplasia. A de novo 1.4 Mb interstitial deletion was identified at 8q13.1-q13.2 on chromosomal microarray. This copy number variant involves 18 RefSeq genes, with 11 OMIM genes and no OMIM morbid genes. Genes within the breakpoints may be involved in the regulation or development of cerebellar development. This case identifies candidate loci for inferior cerebellar vermis development and cerebellar malformation syndromes, as well as lateralising limb anomalies.

CYTOGENETICS

32. CONSTITUTIONAL BALANCED TRANSLOCATION t(8;16)(q11.2;q24.3): A BRIEF CASE STUDY

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A peripheral blood sample from a 33-year-old female (who previously had 2 miscarriages and 1 TOP) was received for chromosome karyotyping and FISH study to exclude mosaicism for an '8p chromosome marker'. An 8p chromosome marker had been reported by another laboratory following amniocentesis in a recent pregnancy with ultrasound abnormality, which was later confirmed in subsequent Products of Conception (POC). Karyotyping was performed on PHA stimulated blood, cultured for 72 hours with Thymidine/Cytidine synchronization. FISH was performed on metaphase chromosomes using the following probes: GS-580L5 for 8p sub-telomere, pZ8.4 for 8 centromere, and GS-121I04 & GS-240G10 for 16p and 16q sub-telomeres, respectively. The karyotype showed 46,XX,t(8;16)(q11.2;q24.3) indicating female with an apparently balanced translocation between chromosome 8 at band q11.2 and chromosome 16 at band q24.3. FISH results showed: ish t(8;16)(q11.2;q24.3)(GS-580L5+,pZ8.4+,GS-240G10+;GS-121I0+,GS-240G10-). The reciprocal translocation on G-banded karyotype was confirmed by FISH using sub-telomeric probes. There was no evidence of mosaicism for an '8p chromosome marker", per se. However, the derivative chromosome 8 reported in the amniotic fluid and the POC was most likely inherited from the mother as a result of 3:1 tertiary 'trisomy' nondisjunction of the translocation'. Chromosomally unbalanced gametes can arise due to chromosome malsegregation during meiosis I and therefore carriers of balanced translocations have an increased risk of infertility, miscarriages or abnormal liveborn children.

33. 46,XY/46,XX CHIMERISM IN A HEALTHY MALE

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A 31-year-old man was referred to cytogenetics for karyotype as part of a pre-IVF work-up. The couple were being treated for undiagnosed infertility problems. Chromosome analysis was performed on peripheral blood lymphocytes. A mixture of 46,XY and 46,XX cells were seen. The man had a normal male phenotype, normal testosterone levels and normal semen analysis. The only findings of note were linear and swirled streaks of pigmentation over the skin of the torso. Several possible explanations were considered to explain these observations. Further studies included FISH with sex chromosome probes on a buccal cell specimen and genotype analysis of 11 polymorphic microsatellite markers from various loci. True chimerism was shown to be the most likely explanation for the results obtained. Further studies with FISH for SRY showed that this gene was present on the Y chromosome in the XY cell line only. The karyotype was reported as: chi 46,XY[79]/46,XX[22]. Several theoretical mechanisms have been put forward to explain the generation of a chimera. These are based around lost twins or rare events in ovum formation and fertilization. Three or 4 gametes may contribute to the genotype. Most chimeras have been ascertained after investigation for clinical abnormality usually involving gonadal and sexual development. Some cases have now been fortuitously ascertained in apparently normal individuals undergoing cytogenetic testing. The incidence of true chimerism is unclear and

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the possibility of a normal out come should be considered when counseling parents of an affected foetus.

34. CHROMOSOMAL MICROARRAY TECHNOLOGY AND ITS USE IN PAEDIATRIC PRACTICE — KNOWLEDGE, CONFIDENCE AND ATTITUDES OF PEDIATRICIANS

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Chromosomal microarray (CMA) is a recently developed test that is increasingly used by pediatricians for investigating the cause of abnormalities present at birth and developmental delay in children. CMA detects small areas of missing or duplicated material across all chromosomes. The test's higher resolution compared to conventional chromosome testing has enabled increased diagnostic yield. Despite these benefits there are challenges associated with CMA testing, particularly the risk of obtaining results of unknown or uncertain significance. Given these developments, it is timely to evaluate pediatrician experience with this new technology. The purpose of this cross-sectional study is to examine knowledge, confidence level and attitudes of pediatricians with respect to the utilization of CMA testing. In this quantitative study, general pediatricians and paediatric neurologists from Sydney Children's Hospital, The Children's Hospital at Westmead and St George Hospital (estimated 100) have been invited to complete an online questionnaire. Questions have been specifically designed to elucidate clinicians' attitudes regarding indication for CMA use; interpretation of CMA results; level of knowledge of CMA technology; experience with variants of uncertain significance and incidental findings; and their resource and support needs. The results of this study may assist in identifying gaps in knowledge, clinicians' confidence levels and their support and resource needs. These will inform the development of targeted educational resources for the non-genetic professional ordering CMA tests.

35. A NEWLY DESCRIBED RECURRENT 2q21.1 MICRODELETION SYNDROME IN A FAMILY WITH SIX AFFECTED MEMBERS: EXPANSION OF PHENOTYPE

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In 2012, Dharmadhikari et al., described a 2q21.1microdeletion in six unrelated individuals as a distinct recurrent microdeletion syndrome. Clinical features included developmental delay, intellectual disability, attention deficit hyperactivity disorder, epilepsy, aggression, and other neurodevelopmental abnormalities. Gimelli et al. 2013, described another patient with a similar phenotype. The candidate genes for the phenotype in this region are GPR148, FAM123C (AMER3), ARHGEF4, FAM168B, and PLEKHB2. We describe a family with six affected individuals all having the same 0.41 Mb 2q21.1 microdeletion. Their microdeletionwas very similar to the previously reported cases. One of our patients has an additional 17p12p11.2 duplication. The behavioral/neurological phenotype in our patients was similar to previously described patients. Our patients presented with intellectual disability and developmental delay, severe receptive and expressive language disorder, muscle hypotonia and behavioral problems. In addition we found dysmorphic features such as single palmar creases, pectus excavatum, short hands and feet, and camptodactyly with variable penetrance. In this report, we describe additional phenotypic features in this microdeletion syndrome. Keywords: 2q21.1 microdeletion, intellectual disability, candidate genes.

36. PRENATAL DIAGNOSIS OF AN UNBALANCED COMPLEX CHROMOSOME REARRANGEMENT: USING OLD AND NEW TECHNIQUES

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Complex chromosome rearrangements (CCR) are defined as rearrangements involving three or more breakpoints. The most common is the three-way exchange involving the translocation of chromosome segments from three separate chromosomes. CCR are relatively rare, but most of those identified are found to be familial. Due to the possibility of malsegregation or recombination at meiosis, there is a high risk of chromosome imbalance in the conceptus, and hence a high incidence of miscarriage, infertility or fetal abnormality. Here, we report the diagnosis of an 4:2 malsegregation of a maternal t(9;11;13) in a prenatal sample using conventional cytogenetic, FISH and microarray techniques.

37. A CASE OF MOSAIC WHOLE GENOME UNIPARENTAL DISOMY WITH BECKWITH-WIEDEMANN SYNDROME (BWS), BENIGN TUMOURS AND RESPIRATORY FEATURES

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We describe a patient demonstrated to have a calcified renal mass on antenatal screening. Postnatal assessment revealed hepatomegaly, facial and limb hemihypertrophy, macroglossia, DDH and severe hyperinsulinaemic hypoglycaemia. Postnatal ultrasound confirmed the renal mass and demonstrated multiple hepatic lesions. Urinary catchecholamines, AFP and conjugated bilirubin were elevated; however, MRI demonstrated benign hepatic hemangioma and MIBG uptake was not consistent with neuroblastoma. Microarray demonstrated whole chromosome uniparental isodisomy in a 70% mosaic pattern. Due to the features in keeping with paternal imprinting of chromosome 11 (BWS), it is likely that this is paternal in origin. This likely represents chimerism with two cell lines derived from two independent fertilizations fused in a single embryo; one line a normal biparental fertilization and the other a paternal fertilization of a nullisomic oocyte with subsequent haploid rescue. There is a risk of chromosomal imprinting disorders and recessive disease traits from the imprinted genome. Subsequently, the patient developed an oxygen requirement felt to be caused by viral pneumonitis, but with no growth from nasopharngeal aspirate. Microlaryngoscopy showed severe laryngomalacia. Bronchoscopy was performed and samples sent for microscopy, virology and histopathology. Mosaic whole chromosome isodisomy has been previously described. The clinical phenotype has been previously described to include benign tumours, and features of BWS, to our knowledge there have not been previous reports of laryngomalacia or interstitial lung disease. At present it is unclear if these are the result of the chimeric isodisomy, a recessively inherited trait, or an incidental finding.

38. A FAMILY WITH CHROMOSOME 1q21.1 DUPLICATION WITH VARIABLE PHENOTYPIC EFFECT

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We present a family with Chromosome 1q21 duplication where three relatives are affected. The combined phenotype include facial

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dysmorphism, congenital cardiac disease and limb abnormalities. Microarray showed 1q21,1 (144943150-146307015) duplication. 1q 21duplication is found in general population as well as in patients referred for genetic testing. This duplication is found to be associated with CHD, especially TOF. One particular gene of interest here is GJA5 gene, which encodes for the cardiac gap junction protein Connexin 40. Another interesting gene in this area is a paralogue of HYDIN gene of Chr 16q22.2. This is exclusively expressed in brain and may explain the head size abnormalities in many reported cases. The duplication in this case is very close to but not involving the TAR region. Phenotypic features including micro/macrocephaly, developmental dalay and facial dysmorphism (deep set eyes, hypo/hypertelorism) has been described in cases with this duplication. Key words: 1q21.1 duplication, phenotype.

GENETIC COUNSELING AND GENETIC EDUCATION

39. THE EFFECTIVENESS OF EDUCATION IN A JEWISH SCHOOL GENETIC SCREENING PROGRAM

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Individuals from the Ashkenazi Jewish population are at increased risk of having a child affected by specific serious, often fatal, genetic conditions such as Tay-Sachs disease (TSD, OMIM #272800). Jewish high school community genetic screening programs were established in Sydney in 1995 for TSD. An education session is provided to all Year 11 students outlining the clinical features and genetic basis of the conditions for which screening is offered. Following informed consent, testing is currently offered for TSD and four other genetic conditions on-site a few days after education. This year, introduction of Next Generation Sequencing (NGS) will allow an increase to nine tested conditions. Mathematical modeling predicts an increase in both the frequency of detected carriers and Variants of Unknown Significance (VUS). The education session will be altered to reflect these changes. We will use validated questionnaires to evaluate the impact of introducing NGS by measuring student outcomes: knowledge, fears, and attitudes. Previous research indicates that when the number of conditions included in a program is increased, decreased knowledge and heightened anxiety can result. Our research will examine whether the education offered with NGS provides students with sufficient understanding to enable informed consent to genetic testing for nine conditions, while allaying anxiety and supporting informed reproductive choices. This research will help identify pedagogical changes of possible benefit to future screening rounds. We will also examine participants' baseline level of knowledge to determine whether the program has increased understanding about genetic conditions in the community over the past 19 years.

40. ACCESS, UPTAKE AND COMMUNICATION OF GENETIC TEST RESULTS IN AUSTRALIAN FAMILIES WITH LONG QT SYNDROME

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Familial long QT syndrome (LQTS) is the most common primary arrhythmogenic disorder. The clinical diagnosis can be difficult, and genotype plays a vital role in early disease identification. We sought to assess factors that impact on communication of genetic results, uptake and access to testing. Participants were recruited from the Australian Genetic Heart Disease (AGHD) Registry and the Genetic Heart Disease Clinic, RPA Hospital Sydney. Sociodemographic, clinical, genetic and family history data was obtained via the AGHD Registry. A survey including the Hospital Anxiety and Depression Scale (HADS), Intolerance of Uncertainty Scale (IOU) and a number of short and open-ended questions about communication was sent to participants. Fifty eight participants have so far been approached with 45 (78%) surveys returned. Of those with a genetic result, 91% self reported all first degree relatives were informed. Probands reported 'feeling obligated' (78%) and 'knowledge results could help relatives' (84%), played a large role in the decision to share. Ninety percent reported thinking a result would 'help relatives make healthcare decisions' made sharing easier. Probands with a pathogenic result were less likely to be satisfied with their understanding of LQTS (78% vs. 30%, $p = .037$). Other factors including family history of sudden cardiac death and sociodemographic characteristics showed interesting trends, but small sample size has limited the power of these analyses. Data collection is ongoing. Preliminary results suggest communication of genetic results is good in Australian LQTS families, and this research may help to inform pre and post-test genetic counseling.

41. CHALLENGES IN GENETIC COUNSELING FOR PRENATAL MICROARRAY RESULTS OF UNCERTAIN CLINICAL SIGNIFICANCE

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Genome-wide microarrays are increasingly being used in the prenatal setting due to their ability to detect a higher number of chromosomal aberrations than a standard karyotype. With the introduction of such technology it must be acknowledged that there is a greater possibility of detecting results of uncertain clinical significance. Few studies have explored the experiences of patients who have received results of uncertain clinical significance and the challenges for the services delivering such results. This poster will focus the recent literature on array technology used in the prenatal setting and will provide a case example of a woman who was given a result of uncertain clinical significance after undergoing a CVS for increased risk of Trisomy 21. By examining the counseling and logistical challenges presented in this case, suggestions can be made as to how to effectively implement this new technology into the prenatal setting.

42. HEREDITARY BREAST AND OVARIAN CANCER — A RELATIONAL INVESTIGATION INTO THE MORAL CONCERNS OF GENETIC INFORMATION DISCLOSURE

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Since the introduction of genetic testing for hereditary diseases, genetic counseling has come to be an invaluable aspect of clinical genetics. Germline mutations in the breast and ovarian cancer genes *BRCA1* and *BRCA2* account for the majority of cases of hereditary breast and ovarian cancer (HBOC). *BRCA1* and *BRCA2* germline mutations alone are attributable to 3–5% of *all* breast cancer and 5–10% of *all* ovarian cancer cases, thus genetic testing for these mutations is now a well-established practice in reducing HBOC mortality and morbidity. However, the generation of genetic information via the process of genetic testing places the patient in a

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complex landscape of moral issues. One such issue is genetic information disclosure to family. In this study we ask: How do HBOC patients navigate the complex moral issues in genetic information disclosure? ≤20 genetic counselors Australia-wide will be interviewed in-depth to gain a health service provider's perspective of the phenomenon. Using modified grounded theory methods, interview data will be coded and analyzed to present results as in-depth grounded themes. It is hypothesized that patients engage their moral concerns in a relational framework, considering their personal autonomy, moral agency and genetic responsibility primarily in relation to others. This study will join the growing body of research in genetic counseling attempting to understand the subjective patient experience in a way that encompasses their interpersonal responsibilities and concerns. This study will help improve genetic counseling practice by enabling counselors to accurately reflect the patient's reality.

43. A CASE REPORT: DECEPTION — FALSE FAMILY HISTORIES AND THE ETHICS OF CONFIDENTIALITY

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When a genetic counselor performs a cancer risk assessment, the primary tool is the reported family history. Genetic counselors recognize that family histories may be misreported and so aim to explore all practical avenues to confirm the family history. This involves obtaining consent from the relevant family members to access their medical records. Generally, errors in the family history arise through family members being mistaken or confused; only rarely do they occur through deliberate falsification. However, deliberate falsification appeared to be a significant issue in this case. As arranged by my patient, her family history of cancer (primarily breast and ovarian) was provided by her mother. However, the mother had been seen at the genetics service 16 years previously and, on comparison with these notes, irreconcilable discrepancies in the two family histories were noted — particularly in the mother's personal history of cancer. Without the mother's written consent to share information, we were left with questions as to what could be disclosed to my patient and at what point might a breach of confidentiality be warranted. The particular characteristics of this case allowed us to avoid a controversial outcome; however, it was necessary to explore the ethics of confidentiality to ensure we were properly prepared. Some of the points to be considered when contemplating a breach of confidentiality are presented in the context of this case.

44. INFORMING BEST PRACTICE IN HUNTINGTON DISEASE PRESYMPTOMATIC TESTING

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International guidelines for pre-symptomatic genetic testing for Huntington disease were developed in 1994 and recently reviewed in 2012. It was therefore considered timely to evaluate the pre-symptomatic testing process at the Hunter Genetics service, and to explore the experiences and views of clients who had undergone testing. Fourteen participants who had undergone pre-symptomatic testing from 2007–2012 were interviewed using semi-structured questions. Telephone interviews were audio recorded, transcribed, de-identified and coded using NVivo10 data management software. Inter-coder reliability >90% was achieved. Thematic analysis resulted in the identification of six main themes: (1) motivations for testing; (2) access to services; (3) information and client-centred care; (4) perspectives on the guidelines; (5) support person and (6) the afterwards. Motivations for testing were similar to previous studies, such as reducing uncertainty and family planning. Participants felt they were seen promptly by the service, that sufficient information about testing was provided, that care was client-centred and that their needs were met at the time of testing. Use of a support person varied. Participants who only involved their support person at the results-giving session described a greater emotional impact of the result on their support person. A small number of individuals reported a significant long-term psychological impact after receiving a negative test result. Recommendations for practice include the involvement of a support person(s) from the initial session, and long-term follow-up for all individuals undergoing pre-symptomatic testing, regardless of result.

45. EXPLORATION OF THE ACCEPTABILITY OF HYPOTHETICAL TREATMENT-FOCUSED GENETIC TESTING (TFGT) IN WOMEN NEWLY DIAGNOSED WITH BREAST CANCER IN MALAYSIA

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Introduction: In medium-resource and developing countries such as Malaysia, knowledge about treatment-focused genetic testing (TFGT) is still limited. Given its potential to alter first-line surgical decisions in women newly diagnosed with breast cancer, it is essential to explore the perspective of Malaysian women diagnosed with breast cancer towards TFGT. AIM: To explore the hypothetical acceptability of TFGT among Malaysian women diagnosed with breast cancer under the age of 50 years. *Methods:* Currently, 11 out of 20 Malaysian women with a diagnosis of a stage 1 or 2 breast cancer (aged <50 years) within the last 6–18 months, have been interviewed. Face-to-face semi-structured interviews were conducted using a topic guide adapted from an Australian study to be relevant to a Malaysian population (Zilliacus et al., 2012). The audio-recorded interviews will be transcribed verbatim, and data will be thematically analyzed using NVivo 10.0 software. *Results:* Themes identified will be reported including a comparison to the results of a similar Australian population study done by Zilliacus et al. (2012). Issues additional to hypothetical acceptability, to be explored include: (1) perceived advantages and disadvantages of TFGT; (2) its potential influence on surgical treatment decisions; and (3) preferences in terms of information needs, timing of the TFGT offer, and the format of information delivery. *Rationale of the Study:* The results will inform how best to offer TFGT in Malaysia, and to determine the potential utility of TFGT in routine practice in this country.

46. EXPLORATION OF THE ACCEPTABILITY OF PRENATAL TESTING FOR WOMEN FROM THE INDIAN COMMUNITY IN SYDNEY

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Advances in prenatal genetic testing are changing the face of today's prenatal care. Prenatal testing (PNT) is widely available to couples in the reproductive age group to avoid having a child with a serious intellectual or physical disability. While PNT is available to both women from the majority culture and those from culturally and linguistically diverse (CALD) backgrounds in Australia, knowledge about testing may be limited in certain CALD communities, including the Indian community. This study is the first qualitative study of

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its kind examining Australian-Indian women's attitudes and beliefs towards conventional and new prenatal tests, which are designed to identify fetuses with high probability of a serious genetic condition. Drawing on empirical research, this study seeks to explore factors that determine acceptability of PNT with a view to understanding which components reflect deeply held cultural (including religious) views and which factors represent views of the majority culture. Semi-structured interviews incorporating hypothetical scenarios will be conducted with 30 women of childbearing age recruited through the Indian Association, NSW, to explore their views. This community may be characterized by heterogeneous belief systems, which may be different from those of the majority culture. These beliefs are likely to influence uptake and understanding of prenatal testing in Indian women. On conclusion of the study, it is hoped that the findings will influence service provision and provide the basis for the development of appropriate educational resources on prenatal testing catering to the needs of Indian women in Sydney, Australia.

47. PRESCREEN COUNSELING FOR RECESSIVE ASHKENAZI JEWISH DISORDERS ACROSS 18 YEARS

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Australian community testing programs for Tay-Sachs disease carrier testing first began in Sydney in 1992. By 1995, the program had extended to provide access into Jewish Day Schools. The program currently has two routes of access: testing for 5 conditions free of charge offered to Year 11 students in Jewish Day schools, and subsidized testing for 9 conditions at an outreach centre at Wolper Jewish Hospital. Over time, the disorders tested changed, as did the prior understanding, pricing and the information given to clients. Posting of pretest information was superseded by emailing in 2003, and an extensive email with information on each of the disorders, genetic inheritance, and links for further information was introduced. In 2013 this was reduced to a shorter email with the consent form and price list attached. Testing is now routine for couples in the Jewish community. Client knowledge about each of the individual disorders is limited, and couples' testing choices tend to be based more on test cost and risk evaluation. In future, with the arrival of Next Generation Sequencing, as the number of offered conditions for testing increases further, the pretest information will still need to cover recessive mode of inheritance, result retrieval, storage of sample and data, and procedures and choices if testing positive, in a framework of informed consent, but with necessarily reduced information about each individual disorder. The health professional will need background knowledge but more of the consultation is likely to be spent discussing decision-making than specific disorders.

48. PSYCHOSOCIAL IMPACT OF FAMILY HISTORY SCREENING IN AUSTRALIAN PRIMARY CARE: A QUALITATIVE EVALUATION

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The potential harms and benefits of family history screening in general practice require investigation before routine adoption. To explore the psychosocial impact of family history screening for heart disease, diabetes and cancer in primary care. Twenty-eight semi-structured telephone interviews were conducted with patients already enrolled in a family history screening study through their general practice. Qualitative constant comparative analysis was undertaken of transcript data. Common themes included the way in which the family unit, individual stage of life and a number of external triggers interact and contribute to how an individual comes to terms with familial disease risk. Unique findings emerged relating to the Australian perspective of participants. Living in Australia created a barrier to effective communication amongst family members about family health, and family history collection. In addition to the vast geographical distance both within Australia, and between Australia and other countries, there was an additional sense of isolation described within an historical context. The family history screening questionnaire was considered user-friendly and a worthwhile approach to supporting disease prevention in primary care, although some participants did not retain an accurate understanding of their familial cancer risk. A person's response to family history screening is reliant on a complex interplay of family, personal and external factors, which in turn are driven by their stage of life. The impact of immigration and geographic isolation from family members may further complicate a person's response to undertaking family history screening.

49. ONLINE PERSONAL GENOME TESTS: FIRST QUALITATIVE DATA ON AUSTRALIAN CONSUMER'S PERSPECTIVES AND EXPERIENCES

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With access to genetic information increasingly available outside of the clinical setting, there is also a shift in the ways in which genetic information is understood and used in daily life. In the public sphere, this is consistent with an interest in genetic information about the 'self'; reflected by the growing demand for direct-to-consumer personal genome tests. Unfortunately, there is often a discrepancy between expectations of what information personal genomic tests may provide and what explanatory power they currently have — as we lack much of the scientific data required to interpret the information these tests divulge. At present, it is unclear what expectations Australians have of personal genome tests and how these expectations and needs equate with what these tests can offer. In this paper we present the first qualitative data on Australian consumers' perspectives of direct-to-consumer personal genome testing (DTCPGT). Our findings suggest the decision to undertake DTCPGT is influenced by a number of personal, familial and societal factors. Our results will be discussed in light of ongoing debates over direct-to-consumer personal genome testing and the impact this technology is having on the consumer, including how they perceive the 'risks' to their health and security. We will suggest how personal genomic information can influence understandings of genetics and the role personal genetic information could and should play in Australian life.

50. DO TELEHEALTH GENETIC COUNSELING SERVICES FOLLOW THE RECIPROCAL ENGAGEMENT MODEL OF GENETIC COUNSELING? EXPLORING ATTITUDES OF GENETIC HEALTH PROFESSIONALS IN AUSTRALASIA, CANADA AND THE USA

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The main objective of this study was to identify the tenets of the reciprocal engagement model (REM) that genetic health professionals (GHPs) perceive themselves as effectively or ineffectively using during in-person and telehealth genetic counseling. Additional aims included comparing perceived modes of practice of participants in Australasia, Canada and the United States, as well as to identify

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associations between comfort level using telehealth and use of the REM during telehealth genetic counseling sessions. A survey was created to address the aims of this study. The results of the study showed that GHPs perceived themselves as using the REM during both in-person and telehealth genetic counseling services. However, participants were found to have a significantly decreased level of agreement using the REM during telehealth sessions compared to in-person. In general, participants' responses did not vary based on location. In addition, results from this study indicated a positive association between comfort level using telehealth and level of agreement using the REM for half of the statements discussing telehealth use of the tenets. The main findings of this study show that GHPs perceive themselves as using the REM of genetic counseling during both telehealth and in-person sessions, although to a lesser degree when using telehealth. These results indicate that although GHPs perceive themselves as using a normative guide of practice during in-person and telehealth sessions, there are improvements to be made in telehealth practice.

51. EXPERIENCES OF WOMEN WHO CONCEIVED WITH PRE-IMPLANTATION GENETIC DIAGNOSIS WHEN DISCLOSING CONCEPTION TO THEIR CHILD

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Background: Preimplantation genetic diagnosis (PGD) is a technique which allows genetic testing on embryos created with in vitro fertilization (IVF). Historically, this has been used for couples with a history of genetic disease, but now a form of PGD known as preimplantation genetic screening (PGS) is often offered to other couples using IVF, as a way to detect any chromosomal imbalances in the embryo. Little is known about if or how parents who use PGD share this information with their children, or if they would benefit from additional support in disclosure. *Methods:* Previously collected questionnaires from 99 women who conceived with PGD/PGS and IVF and 199 women who conceived with IVF alone were analyzed using inductive content analysis. Then, a questionnaire was developed based on those results and 6 women who conceived with PGD/PGS and IVF and had disclosed conception participated in in-depth interviews, which were transcribed verbatim and analyzed with thematic analysis. Both samples heavily represented the opinions of women who had undergone PGS. *Results:* Among women who conceived with PGS, disclosure of IVF tends to occur early, but the use of PGS is discussed later in the child's life, or not at all. Women who conceived with PGS questioned the relevance of that information for their child. Women who underwent PGD for familial genetic conditions may have far greater support needs than women who used PGS, as the genetic information has great relevance for the child.

52. THEY JUST TOLD US . . . HOW SHOULD WE MANAGE INCIDENTAL IDENTIFICATION OF CARRIER STATUS IN CHILDREN?

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According to the Human Genetic Society of Australasia guidelines, 'Minors should only have carrier testing performed when the resulting information will be used to help with their health management in the immediate future.' However, they do not address the specific issue of how to manage the incidental identification of carriers, which may occur during testing intended to identify affected individuals such as prenatal testing and newborn screening. In this paper, I report on incidental carrier status communication from the perspectives of parents and genetic health professionals. These findings have emerged from a qualitative study examining requests for and communication of carrier results with 17 key informant genetic health professionals around Australia and 28 parents of children with one of 3 genetic conditions (cystic fibrosis, hemophilia and Duchenne muscular dystrophy). Inductive content and thematic analyses were used to analyze the data. The data showed that practices relating to disclosure of this information to parents are variable. In some cases, parents who have not asked to know the carrier status of their children are given this information. Parents reported this had occurred either through prenatal testing or when diagnostic genetic testing was performed to confirm a sibling was not affected following diagnosis of their other child. This paper will draw on the data from these interviews to discuss the appropriateness of reporting carrier results to parents who did not specifically request testing and how this is incongruent with recommendations not to grant carrier testing when parents explicitly request testing for their children.

GENOMICS, NEXT GENERATION SEQUENCING AND EXOMES

53. WHOLE EXOME SEQUENCING IDENTIFIES A NOVEL COMPOUND HETEROZYGOUS MUTATION IN *PNPT1* IN AFFECTED SIBLINGS WITH AN UNKNOWN, PRESUMED GENETIC DISORDER

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The recent advances in whole exome sequencing (WES), have led to the identification of many mutations responsible for rare Mendelian diseases. Here, we performed WES in a non-consanguineous family with two affected boys presenting with severe intellectual disability, sensorineural deafness, optic atrophy, an axonal neuropathy and chronic lung disease. WES was performed using the Illumina HiSeq2000 platform. Target regions were captured using the Agilent SureSelect Human All Exon 50Mb Kit. The analysis of WES identified rare functional compound heterozygous missense variations in exons 9 (p.Q254K) and 19 (p.A510P) of *PNPT1* (Polyribonucleotide nucleotidyltransferase 1) in both affected boys. *PNPT1* encodes for the protein PNPase, which is involved in the transport of small RNAs to mitochondria. Mutations in *PNPT1* have previously been reported to affect RNA import into mitochondria, mitochondrial protein translation, combined oxidative phosphorylation deficiency 13 (MIM 614932) and autosomal recessive deafness 70 (MIM 614934). In silico analysis predicted the identified variations to be damaging

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with both variations being located within highly conserved regions of the gene. In vitro analysis revealed a clear reduction in PNPT1 protein and mRNA expression in patient fibroblasts compared to control cells. Furthermore, patient fibroblasts showed significantly reduced protein levels of the mitochondrial respiratory chain (MRC) complexes COI & COIV, reduced COI & COIV enzyme activity, and a 33% reduction in total mitochondrial protein synthesis. Using WES, we have identified novel, probably pathogenic variations in *PNPT1* in this family. Lentiviral rescue studies are being undertaken to provide further evidence of pathogenicity.

54. REPORTING FINDINGS OF MASSIVELY PARALLEL SEQUENCING: A MODEL FOR GENETIC SCREENING OF AUTOSOMAL RECESSIVE CONDITIONS IN AT-RISK COMMUNITIES

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Community genetics screening for autosomal recessive (AR) conditions has been shown to be an effective strategy for decreasing the incidence of affected births in the at-risk Ashkenazi Jewish (AJ) community. We describe the impact on rates of carrier identification of changing laboratory techniques from conventional DNA testing for specific conditions to an expanded panel using massively parallel sequencing. We developed a simple mathematical model based on binomial probability for predicting the proportions of tested individuals who will be asymptomatic AR genetic carriers of autosomal recessive conditions. We validated the model using data from an Australian AJ community genetics program. The model correctly calculates observed rates of AR carriers for both conventional DNA testing and massively parallel sequencing. Increasing the number of tested conditions results in rapidly rising proportions of genetic carriers detected. Our model correctly explains observed rates of AR genetic carriers. The primary driver of these rates is the number of tested conditions. Future increases in the number of included conditions will increase the number of genetic carriers detected and have implications for downstream health costs and resources.

55. VALIDATION OF NEXT GEN SEQUENCING PLATFORMS FOR HYPERTROPHIC CARDIOMYOPATHY

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Hypertrophic cardiomyopathy (HCM) is a major cause of sudden unexpected cardiac death across all age groups but particularly in young athletes. Many patients are asymptomatic and diagnosis in a family often follows the sudden unexplained death of an affected family member. Genetic mutations account for a significant percentage of cardiomyopathies, with mutations in the genes encoding the thick filament components myosin heavy chain and myosin binding protein C (*MYH7* and *MYBPC3*) responsible for 75% of inherited HCM cases. Most are private mutations and molecular genetic diagnosis is important for at-risk individuals within these families. Carriers may be offered increased surveillance or offered precautionary measures such as the use of a pacemaker. Screening for mutations in South Australian patients is currently provided by overseas/interstate laboratories. The cost for these tests is considerable, although cascade screening of at risk individuals is routinely offered 'in house' to reduce ongoing costs, including both molecular screening and cardiac surveillance (echocardiogram, ECG, CMR). We have recently trialled the Agilent HaloPlex Cardiomyopathy NGS panel with the intention to offer screening for 34 genes including *MYBPC3* and *MYH7*. Initial blinded trials using the HaloPlex system have been successful, with the detection of all previously identified variants. Validation is currently being undertaken, with parallel screening for samples referred for HCM. The results of these analyses will be presented and discussed.

56. KIDS HEART RESEARCH IN WESTERN AUSTRALIA: CAUSES FOR CONGENITAL HEART DISEASE

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Significant research is proceeding into the understanding of genetic causes of CHD. With 1% of children born with CHD worldwide, CHD contributes significantly to the morbidity and mortality of newborn children. Australia experiences over 2000 new CHD diagnoses a year and it is thought that up to 20% of CHD can be contributed to known underlying genetic mutations or environmental teratogens, whilst the remaining cases are multifactorial — representing an interplay between genes and the fetal-maternal environment. Environmental risk factors emerging as significant for causing CHD are maternal diabetes, anticonvulsant medication and anti-depressant medication. Other loose associations are being drawn between CHD and obesity, maternal febrile illness, periconceptional stress, while folic acid seems to confer protection. We have commenced a project in January 2014 to capture information on children undergoing surgery or diagnostic evaluation for CHD at Princess Margaret Hospital for Children. With appropriate informed consent, DNA is being collected on children and their parents with the aim of undertaking whole genome studies to elucidate the genetic causes of CHD. Environmental risk factors in family and children are being recorded in order to better understand this contribution to the cause of CHD. We are aiming to recruit 120 children, plus their parents, per year for inclusion in the study. The study methods and preliminary results will be discussed in this presentation.

57. TESTING FOR AORTOPATHIES AND RELATED DISORDERS USING MASSIVELY PARALLEL SEQUENCING (MPS)

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The number of genes associated with aortic dilatation and dissection is expanding. The clinical phenotypes associated with some of these genes include Marfan syndrome, the Loeys-Dietz syndromes and Ehlers-Danlos syndrome of the vascular subtype. Considerable phenotypic overlap between conditions presents a challenge as to

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knowing which gene to test first. Massive parallel sequencing (MPS) or next generation sequencing (NGS) allows multiple genes to be sequenced simultaneously at a relatively low cost. Validation of a MPS strategy for an aortopathy panel of genes for diagnostic testing is essential. Seventeen genes were selected for testing, due to their known association with aortopathy/connective tissue disorders. Two different approaches were used to achieve targeted sequencing (Illumina TruSeq Custom Amplicon and Illumina TruSight Exome) for our patient cohort, some of whom had been previously tested for mutations in *FBN1*, *TGFBR1, TGFBR2*, *ACTA2, SMAD3, TGFB2* and/or *COL3A1*. Both targeting approaches were able to detect most of the known variants. Insufficient coverage was the primary cause for variants not being detected by MPS. Novel candidate disease-causing variants were also detected in genes that were not previously tested due to costs and/or time constraints. Overall, the TruSight Exome provided greater coverage compared to the TruSeq Custom Amplicon panel. Massively parallel sequencing is an important clinical tool for diseases with vastly overlapping phenotypes. Adoption of this form of testing will improve the genetic diagnosis of aortopathies and related connective tissue disorders.

58. SCREENING FAMILIAL HEMIPLEGIC MIGRAINE GENES USING NEXT GENERATION SEQUENCING

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Familial hemiplegic migraine (FHM) is a rare autosomal dominantly inherited subtype of migraine with aura with mutations in the genes CACNA1A, ATP1A2 and SCN1A shown to cause this disease. Testing for FHM using Sanger sequencing is both difficult and expensive due to the number and size of sequences to be investigated. In this study, we have utilized Next Generation Sequencing (NGS) using the Ion Torrent personal genome machine (PGM) system to screen the coding sequence, exon-intron boundaries and UTR regions of these three genes. In samples from patients previously screened by Sanger with no mutation detected in the standard exons used for sequencing, both novel and known mutations were detected through the use of NGS technology. These results indicate that the NGS approach can be applied to the diagnosis of FHM and importantly, provides an improved method for use in routine clinical molecular diagnostic testing. The newly detected mutations identified in this study might have relevance to the cause of the disease in these patients; however this needs to be tested functionally.

59. SCREEN FOR COMMON MUTATIONS IN THE HBB GENE USING A PRIMER EXTENSION ASSAY WITH DETECTION BY MALDI-TOF MASS SPECTROMETRY

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Background: The wide spectrum of hemoglobin beta (HBB) gene mutations in the Australian population reflects our ethnic mix and high rate of intermarriage. Between September 2008 and November 2012, 46 different HBB mutations were detected in our laboratory, largely in antenatal patients presenting late in the first trimester. The purpose of this study was to develop a fast and cost-effective screening strategy for screening HBB mutations in the antenatal population. *Methods:* A three amplicon multiplex PCR covering the coding and flanking intronic regions of the HBB gene was designed. Two additional allele specific primers were incorporated to amplify the HBB:g.71741_7230delinsAAGTAGA mutation common in our Indian population. Following PCR, residual dNTPs are removed by shrimp alkaline phosphatase digestion. A second primer extension PCR assay incorporates allele specific internal primers that lie immediately adjacent to the nucleotide variant. Mass modified ddNTPs (Sequenom IPlex Pro reagents) bind to the complementary base, terminating extension. WT and mutant alleles are then identified by mass following MALDI-TOF. *Results and Discussion:* The primer extension assay screens for 15 mutations that cover 80% of the common mutations seen in this laboratory. No false positives or false negative results were detected in 40 patients tested over 3 months covering all mutations in the screening panel. The assay will tolerate 30–300 ng of DNA in a 10 microlitre PCR format. Approximately 55% of the patients received by this laboratory will have one of the mutations on this panel, reducing assay costs and turn around time for patient analysis.

60. APPLICATION OF NGS TO IMPROVING DIAGNOSTIC YIELD FOR INFANTILE-ONSET EPILEPTIC ENCEPHALOPATHIES

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Epileptic encephalopathies (EEs) are characterized by severe, frequent seizures with a detrimental effect on development. The majority of EEs have a genetic basis, with significant genetic heterogeneity (>300 genes). A small subset are treatable. Rapid investigation of children with EE is mandatory to guide therapeutic choices and reproductive counseling. To date, the investigative process requires invasive neurometabolic procedures and financially prohibitive sequential genetic testing, with a low diagnostic yield (<10%). Next-generation-sequencing (NGS) provides a compelling tool to explore the genomic basis of undiagnosed EE. Recent literature has demonstrated the utility of NGS in epilepsy diagnostics, with a diagnostic yield of up to 50%. However, many studies have a paucity of phenotypic information limiting genotype-phenotype correlations and clinical prognostication. We investigated a cohort of children affected with infantile-onset EE, categorized by clinical and electrophysiological criteria. Whole exome sequencing was performed on 10 affected children using the HiSeq 2500 platform. We performed short read sequence alignment using BWA, and variant calling using the GATK HaplotypeCaller following best practices approaches. Potential causative variants were identified by a tiered approach: (1) comparison with a list of EE genes, (2) comparison of all potentially pathogenic variants with current literature (3) trio analysis to identify de novo variants. Sanger sequencing was used to confirm candidate mutations. We identified pathogenic/likely pathogenic variants in 5 out of 10 children, including a variant in a newly described EE gene. This study highlights the importance of NGS in Mendelian disorders as a rapid diagnostic technique.

61. A SUCCESSFUL APPLICATION OF NEXT GENERATION SEQUENCING TO THE DIAGNOSIS OF ADAPTIVE PRIMARY IMMUNODEFICIENCY

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Adaptive Primary immunodeficiencies (A-PIDs) include more than 100 known single gene diseases that affect lymphocyte function, and are associated with predisposition to infection. Next

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generation sequencing (NGS) technologies have demonstrated increasing utility in the diagnosis of single gene disorders including A-PID. We aimed to assess the clinical utility of the haloplex target enrichment system, in the identification of disease causing mutations in genes associated with A-PID. DNA was collected from 6 patients with A-PID caused by known mutations in genes associated with X-linked and autosomal recessive A-PIDs, and from 6 patients with an A-PID where the causative gene had not been identified. Samples were analyzed using a haloplex panel including 100 known primary immunodeficiency genes. All 6 known disease-associated variants were identified. One of the unknown patients received a new diagnosis of *RAG1* deficiency, associated with a previously reported homozygous 2 base pair deletion. No diagnosis was made in the other 5 patients. A number of variants were discounted as artefacts by parallel analysis of multiple patients. The haloplex system provided good coverage of adaptive Primary immunodeficiency genes with an excellent diagnostic yield. We recommend parallel analysis with multicalling of samples as a method to reduce systematic errors within NGS processes.

62. EXOME SEQUENCING IN MENDELIAN CLEFT PALATE FAMILIES: RESULTS OF AN INTERNATIONAL OROFACIAL CLEFTING CONSORTIUM

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Cleft lip/palate is one of the most common human malformations occurring, with an incidence of 1 in 700 live births. The majority of affected individuals occur in single member-affected families. This has made the identification of the genetic etiology of orofacial clefting challenging. We have undertaken genomic studies in larger multi-affected families who could be enriched for Mendelian forms of orofacial clefting, in order to identify causative high penetrance alleles. A minimum of two exomes have been performed in each family on the Illumina platform in 20 families with patterns of inheritance consistent with dominant, recessive and X-linked traits. The results have identified pathogenic mutations in some known genes including *ARHGAP29* in approximately 15% of the cohort. Novel genes have been identified in two families for which investigations are ongoing. In addition we discuss adjunct methodologies to reduce the number of variants requiring confirmatory Sanger sequencing, such as the programs GEMINI, homozygositymapper, dominantmapper and SNP arrays.

63. APPLICATION OF WHOLE EXOME SEQUENCING TO INVESTIGATE RETINITIS PIGMENTOSA

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Retinitis Pigmentosa (RP) describes a collection of degenerative eye disorders involving the retina, with a prevalence of approximately 1 in 3,500. There is progressive degeneration of the photoreceptors resulting in loss of night vision, bilateral visual field defects and eventually complete blindness. Although mutations in over 100 genes have been described in RP, diverse clinical features and the genotypic heterogeneity result in a low diagnostic yield. This study aimed to develop a next-generation sequencing pipeline to identify mutations in RP, using whole exome sequencing (WES) followed by analysis for variants in known RP disease genes. WES using the TruSeq exome enrichment system (Illumina Inc., San Diego, CA, USA), was applied to 12 probands diagnosed with RP, 6 each from families with autosomal recessive (AR) and autosomal dominant (AD) patterns of inheritance. Reads were aligned to the reference human genome (hg19) (BWA), and variants were identified (GATK) and annotated (ANNOVAR). Pathogenicity was predicted and detected variants were confirmed and segregation determined using Sanger sequencing. Four pathogenic variants were identified in 4 of the families with AD RP. Clearcut variants were identified in 2 of the AR families and variants in the other AR families are undergoing further segregation analysis. WES in patients with RP provides a cost-effective approach for identification of causative genetic factors. Where a variant in a known disease gene is not identified, the proband data can be used in subsequent family analyses for novel disease gene identification. WES is a useful approach for RP genetic diagnosis.

64. *ARMC5* MUTATIONS ARE COMMON IN FAMILIAL BILATERAL MACRONODULAR ADRENAL HYPERPLASIA

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Context: Bilateral macronodular adrenal hyperplasia (BMAH) is a rare form of adrenal Cushing's syndrome. Familial cases have been reported but, until recently, and at the time we conducted this study, the genetic basis of BMAH was unknown. Recently, germline variants of armadillo repeat containing 5 (*ARMC5*) in MAH, and somatic second hit mutations in tumour nodules were identified. *Objective:* To identify the genetic basis of familial BMAH. *Design:* We performed whole exome capture and sequencing of two affected individuals from each of four BMAH families (AIMAH-01, AIMAH-02, AIMAH-03 and AIMAH-05). Based on clinical evaluation there were seven, three, three and four affected individuals in these families, respectively. Sanger sequencing of *ARMC5* was performed in one other BMAH kindred, AIMAH-06. *Results:* Exome sequencing identified novel variants: Chr16:g.31477540, c.2139delT, p.(Thr715Leufs∗1)

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(AIMAH-02) and Chr16:g.31473811, c.943C>T, p.(Arg315Trp) (AIMAH-03) in *ARMC5* (GRch37/hg19), validated by Sanger sequencing. AIMAH-01 had a recently reported mutation Chr16:g.31476121, c.1777C>T, p.(Arg593Trp). Sanger sequencing of *ARMC5* identified a previously reported mutation, Chr16:g.31473688; c.799C>T, p.(Arg267∗) (AIMAH-06). The genetic basis of BMAH in AIMAH-05 was not identified. *Conclusions:* Our studies have detected *ARMC5* mutation in four of five BMAH families tested, confirming that these mutations are a frequent cause of BMAH. Two of the four families had novel mutations, indicating allelic heterogeneity. Preclinical evaluation did not predict mutation status. The *ARMC5* negative family had unusual prominent hyperaldosteronism. Further studies are needed to determine the penetrance of *ARMC5* mutation-positive relatives of affected patients, the practical utility of genetic screening and genotype-phenotype correlations.

65. AN ASSOCIATION OF MEGACYSTIS MICROCOLON WITH INTESTINAL HYPOPERISTALSIS WITH DIURESIS AND HYPERALDOSTERONISM

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We describe a patient with a diagnosis of megacystis microcolon with intestinal hypoperistalsis (MMIH) and associated polyhydramnios, hydronephrosis and hyperaldosteronism. The diagnosis of megacystis microcolon was first suggested during pregnancy. Due to polyhydramnios, three amnioreductions were performed. An MRI study to elucidate the cause revealed megacystis and hydroureter and MMIH was suggested as a possible diagnosis. Postnatal diuresis was noted, with an output in the first week of life ranging between 6–24 ml/kg/hr. There was associated hyponatraemia requiring intravenous sodium replacement; however, the potassium level stayed stable throughout. On further investigation, there was a transient reduction in the plasma cortisol and massively elevated serum aldosterone, with elevated renin. Gastrointestinal contrast studies revealed malrotation and confirmed the presence of microcolon. Abdominal ultrasound confirmed the megacystis and hydronephrosis; however, the kidneys appeared normal post decompression. MMIH is a rare but well-described condition, thought to be inherited in an autosomal recessive fashion. It has a poor prognosis; as a rule patients are TPN dependant for life unless intestinal transplantation is performed. To our knowledge, it has not been previously described in association with this degree of postnatal diuresis or hyperaldosteronism. At present it is unclear whether this is a new phenotype of the underlying genetic abnormality, a new condition with different genotype to MMIH, or two unrelated conditions presenting concurrently. We have sent blood samples for whole exome sequencing as part of a study into MMIH and hope to have results to present.

MOLECULR GENETICS AND NEUROGENETICS

66. OPTIC ATROPHY IN AN OMANI GIRL WITH A NOVEL MUTATION IN *DDR2,* A DISCOID COLLAGEN II RECEPTOR: EXPANDING THE PHENOTYPE OF SPONDYLO-META-EPIPHYSEAL DYSPLASIA WITH SHORT LIMBS AND ABNORMAL CALCIFICATIONS(SMED-SL)

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Spondylo-meta-epiphyseal dysplasia with short limbs and abnormal calcifications (SMED-SL, OMIM 271665) is a rare autosomal recessive disorder characterized by acromesomelia, distinctive facies, normal intelligence and radiological findings of short tubular bones, abnormal epiphyses and metaphyses, platyspondyly and premature progressive calcifications. We report a 7-year-old Omani girl with clinical and radiological features of SMED-SL and progressive visual impairment due to bilateral optic atrophy. Her 10-year-old affected brother lacks the ocular phenotype confirming the intra-familial variability of this disorder. Diagnosis was confirmed by identifying a novel homozygous truncating mutation in exon 18 of the DDR2 gene; (MIM 191311).The DDR2 encodes the discoidin domain containing receptors, a subfamily of tyrosine kinase receptors, which recognize collagen II as their ligands. Optic atrophy has previously been noted in a SMED-SL patient from the United Arab Emirates, but was reported to be consequent to glaucoma. Our case had rod-cone dystrophy, and we postulate that the optic atrophy is due to a primary degeneration consequent to the effect of DDR2 mutation on collagen integrity. Primary optic atrophy and retinal dystrophy with SMED-SL has not been previously reported. The (c.CT2468Del) mutation in our patient resulted in a frame-shift leading to premature termination of translation and a predicted amino acid change at position S823 (p.S823Cfs∗2). Functional studies of the mutant protein confirmed its localization in the endoplasmic reticulum and a deficiency in collagen-induced receptor activation, suggesting that protein trafficking defects is the major cellular mechanism of the loss of DDR2 functions. We recommend ophthalmology screening in patients with SMED-SL.

67. PROTEIN C GENE VARIANTS DETECTED IN A COHORT OF NEW ZEALAND PATIENTS WITH PROTEIN C DEFICIENCY

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Protein C is a key part of the coagulation cascade where its active form inhibits coagulation by proteolytic cleavage of activated factors V and VIII. Protein C deficiency in mild cases is associated with an increased risk of thrombosis and in severe cases is associated with neonatal disseminated intravascular coagulation. While protein C deficiency can be caused by variants in the *PROC* gene, it can also be an acquired deficiency, and determination of the specific cause is valuable for correct diagnosis, ongoing treatment and family studies. The Molecular Haematology lab at LabPLUS, Auckland Hospital receives requests for the genetic analysis of protein C deficient patients from across the North Island of New Zealand. Between 2009 and 2014 we detected 10 different *PROC* variants in 22 patients with protein C deficiency. Of these variants, 6 were previously unreported, including two families with protein C deficiency segregating with the detected *PROC* variant across two generations. One protein C variant (p.Ser292Arg) that has not been previously reported was detected in nine patients, with seven of these being apparently unrelated. The high number of occurrences of this previously unknown variant raises the possibility of a founder effect in the local Maori population. To improve the awareness of the local *PROC* variant environment in New Zealand we provide here a summary of the PROC variants detected and their associated protein C phenotypes.

68. LARGE *CYP1B1* GENE REARRANGEMENTS ARE NOT RESPONSIBLE FOR PRIMARY CONGENITAL GLAUCOMA IN A SA PATHOLOGY TESTED ANZRAG PATIENT COHORT

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Primary Congenital Glaucoma (PCG) is a rare developmental disorder of the eye typically diagnosed within the first few years of life. If left uncontrolled, PCG can result in irreversible vision loss. The incidence in Australia is estimated to be 1 per 30,000 births, with evidence that ~20% of cases demonstrate an autosomal recessive mode of inheritance attributed to pathogenic variants within the *CYP1B1* gene. Our laboratory at SA Pathology is the only accredited service within Australia to offer inherited Glaucoma testing specific to PCG. To date, our molecular genetics assay of the *CYP1B1* gene has been limited to bidirectional genomic DNA sequencing of both coding exons and the respective intron exon boundaries. This choice of methodology is consistent with the literature that small sequence changes are primarily responsible for the reported cases of familial PCG. A recent study however has found that large intragenic deletions involving *CYP1B1* may also contribute to the PCG phenotype. To investigate this further, samples from 50 PCG affected patients recruited through the Australian and New Zealand Registry of Advanced Glaucoma (ANZRAG) and in which sequencing results did not fully explain their phenotype, were subjected to further study using Multiplex Ligation-dependent PCR Amplification (MLPA). Our results demonstrate that genomic deletions do not appear to be a common event involving the *CYP1B1* gene of ANZRAG recruited PCG patients presenting to our laboratory for testing.

69. THE *KCNT1* PHENOTYPIC SPECTRUM INCLUDES ADNFLE, MULTIFOCAL EPILEPSY AND SUDDEN UNEXPLAINED DEATH IN EPILEPSY

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Recently, we employed exome sequencing to identify a new gene, *KCNT1*, in epilepsy and its comorbidities. Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) and malignant migrating focal seizures of infancy (MMFSI) are phenotypically distinct disorders both caused by mutations in *KCNT1*. This suggests that a larger spectrum of disorders associated with mutations in this gene, which may include focal epilepsies with psychiatric disorders, exists. To investigate the role of *KCNT1* in the focal epilepsies, patients with ADNFLE, focal epilepsies, focal epilepsy with psychiatric co-morbidities and MMFSI were analyzed for mutations in *KCNT1* by high-resolution melting-curve analysis or by targeted next-generation sequencing. Heterozygous missense mutations were identified in two families and six sporadic MMFSI patients. One family had focal epilepsy, multifocal epilepsy with learning difficulties and MMFSI. The mutation in this family was previously reported in a family with ADNFLE. The second family had ADNFLE, learning difficulties and psychiatric disorders. One member of this family had cardiac arrhythmia and another had sudden unexpected death in epilepsy (SUDEP). Six of the eight *KCNT1* mutation-positive patients and families had mutations that have been previously described. We have extended the phenotypic range associated with *KCNT1* mutations to include patients with cardiac arrhythmia and SUDEP. We have identified the same mutation in patients with ADNFLE and MMFSI, rendering genotype-phenotype correlations with *KCNT1* difficult. We have found recurrent mutations, suggesting that mutational 'hot-spots' occur in *KCNT1*.

70. ESTABLISHING A HUMAN INDUCED PLURIPOTENT STEM CELL MODEL OF PCDH19-FEMALE LIMITED EPILEPSY

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PCDH19-Female Limited Epilepsy (PCDH19-FLE) is an early onset epilepsy that affects almost always females and is associated with a spectrum of developmental, behavioral and intellectual problems. It is caused by mutations in the X-linked gene, protocadherin 19, with over a 100 different, likely loss-of-function mutations published. Very little is currently known about the function of PCDH19 protein and the mechanisms leading to PCDH19-FLE. Due to the unusual inheritance pattern for this X-linked disorder we and others postulated that a mosaic population of PCDH19-expressing and PCDH19-null cells in the patient brains is driving the molecular pathogenesis. We decided to address the postulated cellular mechanism of 'cellular interference' by establishing a clinically relevant disease model that employed induced pluripotent stem cell (iPSC) technologies. We extracted skin fibroblasts from three (two affected females and a carrier male) patients with three different PCDH19 mutations and proceeded to reprogram these into the pluripotent state. Interestingly we were only ever able to isolate wild-type PCDH19 expressing iPSCs from affected females, suggesting selection in favour of cells with wild-type PCDH19 or the presence of a dominant X-chromosome. We were, however, able to generate iPSCs from a male with a PCDH19 mutation, which were subsequently differentiated into cortical neurons, predicted to be one of the clinically relevant cell types. To investigate the mechanism of 'cellular interference' of PCDH19-FLE we are currently mixing PCDH19-FLE and control male-derived iPSCs and differentiating these into cortical neurons to replicate the predicted cellular mosaicism of the affected, female or male mosaic patient brain.

71. A SINGLE NUCLEOTIDE SUBSTITUTION IN EXON 64 OF THE DYSTROPHIN GENE DETECTED BY MULTIPLEX LIGATION-DEPENDANT PROBE AMPLIFICATION

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Duchenne Muscular Dystrophy (DMD) is a neuromuscular disease characterized by progressive muscular weakness and degeneration of skeletal muscle. DMD is an X-linked recessive disease caused by mutations within the *DMD* gene, encoding dystrophin. About 60% of these cases are due to single or multiple exon deletions, while 10–15% are the result of single or multiple exon duplications. The remaining affected individuals have point mutations or small deletions/insertions in the *DMD* gene. Multiplex ligation-dependant probe amplification (MLPA) is the most commonly used diagnostic technique to confirm a clinical diagnosis of DMD as it can detect whole exon duplications or deletions within the *DMD* gene which account for nearly three quarters of all mutations. In this study we describe how MLPA lead to a DMD diagnosis for a $2\frac{1}{2}$-year-old boy who did not present with a deletion or duplication. An ambiguous amplification product at exon 64 instigated further analysis. Sequence analysis detected a single nucleotide substitution c.9337C>T. This base substitution is directly adjacent to the ligation site for the exon 64 MLPA probe. This base substitution, c.9337C>T is predicted to result in a nonsense (stop) codon (p.R3113X) leading

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to a premature termination and a truncated dystrophin protein. This same mutation has 14 public entries on the Leiden muscular dystrophy page (http://www.dmd.nl). This result highlights the importance of investigating ambiguous amplification results as well as apparent single exon deletions detected by MLPA.

72. Xq25 COPY NUMBER VARIANTS ARE ASSOCIATED WITH INTELLECTUAL DISABILITY: WHICH GENE/S ARE RESPONSIBLE?

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Intellectual disability (ID) is a common, clinically complex, causally heterogeneous and largely untreatable disorder that affects more than 2% of the world's population. ID is characterized by substantial limitations in intellectual function, memory and adaptive behavior that is diagnosed before the age of 18 years. We have identified five duplications and a triplication encompassing a part of Xq25 that segregate with disease and obligate carrier status in six families with a history of ID. Three genes — *THOC2*, *XIAP* and *STAG2* — are located within the boundaries of the largest duplications. While some mutations in *THOC2* gene cause ID, in this case it is probably not causative as *THOC2* is not duplicated in 3/6 families and THOC2 protein levels are not increased in patient-derived lymphoblastoid cell lines (LCLs) harbouring the *THOC2* gene duplication. XIAP and STAG2 protein levels are increased in the patient LCLs carrying the *XIAP* and *STAG2* gene duplications. XIAP performs diverse biological functions, particularly through its E3 ubiquitin ligase activity and STAG2 is subunit of a cohesin complex that regulates cohesion of sister chromatids and gene transcription. Current clinical, genetic and molecular data suggest that dosage of XIAP, STAG2 or both is associated with ID in our families. Functional studies are underway to firmly establish dosage of which gene is responsible for causing ID.

73. 'OUT-OF-PACE'-INTERNEURON MIGRATION IS DISRUPTED BY THE TWO MOST COMMON *ARX* POLYALANINE EXPANSION MUTATIONS

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Perturbation of subpopulations of inhibitory interneurons results in cognitive impairments such as intellectual disability (ID) and epileptic seizures. The *Aristaless*-related homeobox (*ARX*) is a frequently mutated X-linked ID gene encoding a transcription factor indispensable for interneuron development. Here we report our investigations into the role of *ARX* on the development of interneuron subpopulations using mouse models for the two most common *ARX* polyalanine expansion mutations, $Arx^{(GCG)7}$ and $Arx^{432\text{-}455dup24}$. In agreement with the phenotypes seen in human patients, the $Arx^{(GCG)7}$ mice display epileptic seizures in addition to learning impairment, while epileptic seizures are not observed in the $Arx^{432\text{-}455dup24}$ mice. Using ex-vivo neural stem cells from both mouse models we are modeling interneuron migration *in-vitro,* and our analyses demonstrates retarded migration in interneurons derived from both $Arx^{(GCG)7}$ and $Arx^{432\text{-}455dup24}$ mice. Interestingly, migration of the neurons from the $Arx^{(GCG)7}$ mice were significantly more delayed than the neurons from the milder mutation. To correspond this in-vitro data in our in-vivo mouse models, we are using neuron birth-dating and interneuron marker analysis to capture the migration and differentiation of affected interneuron subpopulations within the developing brains of both mutant mice. These approaches will allow us to identify specific subpopulations negatively impacted by expanded polyalanine tract mutations in *ARX*. Hence, this spatial and temporal map of affected interneuron development in conjunction with ongoing transcriptome analysis in our laboratory will ascertain the *Arx* target genes that are disregulated and contribute to the pathogenesis of intellectual disability and epilepsy in humans.

74. CASTING THE NET TRANSCRIPTOME WIDE: HOW DO EXPANDED POLYALANINE TRACT MUTATIONS IN ARX CONTRIBUTE TO INTELLECTUAL DISABILITY AND SEIZURES?

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Aristaless related homeobox *(ARX)* is a paired-type homeodomain transcription factor with critical roles in embryonic development. Mutations in *ARX* give rise to intellectual disability (ID), epilepsy and brain malformation syndromes. Over half of all mutations in *ARX* lead to expansion of the first two polyalanine tracts. We have demonstrated a marked reduction in protein expression of mutant Arx, in the developing forebrain of mice modelling the two most frequent polyalanine expansions seen in human patients (Lee et al., 2014). Even though both mutant mouse strains display similar Arx protein reduction, each displays distinct phenotypes. In agreement with the phenotypes seen in human patients, the $Arx^{(GCG)7}$ mice display epileptic seizures in addition to ID while seizure are not observed in the $Arx^{432\text{-}455dup24}$ mice. To investigate the mechanisms underpinning these phenotypic differences we undertook a transcriptome wide approach using RNA-seq to analyze gene expression in the developing forebrain of 12.5dpc $Arx^{(GCG)7}$ and $Arx^{432\text{-}455dup24}$ mice. We have compared analysis of the data using a range of currently available pipelines, including Cuff-Diff, Partek and Edge-R approaches. Our analysis has identified genes that are differentially expressed in each mutant genotype when compared to WT and also differentially expressed genes between the polyalanine tract mutations. We conclude that genes commonly deregulated in both mutant genotypes will contribute to the consistent intellectual disability features and genes that are disrupted preferentially in $Arx^{(GCG)7}$ mice are likely to contribute to the more severe phenotype of infantile epileptic seizures.

75. RAPID IDENTIFICATION OF A NOVEL COMPLEX I *MT-ND3* M.10135C>A MUTATION IN A LEIGH SYNDROME PATIENT

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A patient with a clinical phenotype consistent with Leigh syndrome (LS) required definitive genetic diagnosis for inclusion in an ongoing clinical trial of EPI-743, a drug that shows promise for LS patients. High-density SNP arrays, exome sequencing and targeted mitochondrial sequencing (TMS) on DNA from blood samples from the patient, parents and three unaffected siblings was performed to identify the genetic cause of LS. TMS and qPCR was done on DNA from different tissue samples from the patient to detect the mutant load of the identified variant. Spectrophotometric respiratory chain assays were done on muscle and liver biopsies of the patient to assess the functional consequences of the identified variant. SNP array data revealed no potentially pathogenic variants. Exome sequencing data followed by TMS investigation led to the identification of a novel m.10135C>A mutation causing a p.Gln26Lys change in *MT-ND3* (Mitochondrially Encoded NADH Dehydrogenase 3), a gene previously associated with LS. The father and three unaffected siblings had only the wild-type allele, whereas the proband and mother carried the m.10135C>A allele at levels of ∼99.9% and 1% respectively. TMS and qPCR analysis showed homoplasmy for the mutation in all patient tissues tested. Spectrophotometric enzyme assays showed a more pronounced complex I defect in patient muscle compared with liver. Using NGS techniques, an accurate and rapid diagnosis was achieved for a LS patient. TMS and qPCR approaches are an efficient strategy to quantitate mutant loads of pathogenic mtDNA mutations, with clinical utility for genetic counseling and prognostication.

76. SHORTFALLS IN LONG QT SYNDROME (LQTS) ANALYSIS

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Inherited Long QT syndrome (LQTS) is characterized by prolonged QT interval on electrocardiogram (ECG), which predisposes to cardiac arrhythmias that can result in syncopal episodes and risk of sudden death. It is both a clinically and genetically heterogeneous disorder, with mutations in five genes (*KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1* and *KCNE2*) found to be frequently associated. Inheritance most commonly follows an autosomal dominant pattern. An individual with a clinical diagnosis of LQTS and an identified mutation in the *SCN5A* gene was referred, along with additional family members, for confirmation and subsequent cascade testing for the *SCN5A* mutation. The family history includes multiple cases of sudden nocturnal death. The *SCN5A* mutation identified (c.1109C>T) has been previously reported as pathogenic. However, cascade testing revealed that the *SCN5A* mutation does not segregate with the disease in this family, with two affected individuals carrying only the wild-type allele at this position. DNA from the proband was subsequently sent to an overseas laboratory to be analyzed on a Next Generation DNA sequencing panel of genes associated with hereditary cardiac arrhythmias. Three additional variants of uncertain significance were identified, in the *RYR1*, *CACNA1C* and *GYG1* genes. To date, cascade analysis for the *CACNA1C* variant has been performed but this variant also does not segregate with the disease. The variants in the *RYR1* and *GYG1* genes are most likely non-pathogenic and were not investigated further. This analysis highlights the complexity in determining pathogenicity of a mutation, and emphasises the importance of segregation analysis.

77. MYOTONIC DYSTROPHY — 'THE CASES OF THE FLOATING ALLELE AND THE THREE BANDS'

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Myotonic dystrophy type 1(DM1) is an autosomal dominant multisystem disorder that affects the skeletal and smooth muscle, eye, heart, endocrine system, and central nervous system. DM1 is caused by expansion of a CTG repeat in the non-coding region of the *DMPK* gene. Individuals with repeat length of 5–49 do not have symptoms however, children of individuals with repeat length of 35–49 (the permutation range) are at risk of inheriting a larger repeat size and therefore having symptoms (Martorell et al., 2001); affected individuals have CTG repeat length of >50 (GeneReviews). Generally longer repeat lengths correlates with early age of onset and more severe phenotype. Penetrance is high and anticipation is typically seen in maternal transmission however, anticipation in paternal transmission does occur. We present two DM1 cases with unexpected results. Case 1: a 30yo symptomatic male with a family history. Results from PCR and Southern blot analyses did not corroborate; a 'floating' allele was observed which was normal on PCR, but expanded on SB. Which assay was correct? Case 2: a 7-year-old symptomatic male with a family. PCR analysis showed the presence of a normal allele however, southern analysis showed the presence of three 'bands'. Was this a mosaic result due to sample error or was this patient truly mosaic for DM1.

78. RE-INITIATION OF mRNA TRANSLATION AFTER PREMATURE N-TERMINAL PROTEIN TERMINATION MUTATION (c.34G>C/p.E12X) IN *ARX* OF A PATIENT WITH X-LINKED INFANTILE SPASMS

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Mutations in the *Aristaless*-related homeobox gene (ARX), a transcription factor critical in brain development, leads to a range of X-linked intellectual disability phenotypes. We report a boy who at 6 months of age diagnosed with infantile spasms and hypsarrhythmia without brain malformation by MRI. Sequencing of the *ARX* gene identified a novel c.34G>T mutation predicted to lead to protein termination at p.E12X. This phenotype was surprising as a complete loss of ARX function invariably leads to a catastrophic phenotype including malformation of the brain and genitals. A similar c.81C>G mutation identified in two male cousins with early onset infantile spasms predicted to truncate the protein at p.Y27X was shown to lead to re-initiation of ARX mRNA translation resulting in an N-terminal truncated protein (Fullston et al., 2010). Here we show that the novel c.34G>T/ p.E12X mutation also re-initiates mRNA translation at the next AUG codon (c.121-123/ p.M41), producing the same N-terminally truncated protein as the p.Y27X mutation. Interestingly, our in vitro cell assays indicate production of these truncated proteins is at markedly reduced levels. We are investigating if the pathogenesis is due to the reduction in ARX protein expression or to the altered transcriptional activity of N-terminal truncated ARX missing the octapeptide domain known for its co-repression activity. Our study has shown that premature termination mutations very early in *ARX* do not trigger

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nonsense-mediated decay with subsequent loss of ARX protein, but instead reinitiate translation to produce reduced levels of expression of an N-terminally truncated protein.

79. IDENTIFYING FUNCTIONAL MITOCHONDRIAL GENOME VARIANTS ASSOCIATED WITH FAMILIAL MIGRAINE SUSCEPTIBILITY

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Migraine is a neurological disorder and affects approximately 12% of the population, with 3 times more females than males affected, suggesting that X linked and/or mitochondrial inheritance may be involved. Mitochondrial dysfunction in relation to migraine has only been previously researched in a limited number of small studies and remains a largely unpursued area. Genetically isolated populations with very large pedigree structure, such as the Norfolk Island population, are a valuable resource for discovering functionally important variants that contribute towards complex disease susceptibility. The reduction in phenotypic and genetic diversity as a result of geographic isolation reduces the heterogeneity of complex disorders and increases the likelihood of identifying true susceptibility variants. We have generated complete and accurate mitochondrial genome sequences for 315 individuals from the Norfolk Island Core pedigree using a customized cost effective next generation sequencing approach. Preliminary analysis in a subset of 48 individuals showed that the mt sequence variation in the NI individuals differed from the Cambridge Reference Sequence at 296 positions. Of these variant sites, 29 variants were common in the 48 NI individuals (>5%). Many of these common variants are the defining markers of mitochondrial haplogroup B, to which Polynesians belong. Importantly, our preliminary analysis has identified 6 novel mtDNA variants in the NI sample with 5 out of 6 of the novel variants found in individuals who are migraine sufferers. We are currently analysing the remaining mitochondrial data to determine whether those variants play a role in migraine in the extended Norfolk Pedigree.

PRENATAL DIAGNOSIS

80. DETERMINING THE SENSITIVITY OF DIAGNOSTIC METHODS TO MATERNAL CELL CONTAMINATION (MCC) IN PRENATAL DIAGNOSIS (PND)

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Background: MCC is a potential risk factor for misdiagnosis in PND. The purpose of this study was to quantify the percentage of MCC that can be present in a fetal sample without compromising the fetal genotyping result in each of the different assays used for PND in this laboratory. *Method:* Maternal DNA carrying X-linked and autosomal recessive disease was mixed with unaffected fetal DNA (prepared from dissected chorionic villi) in known proportions. Diagnostic methods examined included primer-extension assays with detection by MALDI-TOF mass spectrometry, gap-PCR, MLPA, long range PCR and Sanger sequencing. STR analysis (AmpFlSTR Identifiler kit, Life Technologies) is routinely used in our laboratory to assess MCC and was run in parallel with each assay to assess the theoretical versus the detectable MCC. *Results and Discussion:* An unacceptable level of MCC was defined as the percentage of MCC that results in an equivocal or incorrect genotype in the fetus. This percentage of detectable MCC was assay dependent, ranging from 1.0% with the gap-PCR, 10–15% for long range PCR, 15–20% for Sanger Sequencing and 50% for MLPA. A correct genotyping result was obtained with the primer extension assay up to 20% MCC. STR analysis had a limit of detection of 1.0%, providing a useful tool for quantifying MCC. Results of this study have provided a basis for interpreting a PND result when MCC is present and advising requesting clinicians when repeat invasive procedure is warranted.

81. CONSUMERS' EXPERIENCES OF AND ATTITUDES TOWARDS NON-INVASIVE PRENATAL TESTING IN AUSTRALIA: THE GOOD, THE BAD AND THE UGLY

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Introduction: Non-invasive prenatal testing (NIPT) is a newly available technology for the detection of common fetal aneuploidies. The procedure takes advantage of the presence of cell-free DNA in the blood plasma of women during pregnancy and promises to be a powerful clinical tool. However, as with the implementation of other prenatal testing methods, careful consideration regarding delivery is needed to ensure the integrity of informed consent is maintained, pre- and post-test counseling provided and to guide health policy development. The purpose of this qualitative study is to investigate the consumer experience of and attitudes towards NIPT for common fetal aneuploidies in Australia. *Methods:* Using a descriptive survey design, women who have accessed this technology will be recruited 6 months postpartum via their obstetrician who ordered the test and data obtained using semi-structured telephone interviews. The interview schedule has been designed to explore a range of issues including informed consent; reasons for use; participants' understanding of the testing capabilities and limitations; participants' views towards the need for invasive confirmatory testing following a positive result; degree of pre- and post-test counseling; how results were delivered; and whether participants opted for sex chromosome results and their reasons for requesting this information. *Results:* Results from this study will be reported including any gaps in information provision or decision-making support that could undermine informed consent. This information will be important for the development of counseling and education strategies for healthcare providers, before making NIPT available to the general Australian obstetric population.

82. PRENATAL DIAGNOSIS FOR INCONTINENTIA PIGMENTI

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Familial incontinentia pigmenti (IP) is an X-linked dominant disorder, generally prenatally lethal in affected males. Carrier females have highly variable abnormalities of the skin, hair, nails, teeth, eyes, and central nervous system. Cells expressing the mutated X chromosome are eliminated selectively around the time of birth, so females with IP exhibit extremely skewed X-inactivation. In carrier females, the prominent skin signs occur in 4 classic cutaneous stages: (1) blistering (birth to ~4 months); (2) a wart-like rash (for several months); (3) swirling macular hyperpigmentation (~6 months into adulthood); and (4) linear hypopigmentation. Alopecia, hypodontia, abnormal tooth shape, and dystrophic nails are observed. Neurologic findings including cognitive delays/intellectual disability are occasionally seen. Incontinentia pigmenti is caused by mutations in *IKBKG* (previously *NEMO)*. A recurrent deletion removing exons

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4 through 10 of *IKBKG* is detected in about 70-80% of affected individuals. Sequence analysis of the gene may be undertaken in clinically distinct cases, not carrying the common deletion. The presence of an *IKBKG* pseudogene, however, may complicate this analysis. PND of deletion cases has provided some complications of its own, particularly when the mother is a carrier of the deletion. Molecular results from chorionic villus sampling (CVS) have on occasion resulted in low level detection of the deletion product, resulting in an inconclusive result. Several PND cases have required a request for follow up amniocentisis. We present data from these and other cases and summarise our results. Studies highlight the need for good counseling and clinical guidance for deletion carrier females.

83. WOMEN IN WESTERN AUSTRALIA; PILOT STUDY OF WOMEN'S UNDERSTANDING OF PRENATAL TESTING OPTIONS, INCLUDING THE OPTION OF NON-INVASIVE PRENATAL TESTING (NIPT), IN A TERTIARY HOSPITAL SETTING

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The prenatal diagnostic options available through the public health system are chorionic villus sampling (CVS) and amniocentesis, both of which carry a small risk of pregnancy loss. Non-invasive prenatal testing using cell free fetal DNA (cffDNA) in maternal blood (NIPT) has been eagerly anticipated by both consumers and health care providers and from November 2012 has been offered in Western Australia by private health care practitioners. With the widespread uptake of NIPT and expanded panel of conditions tested for, such as Prader-Willi/Angelman syndrome, Cru-di-chat, 22q11 deletion and 1p36 deletions, the question arises of how far away population screening for single gene disorders is. A proof of concept study at King Edward Memorial Hospital has already commenced to investigate the feasibility of single gene disorder testing through cff DNA in maternal blood. However, prenatal testing in the presence of a family history of a condition and genetic counseling pre-test is an extremely different situation to population screening in a prenatal testing with no family history. The pilot study presented here aims to give preliminary baseline knowledge of what women in Western Australia understood about their prenatal testing options when NIPT is offered, prior to expanded screening becoming available.

84. REFERRING PATIENTS FOR PRE-IMPLANTATION GENETIC DIAGNOSIS: A SURVEY OF CLINICIANS

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Pre-implantation genetic diagnosis (PGD) is an assisted reproductive technique in which embryos are tested for specific genetic abnormalities to enable the selection of embryos unaffected by the condition tested for implantation and pregnancy. This provides an alternative to prenatal diagnosis and potential pregnancy termination for couples at risk of transmitting a genetic disorder to their children. Recent data indicates that women who had not been informed about PGD felt disempowered about not having been given this option. This study aimed to explore the knowledge and attitudes of obstetricians towards PGD and to identify potential barriers to referral. An online questionnaire was emailed to registered members of The Royal Australian and New Zealand College of Obstetricians and Gynaecologists (RANZCOG). This assessed participants' knowledge of PGD and perceived barriers to referral. A total of 372 practicing obstetricians were surveyed. Obstetricans' perceptions of their patients' financial status (40%) and ability to access PGD services (40%) were identified as the main patient barriers to referral. The study also showed variability in obstetrician referral for PGD and/or to genetic services. Variability was also observed regarding perceived appropriateness of PGD for different indications. Obstetricians who had professional development related to PGD were more likely to discuss the option of PGD than those who had not ($c^2 = 6.44$; $p \leq .01$). These results highlight the need for training opportunities, educational resources and recommendations to guide health professionals and ensure that couples eligible for PGD are informed and appropriate referral pathways are in place.

POPULATION SCREENING INCLUDING NEWBORN SCREENING

85. TAY SACHS DISEASE AND RELATED CONDITIONS SCHOOL SCREENING IN MELBOURNE — A 10-YEAR REVIEW

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A number of autosomal recessive conditions have a higher carrier frequency in the Ashkenazi Jewish population. Carrier screening for Tay Sachs disease has been offered in Jewish high schools in Melbourne since 1997 after similar programs were introduced internationally over 30 years ago. Over the course of the program in Melbourne, advances in genetic technologies have allowed for this testing to transition from a blood test to a cheekbrush testing which has resulted in a higher uptake of testing. In 2008 a further six conditions were added to the standard Ashkenazi Jewish genetic testing panel: cystic fibrosis, familial dysautonomia, Canavan disease, Fanconi anemia, Bloom syndrome and Niemann-Pick disease type A. Over 10 years from 2003 to 2013, 3542 students were tested through the program and 268 (7.8%) were identified as carriers of at least one of the conditions. The carrier frequencies varied from 1 in 32 for Tay Sachs disease to 1 in 161 for fanconi anemia. This program has grown from a single disease to a multi-disease screening program. Uptake has increased from 70% to 95% as a result of introduction of cheekbrush testing. It will be important to monitor the uptake of testing by partners of carriers as the cohort have children.

86. *CFTR* MUTATION SCREENING IN A PRIVATE FERTILITY CLINIC - THE NEXT STEP?

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Cystic Fibrosis carrier screening in a private fertility clinic currently has two main reasons for driving screening patients for CFTR mutations; they are increased risk of carrier couples and CFTR mutations causing infertility. We see at-risk carrier couples every 1 in 368 couples on average, based on the current infertile male (1 in 16) and infertile female (1 in 23) carrier rates. We recently had the opportunity to screen 250 consecutive patients on the Illumina MiSeqDx Cystic Fibrosis 139-Variant Assay and also on the MiSeqDx Cystic Fibrosis Clinical Sequencing Assay. The 139-variant panel is made up of clinically relevant and functionally verified variants as defined in the CFTR2 database and would provide an increased coverage to those patients at higher risk of carrying CFTR mutations (i.e. infertile patients). The clinical sequencing assay covers all protein coding regions and intron/exon boundaries of the *CFTR* gene. We

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present the results from the trial and show the increased number of CFTR mutations picked up in the 250 patients DNA samples tested, concordance with the original results and the results of the sequencing assay and what that could mean for future screening in a private fertility clinic.

87. A NEW SCREENING PROTOCOL FOR CONGENITAL HYPOTHYROIDISM EVALUATED

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Congenital hypothyroidism (CH) is a component of all newborn metabolic screening programs. The thyroid stimulating hormone (TSH) cut-off used varies widely, with little evidence base. To evaluate the TSH cut-off used for the NZ newborn screening program. The cutoffs used were 50 mIU/L blood for paediatric endocrine referral and 15-49 mIU/L blood for repeat dried blood spot sampling. Following extensive modeling, paediatric consultation and approval of the MOH technical group these were changed to 30 mIU/L blood for referral and 8 mIU/L blood for babies older than two weeks. The level for repeat sampling was unchanged. We report our experience of the first 12 months. In 2013, 59196 babies were screened. There were 57 positive screening tests, 20 were true cases of CH (3 athyreosis, 4 dyshormonogenesis, 5 ectopic gland, 8 etiology unknown but TSH >100). Four cases were indeterminate, and may represent cases of either CH or transient hypothyroidism. There were 33 false positive tests (repeat $\leq$8 mU/L blood). Three more infants were referred to pediatricians. 1 was a false positive with initial TSH of 31 mU/L blood, follow-up normal. Two were true cases of CH, with second sample TSH of 14 and 12 mU/L blood diagnosed with dyshormonogenesis and an ectopic gland respectively. In 2013, the change in TSH cut-off led to the detection of 2 cases of CH that would previously have been missed and one false positive. The increase in clinical workload and anxiety for families of healthy infants was minimal.

88. CLINICAL DIAGNOSIS OF CONGENITAL ADRENAL HYPERPLASIA IS UNRELIABLE IN BOTH GENDERS - NEW ZEALAND SCREENING DATA FROM 1994–2012

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New Zealand (NZ) was one of the first countries to include congenital adrenal hyperplasia (CAH) in its newborn screening program. Severe CAH is a rapidly evolving, life-threatening disorder that becomes apparent within the first month of life. To evaluate the efficacy of the NZ screening program, infants with CAH born from 1994 to 2012 were identified from screening records. Case characteristics were reviewed. Over this period, 41 infants (25 female, 16 male) were diagnosed with CAH (1: 26,316). Almost 50% ($n = 20$) cases were identified only by screening, including 20% of females. All clinically detected cases ($n = 21$) had abnormal virilization and 25% an affected sibling. Overall, whole blood 17-hydroxyprogesterone sampling occurred at a median of 2 (range 0–8) days, screen notification at 7 (0–19) days, and treatment commenced at 6 (1–30) days. Infants diagnosed clinically (compared to screening alone) were predominantly female (95% vs. 25%; $p < .0001$), were diagnosed earlier (5.2 vs. 8.8 days; $p < .001$), had higher serum Na (136 vs. 131 mmol/L; $p < .001$) and lower K concentrations (5.4 vs. 6.0 mmol/L; $p < .05$). Vomiting and failure to thrive were present in 15% of screening detected cases, none had hypotension or collapse. Serum electrolyte concentrations showed a linear correlation with age at diagnosis: Na: $r^2 = -0.64$, ($p < .0001$) and K: $r^2 = 0.38$ ($p = .0055$). Screening alone accounted for nearly 50% of CAH detected in infancy, including a fifth of girls, indicating that clinical diagnosis is unreliable in both genders. This study adds further argument for CAH screening internationally.

89. COMMUNICATION AND KEY PERFORMANCE INDICATORS: IMPROVING THE QUALITY OF THE VICTORIAN NEWBORN SCREENING PROGRAM

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In 2011, the Victorian newborn screening (NBS) program successfully implemented a policy of written consent. Following on from this measure to improve program quality, the NBS nurse led a review of sample collection timing and quality. The program was assessed against the *Timeliness of Sampling and Testing* guidelines set by the Human Genetics Society of Australia (HGSA). The review identified some areas for improvement; in particular, the timing of sample collection, the quality of samples collected and the transit time to the laboratory. To improve these measures, an awareness campaign was launched by the NBS nurse for 2013. Notification of the new target measures was given to all NBS liaisons and nurse unit managers, across 96 sites. One hundred and seventy-four on-site education sessions were delivered to nursing and midwifery staff to refresh on correct sampling techniques. These sessions were supported by a comprehensive website and a program-specific e-learning tool. After 12 months, the transit time was reduced from 10 to 7 days (95.5 percentile); 95% of samples were being collected at 80hrs of life ($\sim$3 days) and the percentage of poor quality samples dropped from 0.52 to 0.4. Maintaining program quality requires ongoing monitoring and education; high standards cannot be maintained without regular communication and promotion of the performance measures to be met. As part of the newborn screening team, the NBS nurse plays a vital role in the delivery of a high quality service.

90. REPRODUCTIVE GENETIC CARRIER SCREEN: OUR 18-MONTH EXPERIENCE

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The Victorian Clinical Genetics Services (VCGS) has been offering the 'Reproductive Genetic Carrier Screen' (RGCS) for the past 18 months. Screening for cystic fibrosis (CF), spinal muscular atrophy (SMA) and fragile X syndrome (FXS) have been selected because of the relatively common carrier frequency in the population and the significant impact these three disorders have on families. Carriers of these disorders are completely healthy and most are unaware of their carrier status until they have an affected child. Identification of carriers has important implications for individuals with a family history and the general population. Our protocol for CF is to screen for 38 severe common mutations which account for $\sim$90% of carriers in the Australian Caucasian population by Sequenom analysis; SMA screening is copy number analysis by quantitative real time PCR; FXS screening is offered only to females using the Abbott kit. Results are reported as carrier or low risk for CF and SMA, and increased risk [$\geq$55 repeats] or low risk for FXS. FXS testing also identifies individuals with repeats in the grey zone range and individuals with 3 X-chromosomes, these results are not

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reported. To date, over 3,000 individuals have been screened. Approximately 1 in 20 individuals are a carrier of at least one of the 3 conditions and increased-risk couples have been identified for CF and FXS. The program has enabled us to have a better estimate of carrier frequencies for these three disorders in the Australian population.

91. FIFTY YEARS OF SCREENING FOR PHENYLKETONURIA IN NEWBORNS IN NSW

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Newborn screening commenced in NSW in April 1964 with urine screening for phenylketonuria. Only a small proportion of births were screened initially, but in a decade blood-spot screening became universal with coverage approximating 100%. As screening is not mandatory, the rapid improvement in numbers tested could be attributed to funding being provided by the government as a public health initiative. Over the 50 years there have been several analytical techniques used for screening and confirmation including the bacterial inhibition assay, paper chromatography, thin layer chromatography, capillary electrophoresis and tandem mass spectrometry. To date, molecular techniques have had little penetrance in screening for PKU except for confirmation and to tailor treatment regimes. In NSW, tandem mass spectrometry has been used for screening, confirmation and monitoring since 1998. Since 1964 there have been 4.5 million babies offered testing with 438 confirmed to have persistent elevation of phenylalanine requiring medical intervention of at least monitoring of levels. There have been no babies screened who were not detected with PKU. Assessing the key performance indicators over the last decade provides a sensitivity of 100%; specificity of 99.98%; positive predictive value of 63% and an incidence of 1:9340 with babies referred to the clinical team before 12 days of age. Screening for PKU has proven to be reliable and cost effective. With the concomitant infrastructure existing because of screening for PKU alone, it has allowed for the expansion of newborn screening to the over 40 additional inborn errors of metabolism being screened today.

92. ARTERIAL TORTOUSITY SYNDROME, A CASE STUDY

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Arterial Tortuosity Syndrome (ATS) is a rare connective tissue disorder characterized by tortuosity, elongation and aneurysms/stenosis of large and middle sized arteries throughout the body. It is an autosomal recessive condition caused by homozygous or compound heterozygous mutations in SLC2A10 gene. ATS is characterized by elongated face, macrocephaly, high arched palate, micrognathia, arachnodactyly, joint hyperflexibility, joint contractures, cutix laxa, hernias and various vascular anomalies. Vascular features of ATS can present as tortuosity and elongation of the aorta and arteries throughout the body, stenosis of arteries and aberrant origin of arteries coming off aorta. Pulmonary artery stenosis can cause right ventricular or proximal pulmonary hypertension. Heart failure secondary to elevated right ventricular pressure or systemic hypertension can lead to death in these patients. Arterial rupture can also account for premature death. We present an infant with compound heterozygous mutation in SLC2A10 gene. He had arachnodactyly, hypermobility of joints, dislocated distal interphalangeal joints, bilateral club feet, hiatus hernia, partial eventration of left hemidiaphragm and involvement of multiple blood vessels. Multiple vascular abnormalities in this child were tortuous aorta, stenosis of left pulmonary artery and enlarged right pulmonary artery. He also had large calibre of coeliac artery and portal vein. Monitoring of the vessel abnormalities in patients with ATS needs to be tailored to the individual depending on imaging findings and family history of aneursymal growth. The natural history of aneurysm, dissection and stenosis in ATS is not well established but is of high concern.

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