ABSTRACTS – POSTER PRESENTATIONS

(Ordered according to poster schedule)

MONDAY, 7 OCTOBER 2013

POSTER VIEWING SESSION 1

PP 1

Candidate Genes and Novel Polymorphisms for Anxiety Disorder in a South African Cohort

N McGregor (Stellenbosch University)

Anxiety aetiology remains poorly understood. Increasing focus is placed on the neurobiological and environmental interaction mediating disorder pathogenesis, specifically the role of trauma in susceptibility. Neural circuitry involving the striatum has been implicated in numerous anxiety disoders. Identifying genes responsible for changes at neuronal synapses involved in the manifestation of symptoms typical of these conditions, whilst considering trauma exposure as a contributing factor, could prove crucial to the understanding of anxiety disorders.

Adult Sprague Dawley rats were subjected to maternal/ restraint stress and used to mimic the presence of major life events and mild stress in adulthood, respectively. Gene expression in the synaptic plasticity pathway was investigated using PCR array technology. Human homologue candidate genes were characterised in a case-control association study [obsessive-compulsive disorder (OCD), panic disorder (PD) or social anxiety disorder (SAD)] for which trauma history was known. A tagSNP approach was used for genotyping; alternatively targeted next-generation sequencing (tNGS) was employed. Genotyping data was statistically assessed in conjunction with trauma history to test for gene-environment interaction.

Several genes (*Bdnf*, *Mmp9*, *Arc*, *Ntf4*, *Egr2*, *Egr4* and *Grm2*) were identified as aberrantly expressed in rats with anxiety-like behaviours *vs.* controls. tNGS yielded 42 polymorphisms unique to only either patients or controls of which 38 were novel.

Differentially expressed genes in rats with a trauma history can point to candidate susceptibility genes for anxiety in humans. The novel variation identified within this study will be genotyped in the remainder of our cohort to assess for association, in conjunction with trauma data.

PP 2

Genetic polymorphisms of glycine N-acyltransferase in a South African cohort identified with Ion Torrent semiconductor sequencing

R van der Sluis (North-West University), E van Dyk (North-West University), A van Dijk (North-West University)

Human glycine N-acyltransferase (hGLYAT) is responsible for the glycine conjugation of xenobiotics such as benzoic acid. Significant inter-individual variability in glycine conjugation capacity has been demonstrated using human liver samples. Within the GLYAT gene there are 668 known single nucleotide polymorphisms (SNPs), which were found in the Japanese population, French Caucasian population and the 1000 genome project.

The aim of this study was to determine the genetic polymorphisms within the coding region of the GLYAT gene of a South African cohort using semiconductor sequencing. Genomic DNA of healthy South African volunteers was obtained. SNPs identified with the ion torrent variant caller software were validated using Sanger sequencing.

In this study, one novel non-synonymous SNP (Q61L) and 17 known polymorphisms of the GLYAT gene were identified. Of the 17 known polymorphisms, two (S17T and N156S) were non-synonymous and 15 were located in the intron region. It was also found that some individuals had a combination of one or more non-synonymous SNPs within GLYAT. Recently, we investigated the effect of six non-synonymous SNP variants of GLYAT on the enzyme activity. The enzyme activities of the S17T variant were similar to that of the wild-type, whereas the N156S variant was more active.

SNP variations in the human GLYAT gene have an effect on enzyme activity, which may help explain some of the observed inter-individual variation in glycine conjugation capacity. The combination of these SNPs, revealed by the sequencing study, on the enzyme activity of GLYAT still needs to be investigated.

PP 3

Inter-individual variation in glycine conjugation capacity: a metabolic and molecular approach

C Nortje (North-West University), R Van der Sluis (North-West University), E Erasmus (North-West University), A Van Dijk (North-West University)

Human glycine N-acyltransferase (GLYAT), detoxifies aromatic carboxylic acids through glycine conjugation. Inter-individual variability exists in terms of glycine conjugation capacity. A 10-hour (h) challenge test, using aspirin, is currently used to evaluate glycine conjugation. However, this test is not sensitive enough to detect slight variations in glycine conjugation probably because the collective 10h period for urine collection is too long. The GLYAT gene contains approximately 668 polymorphisms. Here the variability in glycine conjugation was investigated by studying the metabolism and genetic variation.

A more sensitive challenge test was developed by collecting multiple urine samples instead of one collective sample over 10h. HPLC-MS/MS techniques were used. Five unrelated volunteers, with known genotypes in terms of GLYAT, participated in the challenge test. The coding regions of GLYAT of 10 individuals who exhibited low glycine conjugation capacity were sequenced using semiconductor next generation sequencing.

Variations in the rate of glycine conjugation between the 5 individuals could be indicated with the shortened challenge test. In the 10 individuals with low glycine conjugation a total of nine known SNPs were identified of which two were non-synonymous (S17T and N156S). A previous study has shown that compared to the wild-type recombinant GLYAT enzyme the N156S resulted in increased enzyme activity while the S17T had no significant effect on enzyme activity.

The shorter challenge test improved the detection of variations in glycine conjugation. A correlation could not be made between a specific genotype and its effect on the phenotype in terms of glycine conjugation.

PP 4

Identification of an α-synuclein triplication in a South African family affected with atypical Parkinson's disease

C van der Merwe (Stellenbosch University), S Bardien (Stellenbosch University)

Parkinson's disease (PD) is a neurodegenerative disorder that affects the dopamine-producing neurons of the substantia nigra in the midbrain. Identification of mutations in the gene encoding α-synuclein (*SNCA*) was the first evidence for a genetic component underlying this disorder. Genomic whole gene duplications and triplications of *SNCA* are inherited in an autosomal dominant manner, and lead to pathogenic overexpression of the wild-type protein. We screened a South African family presenting with features of atypical parkinsonism for genomic rearrangements at the *SNCA* locus.

Multiplex ligation-dependent probe amplification (MLPA) analysis was performed on genomic DNA obtained from blood samples of the proband and four family members that were available for the study. MLPA involves the hybridisation and ligation of exon-specific probes followed by amplification with quantitative PCR. The PCR fragments were electrophoresed on an ABI 3130xl and the data was analysed using Coffalyser software.

Results showed that a triplication of *SNCA* was present in the proband, which means that he harbours four copies of the gene. The affected sister of the proband was deceased before blood samples could be obtained, but screening of four unaffected family members identified two additional *SNCA* triplication mutation carriers who are at risk of developing the disorder.

In conclusion, this genetic test has enabled a definitive clinical diagnosis in this family and will facilitate presymptomatic genetic testing with appropriate genetic counselling of at-risk family members. Furthermore, it is important to raise awareness of this debilitating, progressive neurological condition as it could potentially be misdiagnosed as Alzheimer's disease.

PP 5

Identification of genetic markers for obesity risk and body composition in a South African black population

V Pillay (University of the Witwatersrand and National Health Laboratory Service), Z Lombard (University of the Witwatersrand), N Crowther (University of the Witwatersrand and National Health Laboratory Service), H Soodyall (University of the Witwatersrand and National Health Laboratory Service)

Heritability studies of body mass index (BMI) have demonstrated that a significant proportion (40-70%) of variance is due to genetics (Bodurtha et al, 1990), with genome-wide association studies (GWAS) of obesity-related traits identifying more than 52 risk loci (Loos, 2012) thus far.

The overall aim of this study is to identify genetic markers of obesity risk in a South African black population. The focus is on gene loci associated with obesity in Europeans to confirm if these loci are similarly associated with obesity risk in Africans. This study focuses on the Birth to Twenty cohort, which is the largest and longest running study of child and adolescent health and development in Africa, and one of the few large-scale longitudinal studies in the world. The Illumina CardioMetabochip chip is a custom Illumina iSelect genotyping array designed to cost effectively test ~200,000 SNPs identified through genome wide meta-analyses for metabolic and atherosclerotic / cardiovascular diseases and traits. The chip was designed and will aid in fine mapping loci previously associated with body composition. 2200 middle-aged Bt20 participants will be genotyped using the CardioMetabochip and association analysis conducted, focusing on loci associated with obesity measures (e.g. Body mass index (BMI), body fat %, waist to hip circumference), adjusting for sex and age. The identification of the genetic factors underlying susceptibility for obesity in Africans will contribute to knowledge of the biology of energy balance and highlight avenues for targeted therapeutic intervention.

PP 6

FC Gamma Receptor IIB rs1050501 Polymorphism is a possible risk factor for Systemic Lupus Erythematosus in black South African patients

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Systemic Lupus Erythematosus (SLE) is a complex autoimmune disease. One of the strongest candidate regions is the Fc gamma receptor (*FCGR*) gene cluster. GWAS studies have linked a polymorphism in FCGR2B (rs1050501) with susceptibility to SLE. The minor allele frequency (MAF) of rs1050501 is subject to considerable ethnic variation, with a higher MAF observed in populations from areas where malaria is endemic. This raises the

possibility that decreased FcγRIIb function may provide a survival advantage against this parasitic infection. The aim of this study was to genotype 100 black South African SLE patients and 100 healthy controls for rs1050501, and to establish the role of this SNP as a risk factor for SLE in this population.

The patients were consenting individuals who fulfilled the ACR criteria for SLE. A total of 96 control and 88 patient samples were successfully genotyped for this polymorphism, using a restriction enzyme digest.

MAF was similar in both the control and patient group, however the homozygous state for the minor allele was found exclusively in the patients with a trend towards significance (X^2=10.28; *p=0.005)*. Subgroup analysis of nephritis in the patient group showed an even stronger trend towards significance (X^2=18.11; *p=0.0001).*

The MAF in this study is similar to that found in Africa (0.24), higher than in Caucasian populations (0.10). This would indicate that there are similar implications for susceptibility to SLE and protection against Malaria in our Black patient group as has been previously described.

PP 7

Identification of Genetic variants influencing adherence to Colonoscopic surveillance for high-risk Lynch Syndrome subjects

L Lamola (University of Cape Town), R Ramesar (University of Cape Town), A Vorster (University of Cape Town)

Adherence to medical intervention refers to the patients' choice to follow practices as recommended by their healthcare providers. For Lynch Syndrome, the most commonly inherited form of colorectal cancer (CRC), disease-free survival period is dependent on the adherence to regular colonoscopic-screening. Thus, failure to adhere to this life saving intervention in those who've been shown to be at risk, may be considered as "risk-taking" behaviour. Previously, reasons influencing adherence have been attributed to socioeconomic, sociodemographic and psychological factors. The aim of the study was to determine whether genetic variations in genes responsible for identifiable neuropsychological/behavioural traits, are associated with adherence to colonoscopic surveillance in individuals who are known to be at high-risk for developing colorectal cancer.

The cohort consisted of 188 individuals diagnosed with Lynch Syndrome. Genotype data was generated using PCR based assays in conjunction with variant detection through the SNaPshot® technique. Categorical principle component analysis (CATPCA) and logistic regression was used to assess the relationships between the variables and genetic markers with adherence, respectively. The logistic regression results showed a possible relationship between genotype and level of adherence to colonoscopic surveillance. Although the correlation was significant (p=0.034), the relationship was not strong enough to be used as a predictive model.

The current study identifies the opportunity for investigating the subtle role of genetics in neuropsychology, which may be combined with more comprehensive neuropsychological factors. All this relies much on the better

understanding of neuropsychological and sociodemographic factors as has been done in recent intensive investigations on this cohort.

PP 8

Investigating mitochondrial DNA variants in Chronic Fatigue Syndrome

E Schoeman (North-West University), F van der Westhuizen (North-West University), J Elson (Newcastle University), R Louw (North-West University), E van Dyk (North-West University), L Erasmus (North-West University), F Ng (Newcastle University), J Newton (Newcastle University), S Al-Ali (Newcastle University)

Chronic Fatigue Syndrome (CFS) is a chronic debilitating condition defined as fatigue not alleviated by rest or sleep. Studies suggest mitochondrial dysfunction in patients with CFS on the basis of muscle bio-energetic function. We investigated a possible role of sub-clinical levels of pathogenic mtDNA mutations in CFS.

CFS patients were identified following conventional clinical screening approaches; potentially confounding causes of fatigue where excluded. We sequenced complete mtDNA of 93 CFS patients (UK-based = 52; South African-based = 41) using an Ion Torrent Personal Genome Machine® Sequencer. Primary data analysis was performed using the CLC Genomics Workbench 4.6.1 (CLC bio).

We detected the co-occurrence of three pathogenic mutations (m.7497G>A, m.9185T>C and m.10197G>A) at low-moderate levels in ~10% of the patients. This number is higher than those reported in a large population study investigating the frequency of pathogenic mtDNA mutations in the general population. The mutations are for the most part seen on a mitochondrial haplogroup U background. A highly significant difference in age between the patients with the mutations and those without were also observed.

Mitochondrial DNA variation may play a key role in a subgroup of CFS patients. There might be an effect of haplogroup background in conjunction with these rare mutations. Haplogroup background has been shown to modulate the penetrance of at least two primary mtDNA mutations. The mutations might alter the course of disease; reducing recovery capacity on a haplogroup U background. Questions remain regarding this hypothesis, but the currently presented data suggests that additional investigation is merited.

PP 9

Detection of cgg expansion mutations in fragile x patients using the amplidex™ fmr1 pcr kit.

A Esterhuizen (National Health Laboratory Service)

Fragile X syndrome (FRAXA) is the most common inherited cause of intellectual disability. It is mostly a result of expansion and methylation of the CGG sequences in the *FMR1* gene. PCR is generally viewed as inadequate

for amplification of the large, CG-rich *FRM1* expansions. Resolution of female zygosity is challenging due to preferential amplification of the shorter allele. The gold standard methodology is Sothern blotting (SB), which assesses allele size and methylation status. However, it requires large amounts of DNA, is expensive and labour-intensive. The AmplideX™*FMR1* PCR kit (Asuragen, Celtic Molecular Diagnostics) employs three-primer CGG Repeat Primed (RP) PCR and capillary electrophoresis (CE) to detect *FMR1* CGG expansions and resolve zygosity in females. This study aimed to evaluate and internally validate the kit, with the view to including it in the diagnostic repertoire. DNA of 40 patients referred for FRAXA testing was analysed with the AmplideX™*FMR1* PCR kit (Asuragen) and CE. The results were compared to those previously obtained with other methods; in-house PCR, Abbott Molecular PCR kit and SB. Result correlation was found for all samples tested, with improved informativity in females. Robust amplification and a clear result were achieved in a sample which repeatedly failed on SB. The AmplideX assay was shown to provide reliable information regarding the *FMR1* expansion status of patients, including homozygous females, in the local laboratory environment, thereby significantly reducing the need for SB. The test is robust, sensitive, easy to perform and is now incorporated into the local testing repertoire.

PP 10

The Requirement for a Population-Specific Diagnostic Tool for CF in South Africa

C Stewart (University of Pretoria), J van Rensburg (University of Pretoria), R Masekla (University of Pretoria), R Green (University of Pretoria), M Pepper (University of Pretoria)

Cystic fibrosis (CF) remains the most common potentially lethal autosomal recessive disorder worldwide. It has been estimated that 9% of White, 26% of mixed race and 54% of Black South African CF patients carry unknown mutations in the cystic fibrosis transmembrane conductance regulator gene (*CFTR)*. Our aim is to characterise the CF clinic patient population at the Steve Biko Academic Hospital (SBAH) and to identify novel *CFTR* mutations.

Patient data was anonymised and collated from 47 files at the clinic. 13% of the SBAH CF clinic patients have an unknown or incomplete molecular diagnosis. Of this 13%, 14% are mixed race, 14% are Indian, 29% are White and 71% are Black. Additionally, 71% of these patients have a positive sweat test and 57% suffer from pancreatic insufficiency, suggesting that they may carry novel mutations.

The SBAH CF clinic data highlights a gap in the knowledge required for the molecular diagnosis of CF, especially among Blacks. It is for this reason that it is necessary to sequence the DNA of these patients. DNA was extracted from 11 patients using the QIAamp DNA Blood Midi Kit, spectrophotometrically quantified and electrophoresed. Selected regions of the *CFTR* will be sequenced and the data analysed using CLC Genomics Workbench.

The Faculty of Health Sciences Research Ethics Committee at the University of Pretoria approved this study.

PP 11

The apoptosis pathway and risk of anterior cruciate ligament ruptures

A September (University of Cape Town), M Rahim (University of Cape Town), S Mannion (University of Cape Town), L van der Merwe (Medical Research Council of South Africa), M Posthumus (University of Cape Town), M Collins (Medical Research Council and University of Cape Town)

Introduction: Genetic factors have been implicated with risk of anterior cruciate ligament (ACL) ruptures. Functional variants within the gene encoding caspase-8 (CASP8) were previously associated with Achilles tendinopathy. The aim of this study was to test the association of polymorphisms within the CASP8 gene with ACL injury risk.

Methods: A genetic-association study was conducted on 234 control (CON) and 227 ACL rupture participants who were genotyped for the functional variants rs1045485 and rs3834129. Statistical analyses were performed to determine significant differences ($p<0.05$) in genotype/ allele frequency distributions between the various groups.

Results: The D allele of rs384129 was found to be significantly ($p=0.031$) overrepresented within the ACL group compared to the CON group. A dominant model was noted in which individuals with either the DD or DI genotypes were at greater risk of ACL rupture (OR:1.7; 95%CI 1.07-2.71). No further differences were noted between groups for rs1045485.

Conclusion: These findings provide preliminary evidence implicating CASP8 with ACL risk. Furthermore, it highlights the potential significance of apoptosis in pathobiology of musculoskeletal injuries such as ACL ruptures and chronic Achilles tendinopathy.

PP 12

Searching for the missing heritability: the role of AKAP9 as a genetic modifier in the Long-QT Syndrome

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Long-QT syndrome (LQTS), an inherited arrhythmia disorder characterised by a prolonged QT interval on the electrocardiogram and the occurrence of cardiac events, presents with variable phenotypic expression unexplained by the primary disease-causing mutation. This variation can partly be attributed to genetic modifiers that, despite not being disease-causative, contribute to phenotype. This study investigates variants in a known LQTS-causative gene, A kinase (protein kinase A) anchor protein 9 (*AKAP9*), for potential LQTS modifying effects.

A South African LQTS founder family, carrying an identical-by-descent A341V mutation in a potassium channel-encoding gene (*KCNQ1*) (176 non-carriers and 168 mutation carriers), was evaluated for modifying effects of *AKAP9* variants on heart rate-corrected QT interval (QTc), cardiac events and disease severity. TaqMan SNP Genotyping Assays were used to genotype the *AKAP9* tag SNPs: rs11772585, rs7808587, rs2282972 and rs2961024. Associations of alleles, genotypes and haplotypes with phenotypic traits were statistically assessed, taking the correlation resulting from the degree of relatedness and confounding variables into consideration.

The rs2961024 SNP was significantly associated with an age-dependent change in QTc (*P*=0.006). Furthermore, rs11772585 was significantly associated with both cardiac event risk and disease severity (*P*=0.001 and 0.025). Haplotype analysis supported these findings, with haplotypes containing the specific risk alleles resulting in similar effects to those seen in the individual SNP analysis.

The *AKAP9* gene, in addition to its previously reported role in disease-causation, has been identified as a LQTS modifier. Combined with other similar studies, these results could help establish risk profiles for affected individuals and guide potential treatment strategies.

PP 13

Screening for Parkinson's disease-causing mutations in a group of black South African patients.

C Ntsapi (Stellenbosch University), J Blanckenberg (Stellenbosch University), C van der Merwe (Stellenbosch University), B Glanzmann (Stellenbosch University), W Haylett (Stellenbosch University), S Bardien (Stellenbosch University)

Parkinson's disease (PD) is an incurable neurodegenerative disorder primarily characterized by the death of dopaminergic neurons in the substantia nigra pars compacta. PD is pathologically defined by the presence of Lewy bodies and Lewy neurites in the surviving neurons. In recent years a significant genetic component has been found for PD, with mutations in the *parkin* gene being the most common genetic cause. PD has been poorly studied in sub-Saharan African individuals of African descent possibly due to problems with patient ascertainment, poor access to healthcare and a lack of awareness of PD among health care professionals. The aim of the present study was to determine the genetic aetiology of a group of black South African PD patients.

A total of 25 unrelated black African PD patients were recruited with an average age- at- onset of 53 years and 56% were male. All 12 *parkin* exons were screened using Sanger Sequencing. Multiplex ligation-dependent probe amplification (MLPA) analysis was used to detect copy number variation in a number of PD-causing genes. Data was analysed using Coffalyser software (for MLPA) and BioEdit version 7.0.1. (For Sanger Sequencing).

Two patients were shown to have mutations in *parkin*. One patient had a duplication of exon 2 and a deletion of exon 9, and the other patient had deletion of exon 4 and a point mutation, G430D.

Further studies are required to identify disease-causing mutations in these patients. It is likely that they harbor mutations in novel PD genes due to their unique ancestry.

PP 14

The identification of four parkin substrates: Implications for Parkinson's disease

W Haylett (Stellenbosch University), C Kinnear (Stellenbosch University), J Carr (Stellenbosch University), S Bardien (Stellenbosch University)

A salient feature of Parkinson's disease (PD) is the progressive intraneuronal accumulation of proteins. Hence, the opposing processes of protein accumulation and protein degradation are both implicated in PD. Of particular interest is dysfunction of the ubiquitin proteasome system (UPS), as loss-of-function mutations in the UPS component parkin causes PD. Parkin is an E3 ligase that ubiquitinates protein substrates and targets such substrates for degradation; therefore the loss of parkin may result in the deleterious accumulation of parkin substrates and neurotoxicity. This study aims to identify novel parkin substrates and to investigate the role of parkin in cellular toxicity.

Yeast two-hybrid (Y2H) methodology was used to identify parkin substrates by screening an adult brain cDNA library for protein interactions with parkin. Identified interactions were investigated using *in vivo* 3D colocalisation analysis. SiRNA-mediated gene knockdown will be used to examine the effect of the absence of parkin on substrate protein levels.

Four parkin substrates were identified by Y2H and prioritised for further investigation: myelin basic protein (MBP), 14-3-3 eta, γ-actin and ATPAF1. Of particular interest are MBP, which has previously been identified as a biomarker for neurodegeneration and ATPAF1, which is implicated in mitochondrial energetics. Successful siRNA-mediated parkin knockdown has been verified by RT-qPCR; experiments are in progress to determine the protein levels of the investigated substrates.

This study identified four parkin substrates, which will be studied further. It is anticipated that a comprehensive understanding of parkin function will help clarify PD pathology and provide novel approaches to therapeutic interventions.

PP 15

HLA typing: Conventional techniques versus Next Generation Sequencing

J Mellet (University of Pretoria), C Gray (University of Cape Town), M Pepper (University of Pretoria)

The human leukocyte antigen (HLA) complex is the most diverse region in the human genome and plays a critical role in matching donors and recipients for

transplantation. The large number of polymorphisms in the South African population reduces the probability of finding adequate matches between donors and recipients. Next generation sequencing (NGS) enables sequencing of additional exons to resolve ambiguous typing results, which could improve the degree of matching between individuals.

The purpose of this study was to determine: (1) the efficiency of the 454 Life Sciences NGS HLA typing kit in determining the HLA alleles in South African individuals; (2) the degree of genomic complexity that this technique reveals; and (3) the relationship between NGS and conventional HLA typing techniques. HLA genotyping was performed using 454 NGS on 20 samples that had previously been typed by conventional methods. Samples were genotyped using a Roche designed medium (MR) and high resolution (HR) HLA typing kit. Accurate genotypes were assigned to 95.5% of the loci of interest using the MR kit, compared with 98.5% using the HR kit. The 454 NGS technique is more effective than conventional techniques in assigning HLA genotypes. The results emphasise the importance of sequencing additional exons to resolve ambiguous typing results. In conclusion, although the 454 NGS platform together with the HR HLA typing kit is quicker and might reduce ambiguous typing results, the advantages provided when compared to conventional techniques in routine clinical and diagnostic laboratories appear for the present to be minimal.

PP 16

Working towards models for assessing genetic risk of Achilles tendinopathy

C Saunders (University of Cape Town), L van der Merwe (Medical Research Council), J Cook (Deakin University), C Handley (La Trobe University), M Collins (Medical Research Council and University of Cape Town), A September (University of Cape Town)

Polymorphisms in several genes encoding structural, signalling and associated proteins of the ECM have been associated with risk of developing Achilles tendinopathy (AT). This study aimed to investigate the association of polymorphisms within several collagen genes with AT, and to potentially develop a model to discriminate risk of AT using genetic risk factors.

In this case-control study, 133 South African and 207 Australian control (CON) participants, as well as 94 South African and 84 Australian participants clinically diagnosed with AT (TEN) were genotyped for eight polymorphisms within the *COL5A3*, *COL5A2* and *COL3A1* genes. Gene-gene interactions were explored between these loci and polymorphisms within the *COL5A1, TNC, COL27A1, IL-6, IL-1β* and *CASP-8* genes which were previously associated with AT. Logistic regression was used to generate the optimal model of discriminating AT risk using these polymorphisms, which was evaluated by receiver-operating characteristic curve analysis.

No independent associations were observed between any of the polymorphisms within *COL5A3, COL5A2* and *COL3A1* and AT risk. However, significant gene-gene interactions were noted between genes encoding proteins of the cell-signalling and apoptosis cascade with variants within the

COL27A1, TNC and *COL5A3* genes (P<0.05). A preliminary model to discriminate between TEN and CON was identified and included age, sex, *COL5A1* (rs12722), *COL27A1* (rs946053), *COL5A3* (rs1559186), *IL6* (rs1800795) and *CASP-8* (rs1045485; rs3834129) as significant variables (Sensitivity=82.4%; Specificity=58.0%; AUC=0.775).

These results emphasise the complex, multi-factorial aetiology of AT, and further highlight the complex interactions of the genes encoding ECM proteins in the pathobiology of AT risk.

PP 17

Triplet-Primed PCR: An Introduction into the South African Diagnostic Arena.

D Smith (University of Cape Town), A Wichers (University of Cape Town), A Esterhuizen (National Health Laboratory Service), J Greenberg (University of Cape Town)

Triplet repeat disorders are caused by the expansion of a trinucleotide repeat tract beyond a specific threshold. There are sixteen known triplet repeat disorders, including Huntington disease (HD), Friedreich ataxia (FRDA) and eight types of Spinocerebellar ataxia (SCA). Typical methods of molecular diagnosis are PCR-based, using fluorescent primers flanking the repeat region followed by analysis using capillary electrophoresis. However, in cases of apparent homozygosity a conclusive result can not be delivered, since the result may be due to drop-out of the expanded allele.

Triplet-primed PCR (TP-PCR) assays were optimised as a complementary method of molecular diagnosis for five disorders routinely tested by the National Health Laboratory Services (NHLS) at Groote Schuur Hospital. These included FRDA, Spinocerebellar ataxias type 2 and 7, and HD. TP-PCR utilises a single target-specific flanking primer, along with a tailed primer complementary to the repeat, which binds at multiple loci along the length of the repeat region. A third primer is complementary to the 5' tail sequence of the repeat primer and binds to products from previous rounds of amplification, resulting in a ladder-like electropherogram following capillary electrophoresis.

Protocols for testing for FRDA, SCA7 and HD were successfully established, with the SCA2 protocol still undergoing optimisation. These assays are currently being implemented as standard complementary methods for molecular diagnosis of triplet repeat disorders in the NHLS, in order to contribute to the continual improvement and quality of test results.

PP 18

A stem cell-derived model of retinal degeneration associated with Spinocerebellar ataxia type 7.

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With the aim of developing an appropriate *in vitro* model to investigate the retinal degenerative phenotype associated with Spinocerebellar ataxia type 7 (SCA7), we have investigated induced pluripotent stem cell (iPSC)-based technology. SCA7 is caused by an expanded polyglutamine tract within the Ataxin-7 protein, a component of the TFTC/STAGA transcriptional co-activator complex involved in the transcriptional control of various retinal genes. The pathogenic expansion in Ataxin-7 leads to selective degeneration of photoreceptors and cerebellar neurons, resulting in blindness and ataxia.

Dermal fibroblasts from SCA7 patients and unaffected control individuals were infected with a Sendai virus-based vector carrying four transgenes (*Oct4, Sox2, Klf4 and c-Myc*) to induce reprogramming towards a stem cell fate. iPSCs were expanded and characterized in terms of genomic integrity, pluripotency marker expression and differentiation capacity. Two established protocols were utilized to drive stem cell differentiation towards rod and cone photoreceptors, using additives such as retinoic acid, taurine and recombinant sonic hedgehog. The length of the differentiation protocols ranged from 1 to 6 months.

Differentiated cells derived from both protocols exhibited photoreceptor-like morphological features and gene/protein expression profiles, however the 1 month protocol resulted in a significantly higher differentiation efficiency. SCA7 patient-derived photoreceptors displayed abnormal expression profiles of multiple disease-associated transcripts when compared to unaffected controls. These transcripts included the *ataxin-7* gene, as well as key retinal transcription factors.

This approach has provided novel insights into SCA7 pathogenesis, and illustrates an innovative method for the *in vitro* study of SCA7 patient-derived retinal cells.

PP 19

Two atypical variant e13a2 BCR-ABL1 fusion transcripts in Chronic Myeloid Leukaemia

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Chronic Myeloid Leukaemia (CML) is characterized by the t(9;22)(q34;q11) translocation forming the *BCR-ABL1*fusion gene. In >95% of CML, the breakpoint in *BCR*occurs in introns 13 or 14 and in introns 1a or 1b in *ABL1*. Two common fusion transcripts can result, the e13a2 or e14a2. Atypical variants are rare. Quantitative reverse transcriptase PCR (qRT-PCR) is used for detecting and monitoring *BCR-ABL1*transcripts. However, as only common transcripts are detected, false-negative results can occur with atypical variants. We report here two CML patients with novel atypical e13a2 *BCR-ABL1*transcripts that were undetectable with qRT-PCR.

Peripheral blood samples were tested with FISH and qRT-PCR for *BCR-ABL1*. Following discrepant results, where FISH showed a positive result but

qRT-PCR negative, a qualitative RT-PCR followed by direct sequencing was done to determine the exact fusion present.

Sequencing revealed an e13a2-type transcript with a 13bp deletion of *BCR* exon 13 and a 25bp insertion, corresponding to *ABL1* intron 1b, at the fusion site in one patient. In the other patient, a 41bp deletion of *BCR* exon 13 and a 20bp insertion, corresponding to the complement of an inverted region of *ABL1* intron 1b, occurred at the fusion site. In both cases, this mutation overlapped with the qRT-PCR forward primer-binding site causing the negative result.

Although two novel atypical *BCR-ABL1* transcripts have been described, their biological and clinical impacts are unknown. As similar cases are reported, the nature of these variant fusions, their role in leukemogenesis and optimal treatment strategies may be identified.

PP 20

Comparison of Quantitative Real-Time PCR and Droplet Digital PCR for CCL4 Gene Copy Number Determination

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The genes encoding the CC chemokine, CCL4, a natural ligand of the CCR5 receptor, display copy number variation (CNV), which has been shown to play a protective role against HIV-1 infection and disease attenuation. Findings that CNV, of the genes encoding CCL3, also a ligand of CCR5, influences HIV-1 protection have been controversial, largely because of the unreliability of methods used for CNV determination. This study evaluated the standard method of quantitative Real-Time PCR (qPCR) and Droplet Digital PCR (ddPCR) for CCL4 CNV determination. CNV of the genes *CCL4L1*, *CCL4L2* and combined *CCL4L* was assessed in a cohort of HIV uninfected South African Black (n=23) and Caucasian (n=32) individuals using qPCR and ddPCR. A stronger correlation between the number of *CCL4L* copies and the sum of *CCL4L1* and *CCL4L2* copies generated by ddPCR (R^2 = 0.9915) compared to qPCR (R^2 = 0.6494) was observed. Greater inaccuracy at higher copy numbers was exhibited by qPCR, which is particularly relevant to the cohort of Black individuals, who display a higher range of *CCL4L* copies (3-8) compared to Caucasians (0-4), and a higher population mean (4 and 2 respectively) . As expected, significant differences in copy number were observed between the Black and Caucasian population groups for *CCL4L* (p = <0.0001), *CCL4L1* (p= 0.0060) and *CCL4L2* (p< 0.00010). The method of ddPCR proved to be far superior to qPCR for assessment of CCL4 CNV, the accuracy of which is essential when determining its role in protein production and HIV-1 protective immunity.

PP 21

Targetable disease-associated HTT haplotypes in the South African population

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Huntington disease (HD) is an inherited neurodegenerative disorder resulting from the expansion of a CAG-tract in the huntingtin (*HTT*) gene. Worldwide prevalence varies, with the highest figures reported in populations of European ancestry. HD in South Africa (SA) occurs in black, white and 'Coloured' subpopulations. Similar prevalence estimates have been made for the white and coloured groups and a significantly lower estimate in the black subpopulation.

Recent studies on European and Asian cohorts reported population-specific *HTT* haplotypes, categorised into haplogroups. Based on similar analyses, we recently published results of a preliminary study showing the presence of disease-associated haplotypes specific to SA subpopulations. HD alleles from white and coloured patients occurred predominantly on haplogroup A, signifying a similar European origin; while expanded alleles from black patients were found on haplogroup B suggesting distinct origins of the mutation. In addition, average CAG-tract size of general population alleles could be associated with reported prevalence estimates. These results are to be substantiated in a larger cohort of individuals from the general population of SA.

CAG-tract size is being assessed in over 1000 general population DNA samples. For a sub-set of these, high-throughput genotyping of 96 SNPs will be performed and haplotypes constructed. Disease-associated haplotypes will be analysed in order to identify suitable targets for selective silencing of the expanded allele, and to determine the proportion of SA patients that could potentially benefit. This data is essential for the inclusion of African populations in the current drive for therapeutics already under development for HD patients.

PP 22

Genetic modifiers of CAG-tract instability in the Huntington disease gene

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Huntington disease (HD) is an inherited neurodegenerative disorder that results from the expansion of a CAG-tract in the huntingtin (*HTT*) gene. The CAG-tract is polymorphic and unstable and in affected individuals has expanded to 36 repeats or more. Most HD patients are diagnosed in the fourth or fifth decade of life, however, approximately 5-10% of cases present before the age of 21. These individuals are affected by juvenile HD (JHD) and

a molecular genetic test commonly detects an allele with 60 or more repeats. In atypical cases, JHD occurs in individuals with less than 60 repeats suggesting that factors other than the size of the CAG-tract contribute to onset. While adult-onset HD is characterised by chorea, cognitive disturbances and psychiatric problems, features of JHD include positive family history, rigidity and declining cognitive function. The molecular basis of CAG-tract instability resulting in JHD and the disease phenotype are as yet poorly understood.

South Africa has one of the highest reported rates of JHD. Several families have been identified with both typical and atypical presentation of the disease. Using DNA sequencing of the region encompassing the *HTT* gene, the expanded allele will be tracked from one generation to the next in an attempt to identify *cis*-acting variants that influence CAG-tract instability. The results of this investigation may lead to the identification of determinants of CAG-tract instability and mechanisms of pathogenesis that would be relevant for new forms of therapy.

PP 23

Polymorphisms in the TNFA region associate with HIV-associated sensory neuropathy susceptibility in black Southern Africans

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A common neurological complication associated with HIV infection is HIV-associated sensory neuropathy (HIV-SN). Single nucleotide polymorphisms (SNPs) in the tumour necrosis factor-alpha (*TNFA)* and surrounding genes in chromosome six have been associated with HIV-SN susceptibility in Caucasian, Indonesian, Chinese and Malay individuals. The study aimed to investigate the association between previously identified SNPs, and population-specific tagSNPs, in the *TNFA* region and HIV-SN susceptibility in a black Southern African population.

DNA was isolated from 340 HIV-positive individuals attending the Virology Clinic at the Charlotte Maxeke Johannesburg Academic Hospital, South Africa. 189 individuals (56%) had a clinical diagnosis of HIV-SN. All participants were 18 years of age or older and had been on antiretroviral therapy for at least six months. SNPs previously identified to be associated with HIV-SN, and tagSNPs appropriate for an African population were genotyped using a GoldenGate™ Genotyping Assay with VeraCode microbeads and data was read on an Illumina BeadXpress Reader. Analysis was performed using PLINK software for association analysis.

None of the previously identified SNPs associated with HIV-SN susceptibility in this African population. Six tagSNPs, however, were independently associated with reduced risk of HIV-SN after correcting for other risk factors for the neuropathy and multiple comparisons. These tagSNPs were also present in several haplotypes, which were also independently associated with HIV-SN susceptibility.

The study provides strong evidence in support of previous studies for the role of the *TNFA* region in HIV-SN susceptibility, but emphasizes the importance

of carrying out population-specific association studies to elucidate disease risk.

PP 24

A preliminary investigation of Cardiotoxicity in the treatment of breast cancer in an African setting

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Objectives: To provide insight into the clinical management of breast cancer patients on anthracycline-based treatment with a focus on the prevalence of anthracycline-induced cardiotoxicity (ACT). This pilot study will pre-empt investigation into the genetic basis of ACT whereby treatment may be individually tailored so that cardiotoxicity may be prevented or limited.

Design & Method: A retrospective patient file review was conducted on patients diagnosed between 2011 and October 2012 at Groote Schuur Hospital in Cape Town. One hundred and sixty three breast cancer patients on adjuvant anthracycline-based chemotherapy were analysed. Demographics, factors influencing therapeutic decisions pertaining to cardiac risk, left ventricular ejection fraction (LVEF %) as a measure of cardiac function and chemotherapeutic regimen data were assessed.

Results: The average patient in our study cohort was a female aged fifty-one of Mixed Ancestry/Indian origin. We also observed an aggressive type of tumour notably in young African females. There was a significant trend for diminished cardiac function in patients after 3 cycles of anthracycline-based chemotherapy predominantly in patients on Adriamycin than those on Epirubicin, the less cardiotoxic derivative.15.3% of patients on Adriamycin had their treatment amended whereby Adriamycin was substituted to Epirubicin.

Conclusion: Determining risk of ACT using pre-existing clinical factors was not conclusive. However, this pilot study did show a significant cardiac impairment due to anthracycline-based treatment, which warrants investigation. The curative ability of chemotherapy must be counterbalanced with the absence of ACT therefore determining individual susceptibility to cardiotoxicity may hold the key to event-free breast cancer survival.

PP 25

Adolescent Alcohol Dependence: A Gene-Imaging-Environment Perspective

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Alcoholism is a chronic disorder characterised by the compulsive and excessive consumption of alcohol and has been shown to be one of the most prevalent mental disorders in South Africa. Alcoholism has been estimated to have a heritability of between 40-60% and studies have shown that early

environmental risk factors can determine whether an individual will be more susceptible to alcohol use disorders (AUDs) later on in life. As brain structural volumes have been associated with AUDs, we hypothesise that by investigating the genetic variants previously associated with these volumes, and environmental risk factors, we will elucidate a clearer understanding of the interacting factors contributing towards this complex phenotype.

The aims of this investigation are to determine i) the underlying genetic aetiology of AUDs, ii) the structural changes in the brain which are associated with AUDs, iii) if environmental factors such as childhood trauma is a risk factor for AUDs and iv) if gene x environment interactions predict AUDs.

Our cohort consists of 80 adolescents with an AUD diagnosis and 80 matched controls of Mixed Ancestry ethnicity. A custom-made Illumina microarray was used to genotype the entire cohort. MRI data was collected from a 3T Siemens Magnetom Allegra MR Headscanner using Syngo MR software. Information regarding childhood trauma was determined using the childhood trauma questionnaire (CTQ). Imaging and statistical analyses were performed using SPM8 and SPSS, respectively.

From the microarray analysis, none of the SNPs were significantly associated with AUDs. Gene-imaging and gene x environment analyses is currently ongoing.

PP 26

The Genetics of Adverse Drug Reactions in Common Chronic Diseases: Bipolar Disorder and Lithium Treatment

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Lithium is regarded as the first-line pharmacotherapy for the treatment of patients with bipolar disorder (BPD). Response to lithium has a strong genetic component and lithium-responders have an increased frequency of BPD among their family members. Lithium has a narrow therapeutic index and 75-90% of patients on long-term lithium treatment experience one or more side effects.

The present pilot study explored whether single nucleotide polymorphisms (SNPs) within *GSK3β*, *AKT1*, *ARRβ2*, *GRIA2* and *PPPARGC1α* could be associated with the incidence and severity of lithium-induced side effects. One hundred and five patients with a diagnosis of BPD type I and a history of treatment with lithium were genotyped for ten SNPs within the candidate genes using SNaPshot PCR, Taqman SNP genotyping and RFLP analysis. A questionnaire related to the twenty most common side effects was conducted with all patients.

Results show an association with *rs1130233*G>A in *AKT1* and the presence (p=0.032) and severity (p=0.024) of nausea; and *rs1045280*C>T in *ARRβ2* and the presence of weight gain (p=0.024). *rs8192678*G>A in *PPPARGC1α* was associated with the presence of body aches (p=0.007) and the presence (p=0.030) and severity (p=0.032) of skin problems. After correcting for multiple testing none of these results remain significant.

The results indicate that some of these genes may play a role in influencing inter-individual susceptibility to lithium-induced side effects and may be clinically important in the treatment of BPD. This sets the stage for larger scale studies aimed at understanding appropriate treatment for this debilitating and often fatal disorder.

PP 27

Exome sequencing of a familial form of adult myoclonic epilepsy in two South African pedigrees

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Rapid advances in sequencing technologies in recent years have made it feasible to sequence whole human genomes and exomes in a cost-effective manner. Exome sequencing has been shown to be a particularly powerful technique for the identification of disease genes for Mendelian human diseases, where sequencing of small samples of affected individuals allow for the identification of the respective causal genes.

In the current study, we employ exome sequencing to identify disease-causing mutations in South African individuals diagnosed with familial adult myoclonic epilepsy. This form of epilepsy shows an autosomal dominant pattern of inheritance in two large South African pedigrees, where DNA is available for approximately 30 individuals. Traditional genetic linkage analyses have failed to associate familial epilepsy loci with the disorder in these samples. We therefore subjected six samples (i.e. two affected and one unaffected from each pedigree) to exome sequencing.

Whole human exome enrichment was performed employing the SureSelectXT Human All Exon V4 (51 Mb) kit and 100 bp paired-end sequencing was performed on the Illumina HiSeq2000 to an average coverage of 50X. Bioinformatic analysis involves quality control, short read alignment with Novoalign, sorting and indexing in SAMtools, and recalibration with the Genome Analysis Toolkit.

Once identified, candidate polymorphisms can be genotyped in the remaining cohort to confirm the pattern of inheritance and to implicate them in the pathogenesis of the disorder in these two pedigrees.

PP 28

Familial Telomeric Balanced Reciprocal translocation - two unbalanced siblings

J Kotze (Unistel Medical Laboratories)

Introduction

Balanced autosomal reciprocal translocations occur in about 0.1% of the population and are unique to a family. A female patient aged 2 yrs (Daughter A), presented with failure to thrive, developmental delay, hypotonia and microcephaly, was referred for chromosomal studies.

Method

The patient's peripheral blood specimen was cultured, harvested and slides were stained with GTG-banding. Metaphases were analysed and karyotyped. Microarray comparative genomic hybridization (aCGH) and Fluorescence *in situ* hybridization (FISH) analysis were performed to elucidate the cytogenetic results. The peripheral blood of the family was cultured for conventional cytogenetics and FISH analyses to exclude familial inheritance.

Results

The aCGH result indicated an imbalance in chromosomes 13 and 15 material. FISH analysis performed on metaphase cells using subtelomeric SureFISH probes for chromosomes 13q34 and 15q26.3 confirmed the der(13)t(13;15). Further studies showed that the father carries the balanced t(13;15)(q34;26.3).Cytogenetic and FISH analyses revealed that the sister, daughter B carries an unbalanced der(15)t(13;15). Normal karyotypes were observed in the mother and the son.

Conclusion

Any parent carrying a balanced translocation has a risk of passing on an unbalanced rearrangement to their offspring. Unbalanced rearrangements are more likely to be viable if the breakpoints are located nearest to the telomere, yielding the smallest imbalance. Both cases with unbalanced chromosome complements correlate with the expected clinical manifestations. The presence of this familial translocation would not have been detected without the performance of molecular techniques and therefore a multidisciplinary approach is the most effective for an accurate genetic diagnosis.

PP 29

Investigating the Functional Significance of Antipsychotic Pharmacogenomic GWAS

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Schizophrenia is a complex disorder demonstrating extensive inter-individual variation in symptoms, the antipsychotic treatment of which is only effective in approximately 50% of patients. Although GWAS examining antipsychotic response have identified significant associations, the functional importance of these variants remains unknown. GWAS interpretations tend to focus on gene function, despite the majority of results being located in non-coding regions. Therefore, this study utilised the information available from the ENCODE project to investigate the functionality of non-coding SNPs associated with treatment outcomes.

The literature was mined to identify variants that were significantly associated with antipsychotic response ($P \leq 5 \times 10^{-7}$). The data from the 1000 Genomes and HapMap projects were subsequently utilised to identify variants in LD ($r^2 \geq 0.8$). Thereafter, RegulomeDB was used to assess the impact of the variants on regulatory regions.

Initially, 59 GWAS "hits" were identified, and a further 399 SNPs were in LD with these variants. Twenty-two SNPs were predicted to occur in regulatory regions, scoring significantly with RegulomeDB (≤3). Eleven of these occur in known expression quantitative trait loci (eQTL), and the others show evidence for transcription factor binding with matched transcription factor motifs.

The variants in regulatory regions are being genotyped in 104 South African first episode schizophrenia patients to determine if they are associated with treatment response. Genes that are regulated by these regions will then be evaluated for their likelihood to affect treatment response. This research could contribute to knowledge of African genomes and the improvement of our understanding of regulatory and antipsychotic mechanisms.

PP 30

The use of QF-PCR in the Human Genetics laboratory, NHLS, Groote Schuur Hospital

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The Elucigene QSTR kit implements a quantitative fluorescence polymerase chain reaction (QF-PCR) for the rapid determination of the common chromosomal aneuploidies. The primers target specific highly polymorphic short tandem repeats (STR) on chromosomes 13, 18, 21, X and Y. The application of Elucigene QSTR in conjunction with chromosomal analysis was investigated. DNA was extracted from either peripheral blood or amniotic fluid with the use of the QIAamp mini blood kit (Qiagen), followed by QF-PCR and capillary electrophoresis (ABI *3130XI* Genetic Analyzer). The chromosome copy number was determined by comparison of the peak ratios. Three cases illustrate the practical utility of QSTR PCR to detect and/or confirm aberrant results. Patient one: Chromosomal analysis revealed a karyotype of 45,X,+mar. QF-PCR confirmed the marker to be of Y chromosome origin. Patient two: Using the multiplex QSTR PCR a triploidy was detected which was confirmed on chromosome analysis of the amniotic fluid culture. Patient three: QSTR PCR revealed an imbalance on chromosome 13 and a trisomy 21. Chromosomal analysis of the sample revealed a non-Robertsonian translocation involving chromosome 13 and 21 resulting in a trisomy 21. These findings illustrate the value of QSTR PCR in a diagnostic setting.

PP 31

Genetic variation in the 3'-UTR of CYPLA2, CYO2B6, CYP3A4, NR1I2 and UGT2B7 genes: effects on drug metabolising enzymes regulation by microrna

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Introduction: Variation in the 3'-UTR of drug metabolising enzyme (DME) genes has become a new area of importance in pharmacogenomic studies because of its targeting by non-coding RNAs and, microRNAs. Genetic variation in this region, together with that in the promoter region as well as exonic sequences affects DME gene regulation and ultimately drug response by either creating or disrupting microRNA recognition sites. In this project, we set out to investigate the extent of genetic variation in the 3'-untranslated region and evaluate its possible pharmacogenomics significances.

Methods: The 3'-UTR for *CYP1A2, CYP2B6, CYP3A4, NR1I2* and *UGT2B7* was sequenced for twenty Bantu-speaking South Africans to identify genetic variation. In Silico prediction tools were used to determine the effect of genetic variation on microRNA regulation.

Results: Previously reported and novel genetic variation was identified in the 3'-UTR for *CYP1A2, CYP2B6, CYP3A4, NR1I2* and *UGT2B7* supporting the high degree of genetic diversity among African individuals. The majority of identified genetic variants in DME genes were predicted to interfere with microRNA regulation and could have effects on drug response in treatment with commonly used drugs.

Conclusion: The results from the sequencing provide support for inclusion of genetic variation in the 3'-UTR, in addition to the coding region, in future pharmacogenomics studies to get a clearer picture on correlates of drug response.

PP 32

Exploring the role of genetic variation at the leptin (LEP) and the leptin receptor (LEPR) genes in obesity and hypertension in a black South African cohort

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Obesity and hypertension often occur together and are risk factors for cardio-metabolic disorders. Single nucleotide polymorphisms (SNPs) in the leptin (*LEP*) and leptin receptor (*LEPR*) genes have been shown to be associated with obesity and hypertension, but have not been well explored in African populations. The aim of this study was to identify SNPs in the *LEP* and *LEPR* genes and to examine the potential relationships of these SNPs with obesity and hypertension in a black South African population.

Participants from the African Programme on Genes in Hypertension (APOGH) with various anthropometric and blood pressure (BP) measurements were genotyped for *LEP* and *LEPR* SNPs using the BeadXpress platform.

Relationships between *LEP* or *LEPR* SNPs and obesity, leptin concentration and hypertension were assessed using SAS 9.3 and gPLINK vs2.050, taking into account family relationships, various confounders and correcting for multiple testing.

There were more women (66%) than men and the prevalence of obesity (42%) and hypertension (46%) were high. *LEP* rs17151914 (P=0.0132) and *LEPR* rs6690661 (P=0.0462) were associated with leptin concentration and diastolic BP, respectively in women. The *LEP* rs17151913T-rs6956510G haplotype was associated with an increase in central systolic BP in women (P=0.012) whereas the *LEPR* rs2154381C-rs1171261T haplotype was associated with lower systolic BP in men (P=0.0359).

LEP and *LEPR* SNPs were not associated with obesity but were associated with leptin concentration and diastolic BP in a sex-specific manner. These results indicate that further exploration of the role of *LEP* and *LEPR* in obesity and hypertension in Africans is warranted.

PP 33

Genetic Variation in CC Chemokine Receptor 5 (CCR5) in the South African Population

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CC chemokine receptor 5 (CCR5) is the major human immunodeficiency virus (HIV-1) co-receptor during initial infection. The importance of this receptor in HIV infection and disease progression was recognised with the discovery of the CCR5 delta 32 (Δ32) allele. Individuals homozygous for this mutation lack functional CCR5 receptors and are consequently resistant to HIV infection. Heterozygous individuals display decreased cell surface CCR5, which slows disease progression. Phenotypic expression of CCR5 is heterogeneous and this together with the effect of CCR5 expression on HIV infection and disease progression provided the rationale for investigating both the phenotypic and genotypic distribution of CCR5. The aim of this study was therefore to use the CCR5 phenotypic expression data to select a group of CCR5 low expresser's in South African for analysis of genetic variation in the CCR5 gene. Flow cytometric methods were used to measure the phenotypic distribution of CCR5 in 245 individuals by assessing both the percentage of CD4+CCR5+ T-cells and CCR5 density. Sixty-five individuals, mostly found within the lower CCR5 receptor density range were selected for DNA sequencing. Genotypic data revealed 70 single nucleotide polymorphisms (SNPs), four insertions and the ∆32 deletion. The ∆32 mutation was not detected in the Black African group but was identified in the Caucasian group at a frequency of 18.6 %. Twelve novel mutations were identified in this study with two in the open reading frame (ORF). This study provides invaluable information for characterising the effect of CCR5 mutations on HIV in the South African population.

PP 34

Forensic Analysis to Identify a Lost Child

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An unidentified female body in an advanced degree of decomposition was found. Due to injuries and decomposition it was not possible to identify the body by dental or fingerprint analyses. A blood sample was received from the putative mother and a tissue sample from the female body shortly after the body was found as well as a hairbrush belonging to her lost daughter.

DNA analysis was performed on the putative mother's blood, DNA was extracted from hair on the hairbrush and a femur from the body and analysed. Several different commercial DNA extraction kits as well several other published methods were used with limited success to extract DNA from a femur removed from the body.

A good profile was obtained from the putative mother's blood and a mixed profile consisting of two distinct profiles were developed from the hair from the hairbrush. Using the likelihood ratio method, it was determined with a high degree of certainty that the primary hairbrush profile was from a daughter of the woman who reported a lost child. After several attempts only a partial profile could be derived from the DNA extracted from the femur. The partial profile matched the primary hairbrush profile and, using the likelihood ratio method, it was determined with a fair degree of certainty that the femur profile was from a daughter of the mother. Considering the concordance between mother, hair and femur profiles, it is highly likely that the body was that of the reported lost daughter.

PP 35

The Co-inheritance of Alpha-thalassemia and the Clinical Severity of Sickle Cell Disease in African Patients

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Background: The influence of Alpha-thalassemia (α-thalassemia) on Sickle Cell Disease (SCD) has rarely been investigated in Africa.

Aim: To study the correlation between the α-thalassemia genotype and the clinical severity of Cameroonian SCD patients.

Patients and Methods: A cross-sectional study of hematological indices and clinical phenotypes was performed. RFLP-PCR was performed for the molecular diagnosis of SCD and for the study of the beta-globin gene cluster haplotypes. Multiplex Gap-PCR was performed to investigate the 3.7kb and 4.2kb α-thalassemia gene deletions.

Results: 175 SCD patients and 91 controls were studied, with median ages of 20 and 25 years, respectively. Amongst patients, 57% had >3 painful vaso-occlusive crises per year. The median Hb level was 8g/dl for patients and 13g/dl for controls. Haplotype analysis showed: 36% Benin, 33.5%

Benin/atypical and 18% Benin/Cameroon. Up to 37.1% of SCD patient co-inherited α-thalassemia, as compared to 20 % prevalence of α-thalassemia in the non-affected control. Amongst patients, the genotype distribution was: 30.3% αα/α-3.7, 6.8% α-3.7/α-3.7 and none had 4.2kb deletion. The co-inheritance of α-thalassemia and SCD was significantly, associated with higher RBC count; lower MCV and lower WBC count. Clinically, α-thalassemia was significantly associated with higher age of diagnosis, lower painful crises episodes and hospital admissions. The effect of α-thalassemia on survival, could explain the relatively high proportion amongst SCD patients.

Conclusion: The Co-inheritance of α-thalassemia is associated with a reduced clinical severity of SCD in African patients. The results have implications for anticipatory guidance/counselling of SCD patients and their families.

PP 36

MLPA analysis in patients with suspected Beta-Thalassaemia

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Beta-Thalassaemia (BTH) is an autosomal recessive disorder caused by a decrease or absence in the synthesis of β-globin chains, resulting in lowered amounts of haemoglobin, ineffective erythropoiesis and premature red cell death. BTH occurs at high frequencies in the Mediterranean, Middle East, Central Asia and India. The coding genes are within the β-globin gene cluster on chromosome 11 and consist of five functional genes, *HBE1, HBG2, HBG1, HBD* and *HBB,* and one pseudogene*, HBBP1*, with more than 200 mutations described in the *HBB* gene. Patients are referred to the NHLS, Division of Human Genetics (Johannesburg) for BTH molecular testing. In the majority point mutations or small deletions or insertions are identified.

Thirteen unrelated families, with one clinically suspected proband, were screened. MLPA analysis was performed to confirm the presence of deletions in 4 probands previously indicated to have a possible deletion using linked markers (LM), as well as to screen 9 probands with suggestive haematological indices in whom one (4 probands) or no causative mutations (5 probands) were identified by sequence analysis.

MLPA analysis confirmed the presence and the extent of the deletion in 3 of the 4 families. The deletions varied in size. No deletions were identified in the other 9 probands.

MLPA analysis is a useful additional test to define large deletions in the β-globin gene cluster and allowed for more accurate diagnosis, prenatal, carrier testing and genetic counselling in these families.

PP 37

Application of Array-based comparative genomic hybridisation (aCGH) Microarray CGH in the South African paediatric context.

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Array-based comparative genomic hybridisation (aCGH) is used to detect submicroscopic DNA copy number variants (CNV) not detectable by routine karyotyping. In this pilot series we aim to investigate the application of aCGH in a South African paediatric tertiary hospital setting, to obtain initial information on the detection rate for clinically relevant CNVs and assess issues in the interpretation of results.

The clinical genetics team selects patients from the neonatal and paediatric population of Tygerberg Hospital for diagnostic aCGH using the Agilent ISCA 60K oligoarray. For inclusion the patient needs to be diagnosed with developmental delay/intellectual disability with/without congenital abnormalities (CA), or with multiple CA alone, and have a normal karyotype.

Results generated thus far (12 cases) show 4 (33%) abnormal DNA gains and/or losses, 2 (17%) imbalances of uncertain significance and 6 (50%) no significant imbalance. Of the potentially disease-causing changes, one is novel. Further data on phenotypes and additional patients will be presented.

Early results suggest a high detection rate for disease-causing CNVs compared to the published literature, however the application of stringent selection criteria may play a role in this. The high detection rate of aCGH may offset its high cost, since early aCGH may reduce additional costs related to diagnosis and treatment. However the interpretation of results is not always straightforward and is dependent on data available in international databases (which may not be relevant to local populations).

PP 38

An additional BRCA founder mutation in the South African Ashkenazi Jewish population?

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The approach to mutation analysis in suspected hereditary breast and ovarian cancer (HBOC) families constitutes genetic analysis of the tumour suppressor genes, *BRCA1* and *BRCA2*. While many families carry private mutations, certain population groups (the best example of which is the Ashkenazi Jewish population) have higher frequencies of specific *BRCA1* and/or *BRCA2* mutations, due to founder effect. Three mutations, (two in *BRCA1* and one in *BRCA2*) together, account for 80-90% of disease-causing mutations in the Ashkenazi Jewish population. Routine diagnostics in South Africa (SA) offers screening for these 3 mutations, but full gene sequencing is only available to private patients.

A SA Ashkenazi Jewish family has been identified which carries a mutation in *BRCA2* (c.2808_2811delACAA), other than the common founder one described. However, this is not a private mutation, but a common mutation, which has been described, in several other ethnic groups.

The aim of this study is to determine the frequency of this mutation in a cohort of SA Ashkenazi Jewish patients (N=60) who tested negative for the 3 common founder mutations.

If this *BRCA2* mutation occurs at a significant frequency, identifying it as a fourth founder mutation, it will be included in the routine diagnostic testing for *BRCA* mutations in SA Ashkenazi Jewish patients. Founder mutations not only provide cost-effective, population-specific testing approaches, but also allow for the study of penetrance of a particular mutation in a given population.

PP 39

RAD51C Germline Mutations in South African Breast / Ovarian Cancer Families

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Breast cancer is the second most common cancer affecting women in South Africa. Mutations in two high-risk breast cancer susceptibility genes, *BRCA1* and *BRCA2*, account for approximately 66% of hereditary breast cancer in South Africa. Recently another gene, *RAD51C,* has been identified as a moderate-to-high risk susceptibility gene for breast and ovarian cancer. To investigate the role of *RAD51C* mutations in South African breast and/or ovarian cancer families, we screened 74 patients (negative for *BRCA1* and *BRCA2* mutations) from 69 families for mutations in this gene. Direct Sanger sequencing and Multiplex Ligation-dependant Probe Amplification (MLPA) analysis was performed. Eight different sequence variants were detected (one frameshift, two nonsynonymous, one synonymous and four noncoding variants) in 61 patients. The frameshift mutation (c.93delG p.Gly31GlyfsX9) is one of only 24 disease-causing mutations that have been identified world-wide. Interestingly our family is a breast cancer only family with no reported ovarian cases, whereas all other disease-causing mutations have been reported to occur in families with breast and ovarian cancer. The c.93delG mutation has been reported as a founder mutation in Finnish breast and ovarian cancer families. Haplotype analysis of the South African and Finnish mutation carriers shows a shared common haplotype. The family has been counselled and predictive testing for family members is ongoing. Thus *RAD51C* germline mutations account for 1.4% (1/69) of breast cancer families in South Africa.

PP 40

Identification of an SDHB founder mutation in Afrikaner paraganglioma families

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Paraganglioma (PGL) and pheochromocytoma are rare tumours occurring in the head-and-neck, intra-abdominal and thoracic paraganglia which may lead to significant morbidity. It has been shown that familial paraganglioma is caused by germline mutations in the genes encoding subunits of the succinate dehydrogenase (SDH) mitochondrial complex II. The aim of our study was to identify disease-causing mutations in the SDH susceptibility genes of 12 South African (SA) paraganglioma families. These families predominantly presented with head-and-neck PGL with three families presenting with abdominal tumours. Five of the families had metastatic tumours.

The *SDHB*, *-C*, *-D* and *SDHAF2* genes were investigated for mutations using bi-directional Sanger sequencing and Multiplex Ligation-dependent Probe Amplification (MLPA).

A non-synonymous mutation in the *SDHB* gene [Ile127Ser] was detected in one family and a 7905bp deletion [c.201-4429_287-933del.] removing exon 3 of *SDHB* was detected in 11 Afrikaner families. Interestingly this deletion has been shown to be a founder mutation in the Dutch population. Haplotype analysis of the South African and Dutch patients revealed a common haplotype at the *SDHB* locus. Three families show reduced penetrance and only one of the patients lacked family history.

The identical exon 3 deletions and common haplotype in the Afrikaner patients indicates that this deletion is the first Afrikaner *SDHB* founder mutation, possibly introduced into SA by the Dutch. Ultimately, detection of disease-causing mutations will enable predictive testing of other family members and allow better clinical management of these families.

PP 41

MYOC Variants in Primary Open-Angle Glaucoma in Black South Africans

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Purpose

Mutations in the *MYOC* gene are important as causal factors in some forms of primary open-angle glaucoma (POAG). The aim of this study was to examine the role of three previously identified mutations in the aetiology of POAG in this population.

Methods

The mutations (Gly374Val, Lys500Arg and Tyr453del) were genotyped in 215 black South African POAG patients and 214 controls using the Illumina BeadXpress genotyping platform. Family members of participants identified with the mutations were counselled and screened for glaucoma. Sanger sequencing was used for mutation detection.

Results and conclusions

Black South Africans with POAG may have a *MYOC* mutation that either causes or contributes to their risk for developing POAG in approximately 3.3%. The commonest mutation is a frameshift mutation (Tyr453del) that is incompletely penetrant. It is likely that the mutation causes gain-of-function due to a truncated protein. That the mutation is necessary but insufficient introduces a counselling dilemma. Mutation screening can, however, identify high-risk individuals who can be monitored to detect early signs of the disease. The Gly374Val mutation is predicted to be damaging to MYOC. It is an uncommon cause of POAG in this population. The Lys500Arg mutation is predicted to be benign and tolerated. In the family with the mutation, it did not segregate with the disease suggesting that it is a neutral polymorphism. This study has important implications for the management and counselling of black South African patients with POAG and their families.

PP 42

Association of candidate schizophrenia loci with antipsychotic response in a South African first episode schizophrenia cohort

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Schizophrenia is a complex disorder with diverse symptoms of varying severity. Although GWAS have identified many significantly associated variants, mainly in Caucasians, these findings may not extrapolate to other populations. This study investigated whether the 10 SNPs most significantly associated with schizophrenia risk, identified by the Psychiatric GWAS Consortium (PGC), were associated with antipsychotic treatment response in a South African cohort.

The ten most significant SNPs were genotyped in a first episode schizophrenia (FES) cohort of 104 South Africans. Variants predicted to alter the protein products of genes closest to these SNPs were identified from exome sequencing data of 11 FES individuals and genotyped in the whole cohort. Associations with antipsychotic response, measured by repeated Positive and Negative Syndrome Scale (PANSS) scores over twelve months of treatment, were determined.

Seven non-synonymous variants were identified in the exome data, of which two were novel. Of the 17 genotyped variants, the most significant was one of the SNPs identified by the PGC, which showed associations with changes

over time in negative (P=9.4x10^{-8}), total (P=3.9x10^{-5}) and general (P=0.0033) PANSS. The most significant exome variant demonstrated associations for changes over time in general (P=0.0002), negative (P=0.0032) and total (P=0.001) PANSS, indicating a novel variant associated with schizophrenia.

This study has established a role for some of the PGC findings in schizophrenia treatment response in South Africans, and identified novel variants in this cohort. Combined with future studies, these findings could aid in the improvement of current treatments for schizophrenia in the African context.

PP 43

Whole exome sequencing of South African breast cancer families - Preliminary Results

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Approximately 5-10% of breast cancer (BC) cases occur in women with multiple family members affected by this disease. Of these, approximately 30% are caused by germline mutations in the high-risk *BRCA1* &*2* genes. Germline mutations in other genes (e.g. *RAD51C, PALB2 &ATM*) account for only 3-5% of familial BC cases. Therefore, the genetic etiology of many multiple breast cancer families is unknown. Our research is aimed at identifying variants in novel high-penetrant genes that may cause increased susceptibility for breast cancer. Nine individuals (negative for *BRCA1* &*2* mutations) were selected from 6 families with a history of breast and/or ovarian cancer (≥4 cases). Paired-end library preparation and exome enrichment was performed with the Agilent SureSelect human exon (51Mb) kit by the Beijing Genomics Institute. Illumina sequencing was carried out with the Genome Analyzer IIx system. Paired-end reads were aligned with the Burrows-Wheeler Aligner duplicate reads were removed with Picard followed by local realignment and variant calling with the Genome Analysis Toolkit (GATKvs2.4.9). Variant annotation was done with ANNOVAR. On average 80% of the reads aligned to target regions captured by the enrichment libraries and ~93% was covered by 10X sequence depth. Among the 9 individuals, an average of 11 deleterious [range 6-17] and 86 nonsynonymous [range 69-116] mutations were discovered as novel variants, absent from dbSNP or 1000genomes databases. A list of high-priority genes is being generated for further validation.

PP 45

Implementation of a semantic knowledge base in discovering functional variants in the exomes of Multiple sclerosis case studies.

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Multiple sclerosis (MS) is a prevalent multifactorial inflammatory disease, causing demyelination of axons in the brain and the Central Nervous System. It is hypothesized that genetics may play a crucial role in the development of disease.

We have performed whole exome sequence analysis in four MS cases also presenting with non-anemic iron deficiency which, when rectified through iron supplementation, halted demyelination. We identified 49 'high impact' variants, including 5 frameshifts, and 44 predicted functional missense variants, overlapping between all four cases. As MS is known to be a multi-genetic disease, we developed a variant prioritization strategy that relies on a semantic model of the disease implemented in our BioOntological Relationship Graph (BORG) database. The model links the 'multiple sclerosis' term in the Disease Ontology to terms relevant to the disease in other ontologies, e.g. 'demyelination' or 'abnormal myelination' in the Phenotype Ontology and functions relating to 'myelination' and 'inflammation' in the Gene Ontology. As phenotypes known to be associated with both human genes and those arising in mouse and rat gene knockout experiments are modeled in the BORG, we used a concept of 'guilt-by-indirect-association' to implicate candidates whose roles are unapparent yet biologically plausible.

We present a combination of high-impact variants, many which are novel, in genes involved in myelination and the immune response as putative contributors to the development of MS and also present variants that may explain the iron deficiency phenotype.

PP 46

The molecular aetiology of inherited breast cancer in the South African black population

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Hereditary breast cancer (HBC) caused by mutations in the *BRCA1* or *BRCA2* genes have been well studied in Caucasian and Ashkenazi Jewish populations. Little is known about the genetic aetiology or the clinical epidemiology of the disease in the South African black populations. Founder mutations have been described for numerous populations around the world. The South African black population exhibits a higher than expected incidence of familial breast cancer, often presenting with early age of onset and rapid progression of disease. It is possible that a founder mutation for HBC exists in the South African black population.

This study aimed to investigate the *BRCA1* and *BRCA2* genes for germline mutations in a high-risk South African black breast cancer cohort (33 patients) presenting with breast cancer. Sanger dideoxynucleotide chain termination sequencing analysis and Multiplex Ligation-dependent Probe Amplification (MLPA) analysis was performed to screen for mutations present in either *BRCA1* or *BRCA2.*

Point mutations in *BRCA1* and *BRCA2* account for 9% of the HBC cases in our cohort. Three non-recurrent pathogenic *BRCA* mutations were identified: c.431dupA in *BRCA1*; c.572G>A &c.7712A>G in *BRCA2*. No large gene/exon deletions/duplications were detected. Five novel sequence variants and 51 previously reported single nucleotide polymorphisms (SNP) were detected. Through statistical analysis, three SNPs conferring increased relative genotype risks for developing breast cancer were also identified (rs28897679; rs169547; rs2219594).

BRCA mutations account for some of the HBC risk within our cohort. Other genes predisposing to HBC should be investigated.

PP 47

Development of a genotype risk score for non-alcohol fatty liver disease (NAFLD) as the hepatic manifestation of the metabolic syndrome

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The disease spectrum of non-alcoholic fatty liver disease (NAFLD) extends from steatosis to non-alcoholic steatohepatitis (NASH) with inflammation. Three single nucleotide polymorphisms (SNPs) in the TNF-α (rs1800629), FTO (rs9939609) and PPARγ (rs1801282) genes associated with components of the metabolic syndrome were studied to determine their potential role in the disease process underlying NAFLD/NASH.

A total of 119 patients with fatty liver identified on ultrasound, including 88 histologically confirmed NAFLD patients and 166 controls were genotyped for the selected SNPs using real-time PCR. Relevant biochemical parameters and environmental factors were also assessed using the R software for statistical analysis.

The minor allele frequency of TNF-α rs1800629 was significantly higher in the total NAFLD (p=0.047) as well as NASH subgroup (p=0.030) compared with obese patients without a histologically confirmed NAFLD diagnosis. The onset of fatty liver disease symptoms was on average 5 years younger in the presence of each risk-associated TNF-α rs1800629 A-allele (p=0.028). When considered in the context of a genotype risk score ranging from 0-6, disease onset occurred on average 3 years earlier (p=0.008) in the presence of each risk-associated allele. A significant association was observed between the presence of the TNF-α minor A-allele and increasing C-reactive protein levels (p=0.029), with a favourable reduced effect in the presence of low alcohol intake.

Obesity was excluded as a significant risk factor for progression from NAFLD to NASH. Assessment of the genotype risk score in conjunction with a clinical

and lifestyle assessment may facilitate improved clinical management of patients with NAFLD.

PP 48

Effects of xenobiotic/drug exposure on differential expression of drug metabolizing enzyme (DME) genes : pharmacogenomic implications in cancer prognosis

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Response to medication and survival after cancer treatment vary among individuals. This is largely attributed to genetic variability, in genes encoding drug metabolising enzymes (DMEs). Studies on the effects of anticancer drugs on the expression of DME genes are crucial to understand pharmacogenetic correlates of differential response.

The aim of this study is to evaluate how certain drug-gene interactions affect individual patient response to chemotherapy. Specifically, changes in DME gene expression in response to anticancer drug treatment are the focus of the study. Ultimately, gene expression profiles associated with better prognosis to cancer therapy are sought.

The human-derived cell lines WHCO1, WHCO5 and WHCO6 were treated with 3 clinically relevant drugs: 5-fluorouracil, doxorubicin and cisplatin, as well as known *CYP1* inducers. Following treatment, effects on cell proliferation were determined using the MTT assay. Expression of key DME genes *CYP1A1*, *CYP1A2*, *CYP1B1* and *GSTP1* in response to treatment was elucidated through RNA extraction and real-time PCR, whilst the corresponding protein expression was determined through Western blot analysis.

There were differences in the effects of each of the drugs in each cell line, as represented by IC_{50} values. Experiments to determine gene and protein expression profiles in response to drug treatment are currently being conducted, and data will be available by the time of the conference.

A better understanding of the complex interplay between genes and drugs holds the key to better precision medication.

PP 49

Familial breast cancer in the Indian population of South Africa

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The familial breast cancer (BC) genes *BRCA1* and *BRCA2* play a role in transcription, DNA repair of double-stranded breaks and recombination. Mutations in these genes account for ~40% of inherited BC families and more

than 80% of familial breast and ovarian cancer families. Knowledge of the prevalence of these mutations in a specific population such as the Indians of South Africa (SA) is necessary to provide accurate genetic counselling.

We are currently conducting a study on Indian BC patients requesting testing for the presence of *BRCA* mutations. All the families had extensive pedigrees, which confirmed a familial inheritance. In this pilot study, we studied 35 affected Indian women. Screening commenced with PTT for *BRCA1* exon 11, and *BRCA2* exons 10&11 as no *BRCA* information for this population is known.

This initial screen revealed three (8.7%) deleterious mutations (two in *BRCA1* exon 11 and a single mutation in *BRCA2* exon 11), each currently unique to that specific family. Four (11.4%) additional disease-causing mutations (*BRCA1* exon 22 and *BRCA2* exons 21 and 25) were identified using SSCP/HA as screening method for the smaller exons. The mutation in *BRCA2* exon 21 is the first recurrent mutation, as it was detected for two separate families.

This study examines for the first time the characteristics of hereditary BC in the Indian population of SA. This data is expected to contribute to more accurate tools for clinical management and diagnostic testing of these patients for hereditary BC in the future.

PP 50

Is a familial breast cancer screen on the cards for the Mixed Ancestry population of the Western Cape?

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The incidence of breast cancer (BC) is increasing in South Africa (SA), specifically in mixed ancestry women from the Western Cape. This could be due to the adoption of a more western lifestyle that includes women being physically less active and having fewer children. *BRCA* mutations contribute to the BC burden within this community. Although a founder mutation has been identified, the spectrum of recurrent disease-causing mutations is not known.

We studied 189 BC patients from the Western Cape requesting testing. The selection criteria were an early age at diagnosis, presence of a family history or both. Seventy-two patients (38%) reported a minimum of one relative affected with BC and/or another type of cancer. The majority of patients (62%) were referred due to an early age at onset <<a>50 or bilaterality of the disease.

All were initially screened for the panel of seven common SA mutations that included the mixed ancestry/Xhosa founder mutation (delivered 14 positive test results). The protein truncation test (*BRCA1* exon 11 and *BRCA2* exons 10&11) was performed for 96 (5 positive results), with a total of 24 being fully

screened (2 positive test results). A total of six disease-causing mutations contributed to 10.58% (20) of patients receiving a positive test result.

The results indicated that screening in a step-wise fashion, is cost and time effective as the majority of positive results were obtained using the first two test options. The likelihood of obtaining a positive result was directly linked to the presence of a positive history.

PP 51

Familial breast cancer screening of the Sotho/Tswana population of the Free State: Do we have an effective testing strategy?

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Breast cancer (BC) is a leading cause of mortality for women. Methods to reliably detect the earliest stages have been widely sought. These include using DNA technology to screen for mutations within the familial BC genes, although at a high financial cost. South Africa's health crisis results in the majority of State patients being overlooked regarding genetic screening for familial breast cancer. This is due to a lack of resources and the absence of genetic counsellors to select appropriate candidates.

The aim was to investigate whether the screening methods used for the Sotho/Tswana patients from the Free State were effective to detect mutations within this group. Thirty seven patients were selected based on either an early age at diagnosis (≤45), the presence of a family history or both. Forty three percent reported a positive family history, ranging from very low to moderate risk pedigrees, whereas 48.6% had no family history. Screening commenced with PTT for *BRCA1* exon 11, and *BRCA2* exons 10&11, where after the smaller sections were analyzed using SSCP/HA and direct Sanger sequencing.

A total of four disease-causing mutations were detected (10.8%), all identified using PTT. Three of these are novel, whereas the fourth represented the Coloured/Xhosa founder mutation. Although the SSCP/HA screen of the remaining sections revealed various variants, most were intronic with unknown clinical significance.

Our testing strategy proves to be effective for the Sotho/Tswana population as our first tier mutation detection technique identified all of the mutations currently identified for this group.

PP 52

CCR2, CX3CR1, Rantes and SDFL Genetic Polymophisms and their influence on HIV infection in a Zimbabwean Paediatric Population

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There is growing evidence that single nucleotide polymorphisms (SNPs) in host genes encoding chemokines and chemokine receptors may influence susceptibility to HIV infection and/or HIV/AIDS disease progression. However, not much is documented on the prevalence of these mutations in the Zimbabwean population despite the high burden of HIV/AIDS in the country. This study describes polymorphisms in *CCR2*, *CX3CR1*, *SDF1* and *RANTES* genes in a Zimbabwean paediatric population and their possible association with HIV infection in children born to HIV-infected mothers. One hundred and six children comprising 70 perinatally exposed to HIV, 34 of who were born infected (EI), 36 who were uninfected (EU) and 36 unexposed uninfected (UEUI) controls, all recruited from a follow-up study of mother-child pairs at 7-9 years of age. Genotyping for allelic variants was done using PCR-RFLP, SNaPshot® and Sanger DNA sequencing. The frequencies of the *CCR2* 190A, *SDF1* 801A, *CX3CR1* 745A, *CX3CR1* 839T, *RANTES* In 1.1C and *RANTES* -403A alleles in the HIV-uninfected group (EU+UEUI) group were; 16%, 2%, 9%, 1%, 20% and 44%, respectively. The distribution of *CCR2* 190G/A (P=0.02) genotype, *CCR2* 190G/A-*CX3CR1* 745G/G (P=0.0002) and CX3CR1 745A/A-RANTES In1.1T/T (P=0.05) genotype combinations were significantly different when compared between the HIV-infected and HIV-uninfected groups, both perinatally exposed occurring in 15% v 39%, 0% v 33% and 10% v 0%, respectively. Our findings suggest that chemokine and chemokine receptor gene variants may affect critical pathways in HIV infection singly and in combinations. These combinations may present new therapeutic targets that are more effective against HIV.

PP 53

The influence of ESR1, TNRC9 and MAP3K1 on the expression of breast cancer in the Afrikaner BRCA2 carriers

S Schneider (University of the Free State), N van der Merwe (National Health Laboratory Service and University of the Free State), B Visser (University of the Free State)

The objective is to evaluate the effect of previously identified polymorphisms in several low penetrance genes to elucidate the variation in phenotypic expression observed within *BRCA2* c.8162delG (c.7934del, p.Arg2645AsnfsX3) mutation positive families.

Sixty *BRCA2* 8162delG mutation positive women were selected, of which 30 were affected with breast cancer. Each of these mutation carriers was case matched for age with healthy controls. All 120 participants were genotyped for two polymorphisms (rs2234693 [*PvuII*] and rs9340799 [*XbaI*]) in *ESR1* using PCR and restriction enzyme digestion and polymorphisms rs3803662 in *TNRC9* and rs889312 in *MAP3K1* using TaqMan® genotyping assays.

Of the four polymorphisms analyzed, only rs2234693 (*PvuII*), indicated a possible association with breast cancer (P-value = 0.0896). Haplotypes were compiled for rs2234693 (*PvuII*) and rs9340799 (*XbaI*) with no significant differences between the breast cancer patients and the controls. These results may have been due to the high allelic heterogeneity observed within the Afrikaner population, as well as the small test group used.

The results of the study provided insight into allelic distributions of the SNPs in the Afrikaner *BRCA2* 8162delG mutation carriers specifically. Larger scale genotyping could lead to more significant findings to help elucidate the polygenetic nature of breast cancer with the Afrikaner.

PP 55

CYP3A4 -392G>A SNP Has No Role in Lopinavir Associated response

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Introduction: The use of combination therapy for HIV/AIDS treatment has contributed to the reduction seen in HIV/AIDS related morbidity and mortality. Lopinavir is a protease-inhibitor drug recommended for use within second-line Anti-Retroviral treatment (ART) regimens. It is used in conjunction with ritonavir, another protease inhibitor, which prevents the high first-pass metabolism of lopinavir by CYP3A4. In addition to CYP3A4, OATP1B1, MRP2, PXR and P-glycoprotein are also involved in the disposition of lopinavir. We set out to investigate whether variation in CYP3A4 plays a role in the clinical responses observed in HIV/AIDS patients.

Methods: 70 patients were recruited from Malawi (Queen Elizabeth ART Clinic, Blantyre) and Helen Joseph Hospital. The following clinical parameters were available for the patients: age, CD4 count and viral load. Genotyping for the CYP3A4 -392G>A SNP was performed using PCR/RFLP.

Results: 37.1% of patients were below the age of 40; 23.1% of patients had a viral load greater than 400 RNA copies/mL; and 55.6% of the patients had a CD4 count below 350 cells/mm^3. The genotype frequencies for A/A; A/G and G/G were 0.44, 0.33, and 0.23 respectively. No significant correlation was found between the genotypes and the clinical parameters.

Conclusion: CYP3A4 may play no role in lopinavir pharmacogenetics. There is need to consider other genes.

PP 57

Genetic diversity of the SLC22A2 gene within the Cape Admixed population

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Genomic diversity within sub-Saharan Africa, and for that matter the entire African continent, is relatively under-studied, despite being home to significant portion of human genomic diversity. South Africa in particular contains a wealth of different population groups. The country is indeed home to the indigenous Khoisan, Xhosa, Zulu, Venda, Sotho and Pedi groups, the Afrikaners and the Cape Admixed, the latter being a uniquely admixed population of immigrant Europeans, Asians and the indigenous populations.

Despite this fact, it is well recognized that African populations have been underrepresented in global pharmacogenomics research. The aim of this study was to investigate the genetic diversity of the SLC22A2 gene and to deduce its possible pharmacogenetic implications within the Cape Coloured population of South Africa.

A total of 100 samples in the form of buccal (oral) swabs were collected from healthy, unrelated donors from the Cape Admixed population residing within the Cape Town metropolitan area. A SNaPshot® Multiplex System was specifically designed for the study, successfully optimized and used for genotyping. One hundred genetic profiles were then generated for a total of 10 SNP variants on SLC22A2 gene, using this primer extension-based genotyping method. Important population genetic parameters were calculated, and possible pharmacogenetics implications were determined.

Approximately 80% of the Cape Admixed population displayed a non-variant haplotype. The remaining 20% of the population presented haplotypes in which only one variant allele was detected. Although few variant alleles were detected, they generally occurred singly in a specific haplotype. It may be suggested that the combination of loss-of-function variants might effect a decrease in the protein's transport activity. Furthermore, the allele and genotype frequencies determined within this population bears immense similarity to the global populations they were compared with. This may suggest that the study population may benefit by genetic testing to determine specific dosage.

It may therefore be concluded that the variants displayed within the Cape Admixed population, being uniquely admixed, is indeed comparable to the global population. These findings may contribute in filling the gap of missing important pharmacogenetic data from African populations.

PP 58

Development of two genotyping multiplexes to detect SNP variations in the Organic Cation Transporter (OCT) 1

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Objective: To develop and optimize two inexpensive genotyping systems to detect variations in the SNPs of OCT1.

Basic Procedures: Three buccal swabs were collected from each study participant after ethical clearance was obtained. Genomic DNA was extracted from the buccal swabs, in triplicate, using the in-house phenol-chloroform and salt-lysis DNA extraction protocols and quantified. The polymerase chain reaction (PCR) primers of this study were designed to amplify all 11 exons of OCT1 and the resulting amplicons used as templates for subsequent Single Base Extension (SBE) reactions using the optimized conditions. SNP variants for this study were selected based on possible clinical and pharmacogenetic importance or implications, cited in literature, or predictive functional changes in OCT1 based on data from the PharmaGKB; Ensembl and NCBI dbSNP

databases. The SNP primers of this study were designed to genotype the first 10 SNPs, in multiplex 1 (SBE1), and the remaining 10 SNPs in multiplex 2 (SBE2). SBE 1 and 2 multiplexes were carried out after post-PCR amplification and prior to post-extension purification, followed by genotyping of samples. The optimal annealing temperature and concentrations of template, primers and reaction mix for each multiplex PCR was determined through trouble-shooting.

Main Findings: The optimal concentrations for the primers, reaction mix and template; as well as the optimal PCR conditions for both genotyping multiplexes were successfully determined.

Principle Conclusion: The technique presented herein is effective, efficient, and inexpensive and provides a reliable means for genotyping large quantities of samples relatively quickly. This technique can be further adapted for more extensive forms of assays and research interests.

PP 59

Uptake of carrier testing for ATR-X syndrome: communication and attitudes in a South African family

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Alpha thalassaemia X-linked mental retardation (ATR-X) syndrome is a rare, X-linked intellectual disability syndrome. The prevalence in South Africa (SA) is unknown; however in Cape Town there is one extended family with seven males who were clinically and molecularly diagnosed with this condition. Due to the identification of the mutation in this family, carrier and prenatal testing is available. However, since the availability in 2007, no individuals have presented for testing. The aim of this study was to investigate the reasons why females in this family have not presented for carrier testing.

A phenomenological qualitative approach was used in this study. Eleven semi-structured interviews were performed with female relatives of a male with ATR-X syndrome. The participants were recruited by convenience and snowball sampling and did not have an affected son themselves. Thematic analysis was used to analyse the data.

The majority of the 11 participants viewed their affected relatives positively, which was closely tied to a religious framework; however they still felt that they wanted carrier testing. Elicited reasons for previous non-uptake included being unaware of the availability of testing, who to contact, socioeconomic reasons, low perception of risk and expectations from previous research. Furthermore there was generally a lack of communication and dissemination of genetic knowledge within this family.

These findings assist in understanding some of the barriers to the uptake of genetic testing and dissemination of information in a South African context. This has implications for the way in which research and informed consent is performed.

PP 60

Inversion Chromosome 9: Normal variant OR clinically significant?

M Blaauw (National Health Laboratory Service)

Chromosome inversions are a relatively common structural alteration. Chromosome 9 presents the highest degree of morphological variations among chromosomes. Pericentric inversion of chromosome 9 is a common heteromorphism. Though it is considered a normal variant, many reports in recent literature link it with infertility and recurrent miscarriages.

The aim of this project is to examine the clinical diagnoses of the patients received in the Cytogenetics laboratory, NHLS at Groote Schuur Hospital with a pericentric inversion of chromosome 9.

A survey was conducted on Blood, Amniotic Fluid and Bone Marrow samples from January 2008 to March 2013.

The Blood samples were examined further for reason of referral and clinical significance with the focus on sex abnormalities and recurrent miscarriages.

57 Patients were diagnosed with pericentric inversion of chromosome 9 on Blood samples. 23 were found on Amniotic fluid and only 6 on Bone Marrow samples.

The 4 main reasons for referral on the Blood samples were sex abnormalities, recurrent miscarriage, trisomy and inheritance.

There was only 6 pericentric inversions of chromosome 9 found on Bone Marrow culture, indicating that it is not as random in a normal population as previously thought.

It seems possible that, depending on the specific breakpoints, pericentric inversion of chromosome 9 can be linked to infertility and recurrent miscarriage, since these were the most common reasons for chromosome analysis on Blood referrals.

PP 61

CLOVES syndrome: A report of two cases

E Honey (University of Pretoria)

CLOVES (**C**ongenital Lipomatous **O**vergrowth, **V**ascular malformations and **E**pidermal nevi and **S**keletal anomalies) syndrome presents as a distinct clinical phenotype characterized by complex truncal lipomatous masses. Vascular, acral and other anomalies are commonly seen. It is hypothesized that CLOVES syndrome is caused by somatic mutations arising during early embryonic development and activating mutations in *PIK3CA* has recently been described.

Two patients were referred to the Genetics department of the University of Pretoria with overgrowth and lipomatous masses of the trunk. Neither of the patients fulfilled the diagnostic criteria of Proteus syndrome or any of the other overgrowth syndromes and the diagnosis of CLOVES syndrome was made. Their clinical features, radiological findings and management will be presented

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PP 62

Myeloma fluorescence in situ hybridisation (fish) without plasma cell labelling - are we missing something?

D Taylor (National Health Laboratory Service)

Multiple myeloma is a malignant plasma disorder. These malignant cells carry genetic changes which have both prognostic and treatment implications. The

Groote Schuur Cytogenetics laboratory offers a myeloma FISH panel which includes t(4;14). The presence of this abnormality identifies high risk patients. Literature suggests an occurrence rate of t(4;14) in 15-20% of patients . When we compared this statistic to our data we found a marked difference. t(4;14) was seen in only 4% of our patients. In light of this we designed a study to determine whether or not performing plasma cell labeling combined with FISH would produce clinically relevant differences.

Bone marrow aspirate received for myeloma FISH studies was harvested using the standard 24hr culture method. A slide from the cell pellet was made and processed using standard FISH protocol. The remaining cell pellet was processed according to the protocol for cytoplasmic immunoglobulin staining. For both slides the t(4;14) FISH probe was used.

Using the cIg FISH technique, it became apparent that most of our samples were contaminated with large numbers of normal cells and this lead to the under-reporting of abnormalities, particularly the numerical abnormalities e.g. trisomy/tetrasomy of chromosomes 4 and 14.

Sampling difficulties and the nature of myeloma to be a patchy disease can result in FISH being reported on normal cells. Without plasma cell labeling, normal cells are therefore scored together with plasma cells. This can lead to the reporting of false negative results, which can impact the clinical management of the patient.

PP 63

Familial reciprocal translocations

H van der Horst (National Health Laboratory Service)

Carriers of reciprocal translocations are usually unaware of their karyotype status. Reciprocal translocations are usually harmless and may be found through pre- or postnatal diagnosis. Translocation carriers of balanced reciprocal translocations have an increased risk of miscarriages or having affected children.

In our Cytogenetics laboratory (NHLS) at Groote Schuur Hospital, Amniotic Fluid and Blood samples are received due to abnormalities detected on ultrasound, dysmorphic features, recurrent miscarriages or a known family history of reciprocal translocations. If an apparently balanced translocation is found, it is important to verify the parental chromosomes to establish inheritance.

The aim of this study is to establish whether the reciprocal translocation, or the unbalanced form, was either inherited or de novo. A retrospective study of Blood and Amniotic Fluid samples for karyotyping were done between 2009 and July 2013. Thirteen families were investigated to determine whether the translocations were inherited or de novo as well as whether they were balanced or unbalanced.

The analysis demonstrated that the majority of reciprocal translocations were inherited rather than de novo. There was also a bias on the translocations detected on the blood karyotypes to be in the unbalanced form compared to those detected on Amniotic Fluid karyotyping, which tended to be in the balanced form. Confirming an inherited reciprocal translocation has

implications for counselling of the patient and the parents as well as implications for future pregnancies. Other family members may also be affected.

PP 64

Mutations in GJB2 do not play a significant role in recessive Non-Syndromic Sensorineural deafness in patients from Sub-Saharan Africa

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Mutations in the GJB2 gene encoding connexin-26, could account for 50% of congenital non-syndromic recessive deafness in some Caucasian and Asiatic populations. There is a paucity of published data on sub-Saharan Africans.

We sequenced the coding region of the GJB2 gene, in 180 Cameroonian and 25 South African patients with congenital non-syndromic hearing loss, and healthy controls; and performed bioinformatic analysis of variations in the GJB2 gene; using data from the 1000 Genomes project.

Amongst Cameroonian patients, 26.11% were familial and 5.56% were consanguineous. The majority of patients (70%) suffered from sensorineural hearing loss. Two pathogenic mutations were detected in Cameroon but none in South Africa. Two novel variations, g.3318-41G>A and g.3332G>A, amongst 8 polymorphisms, were detected. The most common variations in both populations were the intronic change g.3318-34C>T and two changes in the 5'UTR, g.3318-15C>T and g.3318-6T>A. Principal Components Analyses (PCA) indicates that independent SNPs in GJB2 clustered in groups, matching geographical location and ethnicity. But, the share of the total variance was only 38%. The second PCA, including only our cohort, indicated 87% of the total variance, but most SNPS only occur once. There were no statistically significant differences between allele, genotype or haplotype frequencies between cases and controls.

Mutations in GJB2 do not play a major role in congenital non-syndromic genetic deafness in Africans. Whole exome sequencing on familial cases is needed to search for genes involved in congenital non-syndromic hearing loss in Africans.

PP 65

Genetic Factors Influencing Inhibitor Development In A Cohort of South African Haemophilia A Patients

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A critical complication of factor VIII (FVIII) concentrate replacement therapy in Haemophilia A (HA) treatment is inhibitor development. Genetic factors predisposing to inhibitors include *F8* gene mutations, ethnicity, family history of inhibitors and FVIII haplotype mismatch. Knowledge of genetic risk factors is important in predicting inhibitor risk and planning exposure to rapidly evolving replacement therapy. There are currently no published studies characterising genetic risk factors for inhibitor development in the South African haemophilia population. The objective of the study aimed to characterise and correlate HA disease severity, inhibitor development, intron 22 inversion mutation (int22) status, ethnicity and FVIII haplotype in a South African severe HA (sHA) cohort. A cohort of sHA patients who had inhibitor and int22 analysis between 1994 and 2011 were reviewed. The int22 positive patients had FVIII haplotype analysis. Disease severity, ethnicity, haplotype mismatch, inhibitor and int22 status were correlated. Of the 229 HA patient records reviewed, 116 (51%) were black and 113 were white. Of these, 82 (36%) patients were int22 positive (of which 46 were black) and 29 (13%) had inhibitors (of which 21 were black). The H1 and H2 haplotypes were the most common in the cohort. In this sHA cohort comprising equivalent black and white patients, black int22 positive patients had the highest frequency of inhibitors compared to black int22 negative, white int22 positive and int22 negative patients. Ethnicity and *F8* gene mutation type were found to be important factors predisposing to inhibitor development. *F8* gene haplotype may contribute to inhibitor development.

PP 66

Molecular Analysis of the RSPO1 and WNT4 genes in South African SRY-negative Intersex patients

P Yu (University of Pretoria), N Oosthuizen (University of Pretoria)

Human gender is characterized by physical appearances of the external and internal genitalia. Establishment of gender relies on both chromosomal assignment of the zygote and environmental factors. Sex-determining chromosomes contain genes that direct foetal development of internal and external sexual organs. Abnormalities in these signals could result in erroneous sexual development. Patients with disorders of sexual development (DSD), in particular, *SRY*-negative Ovotesticular DSD were studied. Two genes associated with the disorder, *R-SPONDIN1 (RSPO1)* and *Wingless-related MMTV* (*WNT4),* were analyzed.

Patients' DNA was screened to determine presence or absence of the *SRY* gene. *SRY*-negative patients were further analyzed. The *RSPO1* and *WNT4* genes were analyzed using conventional PCR, real-time PCR and HRM analysis in combination with sequencing to detect polymorphisms in patient DNA that were absent in published consensus sequences.

Six out of the seven patients studied were *SRY*-negative. Sequencing of the *RSPO1* region showed that patient 2 had a single nucleotide polymorphism G>R at position 4280. In the *WNT4* region, there were several SNP's: patient 5 C>Y at 13376, patient 1 G>R at 24489, patient 2 G>R at 24548, patients 1 and 2 C>M at 24664, patient 4 C>S at 25208, patient 5 A>M at 25522, and patients 1 and 4 C>M and patient 3 C>A at 25636.

Results from this experiment demonstrated a higher incidence of *SRY*-negative Ovotesticular DSD patients in our sample group. Although several polymorphisms have been found in the *RSPO1* and *WNT4* regions, the pathogeneity of these changes remain unclear. Further analyses are currently being done.

PP 67

Differences in microsatellite repeat regions on the Androgen receptor gene in South African individuals with polycystic ovary syndrome

M Nothling (University of Pretoria), N Oosthuizen (University of Pretoria)

Polycystic ovary syndrome (PCOS) is an endocrinological disordermarked by hyperandrogenism and oligo- or anovulation and affects 15% of women of reproductive age worldwide. Exon 1 of the androgen receptor (AR) gene, located on the X-chromosome, contains two microsatellite repeat regions, CAG and GGN, involved in the development of PCOS and androgenic alopecia (premature baldness) - the male PCOS phenotype. This study aimed to determine a possible difference in the number of microsatellite repeats in South African PCOS patients compared to unaffected females, males presenting with androgenic alopecia and unaffected males.

A case-control study was conducted where PCOS patients (n=7) served as the cases, and unaffected females (n=13), bald males (n=5), and unaffected males (n=9) served as controls. The mentioned regions were amplified using HRM-PCR. Samples that showed variation on HRM analysis were sent for sequencing.

Analysis of the CAG region showed averages of 22 repeats in PCOS patients, 21.25 repeats in female controls, 19.25 in bald men and 22 repeats in male controls. The GGN repeat region showed an average of 23 repeats in PCOS patients and bald men, 19.5 repeats in male controls and 19.67 in female controls.

All the preliminary results are within range of the normal number of repeats, reported as 8 - 35 CAG repeats and 10 - 30 GGN repeats in various publications. The low n-number at this point of the study is a possible explanation for our findings. The study is ongoing and we are actively recruiting more cases and controls for further investigations.

PP 68

Smith Magenis syndrome and its reciprocal duplication syndrome: Case report on two South African patients.

E Beckh-Arnold (National Health Laboratory Service and University of the Witwatersrand), L Lambie (National Health Laboratory Service and University of the Witwatersrand), F Essop (National Health Laboratory Service)

Smith Magenis syndrome (SMS) is caused by a microdeletion involving 17p11.2 whereas a duplication of this region results in Potocki-Lupski syndrome (PTLS). SMS has a recognisable phenotype with well-characterised

neurobehavioral and physical features. Similar to SMS, duplication 17p11.2 syndrome also has documented neurobehavioral and physical features, although these tend to be less distinct and differ from the features seen in SMS. In this case report we detail the clinical features in two South African patients and review the literature regarding the genetic mechanism and clinical features of these reciprocal deletion and duplication syndromes. Patient 1 (SMS) is a 5-year-old female with failure to thrive, a cardiac abnormality, developmental delay and behavioural features suggestive of SMS. Patient 2 (PTLS) is a 2-year-old male with unusual features, developmental delay, facial asymmetry and coronal synostosis. Both patients had normal chromosome analysis. Diagnoses were made using the SALSA multiplex ligation-dependent probe assay microdeletion kit (P245 -B1 microdeletion syndromes-1). This testing revealed the genes found to be deleted in patient1 and duplicated in patient 2 at 17p11.2 were *RAI1*, *LRRC48* and *LLGL1*. In conclusion, this report demonstrates the differing clinical features between reciprocal deletion and duplication syndromes and the lack of a distinct phenotype with the duplication of 17p11.2. This emphasizes the importance of using different techniques to assess copy number variants in children with developmental delay.

PP 69

Genetic Analysis of the FGF23 Gene in South African Patients Diagnosed with Hypophosphataemic Rickets

E Pretorius (University Of Pretoria), N Oosthuizen (National Health Laboratory Service and University of Pretoria)

Autosomal dominant hypophosphataemic rickets (ADHR) is an extremely rare form of hypophosphataemic rickets. Mutations occurring in the *FGF23* gene, which encodes the fibroblast-growth-factor-23 protein, leads to clinical presentation of ADHR. We sought to analyse the *FGF23* gene in 50 South African patients diagnosed with hypophosphataemic rickets.

Patients were recruited form the paediatric department at Steve Biko Academic hospital and the Metabolic Bone Clinic at Chris Hani Baragwanath hospital. We used real-time HRM PCR techniques followed by sequencing to analyse the *FGF23* gene in 50 diagnosed patients and 97 controls.

Three patients had a cited synonymous polymorphism, c.569G>K (rs.13312792) while two other patients had uncited synonymous polymorphisms, c.569G>A and c.569G>R. Another patient was heterozygous for an uncited synonymous polymorphism, c.569G>R (A141A) as well as a cited germline pathogenic missense mutation, c.682G>R (rs193922702) where the positive electrical arginine is substituted for a polar glutamine (R179Q). Four patient were found to be heterozygous for a cited missense mutation, c.862C>Y (rs.7955866), where a hydrophilic threonine was substituted for a hydrophobic methionine (T239M). Lastly, a single patient was found to be heterozygous for an uncited missense mutation, c.696G>R, where the negative electrical aspartic acid is substituted for a hydrophilic asparagine (D184N).

Our results show a novel uncited missense mutation as well as a cited pathogenic missense mutation in two separate patients. Our study is ongoing

to determine how the missense mutation affects the protein function of the FGF23 protein.

PP 70

Detection and comparison of polymorphisms within CYP21A2 between South-African patients with 21-OHD and control group with the use of High Resolution Melt (HRM) analysis.

J Lombard (University of Pretoria), N Oosthuizen (University of Pretoria)

Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder that disrupts the biosynthesis of cortisol. 21-hydroxylase deficiency (21-OHD) is responsible for 95% of all CAH cases. The gene that encodes 21-hydroxylase (*CYP21A2*) is found on the short arm of chromosome 6 (6p21.3). *CYP21A2* has a pseudogene called *CYP21A1P*, which has 15 mutations that renders it inactive; *CYP21A2* and *CYP21A1P* are 98% homologous. No published studies could be found on the mutations or polymorphisms of this gene within the South African population.

DNA was extracted from 40 control subjects and 5 CAH patients. A total of 16 primer pairs (pp) (pp16-pp31) were designed specifically for High Resolution Melt (HRM) real time PCR. The primer pairs (pp) cover the entire *CYP21A2* gene and were designed to not amplify *CYP21A1P*. The control group and the patients' DNA were compared to each other with HRM reactions using one primer pair at a time.

Preliminary results of this ongoing study with regard to pp18, 19, 24, 26 and pp28 show significantly high numbers of polymorphisms as described within the literature. The HRM results from mentioned pp's indicate that the majority subjects within the control group share the same genotype as the patients.

Results also identified one patient and 25 controls who do not share the same genotype as any of the other samples when compared according to the specific pp's. These samples might possess unique polymorphisms or mutations. Sequencing of these samples and further study is ongoing to confirm the initial results.

PP 71

Genomic variations associated to Fetal Hemoglobin level in sickle cell disease patients in Cameroon

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Background: High fetal hemoglobin (HbF), a stable and heritable trait, is correlated with reduced morbidity and mortality in SCD.

Methods: to determine the genetic variations which influence HbF and clinical severity of SCD patients in Cameroon, we performed a cross sectional study of clinically stable SCD patients, aged >5 years, involving the following: vaso-

oclusive painful crises, full blood count, HPLC electrophoresis for HbF levels, RFLP-PCR to study haplotypes in the beta-globin-like genes cluster, multiplex Snapshot-PCR to study 11 targeted SNPs.

Results: 655 SCD patients and 204 controls were recruited, median age: 16 years; 30% had >3 painful vaso-oclusive crises per year. The median Hb level was 7.6 g/dl, the median WBC count was $12.2*10^9$ /l. The mean HbF level was 7.5 ± 4.5% (range <0.8% - 22.7%).

Haplotypes analysis showed: 57.3% Benin/Benin, 26.4% of Benin/Cameroon, 5% Cameroon/Cameroon, 5.1% of atypical haplotypes.

All the 270 patients' studies for targeted SNPs, were homozygous CC at the *XmnI-158* locus due to absent Senegal and Indian-Arab haplotypes. The higher median HbF level was associated with the minor allele of SNPs rs4671393 (A) and rs11886868 (T) in the *BCL11A* locus, and rs11886868 (T) tends to be associated with a lower frequency of hospital admissions. rs9399137 (C) *(HMIP* locus) and rs5006884 (T) (OR51B5/6) were both associate with Higher level of HBF level.

Conclusions: Cameroonian SCD displayed a relatively severe clinical phenotype and low to moderate HbF level. Preliminary results confirmed the association of selected SNPs to higher level of HbF and hospital Admissions.

PP 72

Genetics of hereditary hearing loss in Africans

A Wonkam (University of Cape Town), J Noubiap (University of Yaoundé), J Bosch (University of Cape Town), C Dandara (University of Cape Town), F Djomou (University of Yaoundé), G Bengono (University of Yaoundé)

Background: There is paucity of data on genetic of hearing loss (HL) in Africans.

Methods: we studied the etiology of childhood HL in 644 patients with severe HL in Cameroonian and South African Blacks. We sequenced the coding regions of *GJB2 (Cx26), GJB6 (Cx30), GJA1 (Cx43)* genes in selected syndromic and non-syndromic cases.

Results: prelingual HL accounted for 75.1%, with a mean age at medical diagnosis of 3.3±1.2years. The majority of participants had sensorineural (84.9%) and profound or total HL (85.1%). Putative environmental causes, mostly meningitis, accounted for 52.6%.Genetic causes for 14.8% and unknown causes for 32.6%. Amongst genetic cases, 86% were non-syndromic. We found six cases of the type 2 Waardenburg syndrome, two cases of Keratitis-Ichthyosis-Deafness (KID) syndrome, 3 cases of Oculo-auriculo-vertebral (OAV) spectrum, one case of the type 1 Usher syndrome, one case of Pendred syndrome. Consanguineous families accounted for 5.7% of the whole sample, and 15.1% of those with genetic causes.

The analysis of the *GJB2* gene revealed the heterozygous mutation p.D50N in the two patients with KID syndrome. In 200 non-syndromic cases, only two heterozygous mutations in *GJB2* were described in two non related patients. Similarly, no mutations in *GJA1* and *GJB6* could explain the hearing loss in 100 non-syndromic cases.

Conclusion: 1) our data also supports the predominance of environmental factors (meningitis); 2) type 2 Waadenburg syndrome is the most prevalent syndromic cause; 3) Mutations in the *GJB2, JGB6 and GJA1* genes do not play a major role in non-syndromic hearing loss in Africans.

PP 73

Frequencies of HLA class I alleles associated with abacavir and nevirapine immune hypersensitivity reactions in South African Indian and Coloured populations.

S Loubser (National Institute of Communicable Diseases), M Paximadis (National Institute of Communicable Diseases), A Puren (National Institute of Communicable Diseases), C Tiemessen (National Institute of Communicable Diseases)

Pharmacogenomics improves clinical management of patients on therapy by combining patient genotype with known treatment outcome. In HIV infection, abacavir (ABC) and nevirapine (NVP) treatment can precipitate potential life-threatening immune-based complications in susceptible individuals, termed immune hypersensitivity reaction (HSR). HSR is linked to specific human leukocyte antigen (HLA) class I alleles such as HLA-B*57:01 (ABC-HSR) and HLA-B*35:05, HLA-C*04 and HLA-C*08 (NVP-HSR). Pre-screening of individuals for these genes before treatment starts is recommended, however, current HLA-typing methods are time-consuming and expensive. Real-time PCR provides a low-cost alternative genotyping method with a short turnaround time. We have designed gene-specific PCR primers suited for melting-curve analysis using SYBRgreen intercalating dye to identify the HLA class I alleles associated with ABC and NVP HSR. The assay was validated with DNA samples previously HLA genotyped by sequence-based typing and applied to a cohort of healthy individuals of South African Indian (n=50) and Coloured (n=50) ethnicity for comparison to published allele frequencies of South African Black and Caucasian populations. Results indicated that HLA-B*57:01 is present in both Coloured and Indian individuals at frequencies of 8% and 12%, respectively. HLA-C*08 was found in 10% and 20% of Indian and Coloured individuals, respectively, while HLA-C*04 was detected in both population groups at a frequency of 22%. HLA-B*35:05 was detected in 6% of Coloured individuals and was absent in Indian individuals. These data provide new HLA class I allele frequency data for South African Indian and Coloured populations and suggests significant numbers at risk of ABC or NVP HSR.

PP 74

An unusual finding on a blood stained amniocentesis.

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A blood stained amniotic fluid sample was received and processed. Chromosome analysis revealed an admixture of cells. Of a total of 15

metaphases analysed from Primary Culture 1, 9 metaphases revealed a normal 46,XX (female) karyotype while a further 6 metaphases revealed an abnormal 47,XXX karyotype. Its Subculture revealed 1 normal and 1 abnormal cell.

Primary Culture 2 and its Subculture revealed a normal 46,XX in all 14 metaphases analysed.

In view of the abnormal cell line being evident only in Primary Culture 1 and its Subculture, it was assumed that the abnormal cell line was an artefactual *in-vitro* chromosome change, which may be evident in cells, which have been in long-term culture.

Subsequently a foeticide was performed due to multiple abnormalities seen on scan. Fetal heart blood was cultured and all baby's cells revealed a normal female (46,XX) karyotype.

Mother's blood was requested and revealed a 47,XXX in all 17 metaphases analysed. In view of this finding, it was confirmed that the abnormal cell line evident in the amnio was in fact maternal.

The question arises - should we assume that an abnormal cell line is fetal in origin?

This alerts us to the fact that we cannot always assume that an abnormal cell line is an artefactual *in-vitro* chromosomal change if it is evident in just one Primary Culture and its Subculture. Nor can we assume that the abnormal cell line is fetal in origin and the normal cell line maternal, in a blood stained culture.

PP 75

A novel FKRP-related muscular dystrophy founder mutation in South African Afrikaner patients with a DMD/BMD phenotype.

M Mudau (National Health Laboratory Service), F Essop (National Health Laboratory Service), A Krause (University of the Witwatersrand), C Prentice (University of the Witwatersrand)

FKRP-related muscular dystrophy (MD) is an autosomal recessive disorder due to mutations in the fukutin-related protein (FKRP) gene and is clinically similar and often misdiagnosed as a dystrophinopathy. A previously unreported FKRP mutation (c.1100T>C) in exon 4 of the FKRP gene was found in homozygous form in two South African white patients, clinically diagnosed with Duchenne/Becker muscular dystrophy (DMD/BMD). This project investigated whether this mutation is present in other patients with suspected dystrophinopathy and if a founder haplotype exists. The c.1100T>C FKRP-mutation was tested using ARMS PCR in 35 white South African Afrikaner DMD/BMD patients that had tested negative for deletions or duplications in the dystrophin gene using MLPA screening. Linked Marker analysis was done using three linked markers, D19S219, D19S412 and D19S606 flanking the FKRP gene, in the families of affected individuals and normal controls to determine whether c.1100T>C is a founder mutation. Six patients tested homozygous and 4 were heterozygous for the c.1100T>C FKRP mutation. Linked marker analysis showed a likely founder haplotype for the c.1100T>C mutation in all affected individuals different to unaffected

controls. The findings of this study suggests that the c.1100T>C FKRP mutation is likely to be a founder mutation in the South African Afrikaner population. Patients of possible Afrikaans ancestry with a DMD/BMD phenotype who are negative on MLPA should be screened for FKRP. This will be very useful in offering diagnostic, carrier and prenatal testing for the affected individuals as well as accurate genetic counselling for affected families.

PP 76

Mothers' Experiences of Genetic Counselling in Johannesburg, South Africa

M Morris (National Health Laboratory Service and University of the Witwatersrand)

Genetic counselling is now offered in diverse multicultural and healthcare settings. The way in which individuals experience genetic counselling varies between countries due to differences in culture, religion, family and community values and socio-economic backgrounds. Currently, little is known about patients' experiences of genetic counselling in South Africa.

The study aim was to describe and document the experience of mothers who had received genetic counselling at state hospitals in Johannesburg, South Africa. The research design was qualitative in which thirteen women participated. Four voice-recorded focus groups were conducted in suitable African languages, using a question-guide. All data were transcribed and translated, and Interpretative Phenomenological Analysis (IPA) was used for analysis.

Six main themes were identified. These included general lack of awareness of genetic counselling and genetic conditions; experiences of genetic counselling; personal beliefs; challenges in addressing families and communities; dissatisfaction with the healthcare system and a greater need for awareness.

Genetic services and genetic conditions are poorly understood amongst the studied South African population. The experience of genetic counselling varies amongst patients, and genetic counselling styles need to be more flexible. On-going patient support has been indicated and genetic counsellors need to assist in conveying information to community and family members. Challenges in the current South African healthcare system need to be addressed with regard to the role of language interpreters, staff attitudes and support, as well as in-service training. Although findings from this study cannot be generalised to all patients in South Africa, valuable insight was gained.

PP 77

Frequency of CCR5Δ32 deletion mutants in South African Indian and Coloured populations

R Ngqobe (National Institute of Communicable Diseases), S Loubser (National Institute of Communicable Diseases), M Paximadis (National Institute of Communicable Diseases), A Puren (National Institute of Communicable Diseases), C Tiemessen (National Institute of Communicable Diseases)

CCR5 is a chemokine receptor that serves as a co-receptor for entry of R5 HIV strains into CD4+ target cells. CCR5Δ32 is a 32-bp deletion mutant found in a minority (5-14%) of individuals of North European descent and is associated with natural protection against HIV infection. The deletion mutant is absent in African populations and is also rare in Asian populations. In South Africa, little is known about the frequency of the CCR5Δ32 deletion mutant in Indian and Coloured populations. We have developed a real-time PCR assay to detect the presence of CCR5 full-length and deletion mutant alleles using allele-specific PCR primers and SYBRgreen chemistry. The assay was validated using positive control DNA from known CCR5 full-length homozygotes, heterozygotes and a CCR5Δ32 homozygote. The assay was applied to DNA samples available from healthy individuals of South African Indian (n=50) and Coloured (n=50) ethnicity. We identified CCR5Δ32 heterozygotes in both Coloured and Indian individuals at frequencies of 6% and 4%, respectively. No CCR5Δ32 homozygotes were found. The five CCR5/CCR5Δ32 heterozygotes identified were further confirmed using agarose gel electrophoresis. In conclusion, we have developed a quick-screening assay to detect CCR5 allelic variants and have shown that the CCR5Δ32 deletion mutant is present in South African Coloured and Indian individuals at low frequencies.

PP 78

AMLprofiler: Evaluation of a novel diagnostic for acute myeloid leukaemia in the South African setting.

M Alessandrini (University of Pretoria), E Beltchev (National Health Laboratory Service and University of Pretoria), R Pool (National Health Laboratory Service and University of Pretoria), M Pepper (University of Pretoria)

Acute myeloid leukaemia is one of the most common haematological malignancies, which is characterised by proliferation of the myeloid lineage and accumulation of immature haematopoietic cells in the bone marrow. AML is further typified by diverse genetic abnormalities and marked heterogeneity in both response to treatment and survival. Diagnosis is according to a World Health Organisation classification system, whereby clinicians are able to categorise cases into favourable, intermediate and poor risk groups. This has recently been augmented by several novel molecular biomarkers, such as genetic alterations of *NPM1* and *CEBPA* that infer a favourable prognosis,

and increased expression levels of *BAALC* and *EVI1* that infer an unfavourable prognosis.

The AMLprofiler is a novel diagnostic microarray that incorporates seven molecular variables that are used to predict post therapy survival rates. The goals of the study are to evaluate the benefits of the AMLprofiler in the South African context. Several variables are to be compared to the current approaches, including the concordance of results, cost, time and the personnel required.

A total of 65 AML patient samples are to be included in the study, and we have collected and assayed over 20 to date. Our results indicate 100% correlation with findings obtained using standard modalities. Furthermore, several samples were determined to be positive for molecular biomarkers not routinely investigated in South Africa, including *CEBPA*, *BAALC* and *EVI1*. Although the study is still underway, the preliminary data indicates benefit for use of AMLprofiler in South Africa.

PP 79

Ethical Dilemmas in Genetic Counselling: two case histories

M Glass (University of the Witwatersrand and National Health Laboratory Service)

Often in genetic counselling, one is challenged with a conundrum. The aim of this paper is to address two complex cases, question our values and perhaps re-address primary principals of genetic counselling relating to ethical dilemmas.

Ms G and Mr E, have a daughter, and a son, who has sickle cell anaemia. Their daughter was tested for carrier status while checking HLA matching for her brother. Ms G was 10 weeks 3 days pregnant and requested prenatal testing, specifically wanting HLA typing. When challenged, she explained that if the fetus was not a match for her son, she would terminate the pregnancy. Do we offer prenatal testing knowing that Ms G would terminate an unaffected fetus if not an HLA match for her son? How do we address the violation of the autonomy of the daughter, and protect the future autonomy of an unaffected, HLA matched fetus?

Ms M and Mr D came to discuss prenatal testing for sickle cell anaemia; their daughter is homozygous for the βs mutation. Mr D is neither a carrier of the βs mutation for sickle cell, nor of β thalassaemia. There was virtually no risk for the current fetus to be affected. Ms M insisted that Mr D is the biological father of their daughter. Do we assume non-paternity, whilst maintaining Ms M's confidentiality and do we offer unnecessary invasive testing?

Ethical issues in both cases not only address the dilemma of autonomy, but challenge our commitment to beneficence, non-maleficence, veracity and justice.

PP 80

SLC16A2 mutation identified as the cause for XLID in a South African Family

L Lambie (National Health Laboratory Service and University of the Witwatersrand), J Jones (Greenwood Genetic Center), A Christianson (University of the Witwatersrand and National Health Laboratory Service), C Schwartz (Greenwood Genetic Center)

Five to ten percent of intellectual disability in males is X-linked and mutations in 102 genes have been associated. We describe a mixed ancestry South African family, previously thought to have Pelizaeus-Merzbacher-like disease.

Of seven affected males from two generations, five were examined at the Neurodevelopmental Clinic at Rahima Moosa Hospital over a period of 20 years. The pedigree and clinical phenotypes in this family are described. Prior molecular analysis of the *PLP1* (causing Pelizaeus-Merzbacher disease), *XNP*, *ARX* and *MECP2* genes did not reveal mutations. DNA was obtained from 2 affected males and four obligate female carriers. Along with banked DNA from 2 deceased males, samples were sent to Greenwood Genetic Centre, South Carolina, for analysis.

Sanger sequencing of the *SLC16A2* coding region identified a c.1690G>A change in exon 6. This mutation has previously been described in the Human Gene Mutation Database. T3 concentrations were elevated in the two living boys.

Mutations in the monocarboxylate transporter 8 (*MCT8* /*SLC16A2*) gene cause X-linked Allan-Herndon-Dudley syndrome characterised by severe cognitive impairment, childhood hypotonia progressing to spasticity, dyskinesias and seizures. Affected males have abnormal thyroid function tests. A high index of suspicion and testing of T3 levels in males with a similar phenotype is a feasible screening option for this condition in a low resourced setting. Identification of the causative mutation in this family allows for accurate genetic counselling, prenatal diagnosis and appropriate pregnancy monitoring for hypothyroidism in carrier females.

PP 81

Diagnostic, Carrier and Prenatal Genetic Testing for Fragile X syndrome and other FMR-1 related disorders in Johannesburg, South Africa. A 20-year review.

F Essop (National Health Laboratory Service), A Krause (University of the Witwatersrand)

Background: Fragile X syndrome (FXS) is the most common inherited cause of intellectual disability (ID) worldwide caused by the expansion of a CGG repeat in the *FMR-1* gene.

Objectives: This study reviewed the genetic services for FXS and other *FMR-1* related disorders including fragile X-associated tremor/ataxia syndrome (FXTAS) and *FMR-1*-related primary ovarian insufficiency (POI) at the Division of Human Genetics, NHLS and University of the Witwatersrand,

Johannesburg, South Africa, for diagnostic, carrier, and prenatal genetic testing over a period of 20 years.

Subjects and Methods: Records of 2690 patients who had genetic testing for *FMR-1* between 1992 and July 2012 were reviewed. Of these, 2239 with ID had diagnostic testing, 430 carrier or cascade testing and 17 prenatal testing for FXS. Four had FXTAS or POI testing.

Results: Of the 2239 ID patients, 5.7% (128) had a full mutation, 0.5% (12) a premutation and 1.9% (43) an intermediate allele. In 17 prenatal tests, 8 fetuses tested positive for FXS. *FMR-1* CGG repeat distribution analysis in 1532 males negative for the *FMR-1* expansion showed that 29 and 30 CGG repeats were most common (61.1%), with a significant difference in the Black and White populations.

Conclusion: The presence of FXS in all populations as the commonest cause of ID, including the Black population is supported. The *FMR-1* CGG repeat distribution was different to that found in other studies. The number of family members tested by cascade screening is relatively low and suggests that many at-risk family members are not being tested.

PP 82

Review of Paediatric Patients with Classic Galactosaemia in the Metabolic Clinic, Johannesburg, South Africa 2001-2012

L Bhengu (National Health Laboratory Service), J Naicker (National Health Laboratory Service)

Classic galactosaemia, an autosomal recessive condition, is caused by mutations in galactose-1-phosphate uridyltransferase (GALT) gene. Mutations result in GALT enzyme deficiency, which is essential in the metabolism of galactose. Accumulation of galactose-1-phosphate, a toxic metabolite, leads to multi-organ failure and long-term complications. Homozygosity for the Q188R mutation commonly found in Caucasians is associated with a poorer prognosis compared to homozygosity for the S135L mutation, which is found in Blacks.

This study was aimed to assess the presentation, long-term outcome and genotype/phenotype correlation of patients seen in the Metabolic Clinic in Johannesburg between 2001 and 2012.

Twelve patients were diagnosed: 11 blacks, one white, 8 females and 4 males; and 5 (42%) were under 3 months of age. Most had liver dysfunction, neurological deficits, cataracts and sepsis. All had severe GALT deficiency, 7(64 %) blacks were homozygous for S135L mutation and one white child was heterozygous for Q188R/K285N mutations. All improved with a galactose-restricted diet. Follow up of the black children showed developmental delay and poor vision.

These findings suggest that the S135L mutation in black patients has a poor prognosis but late detection and treatment may contribute to a poor early outcome.

Long term follow up with a larger cohort of patients is required to confirm these findings. Due to the unavailability of newborn screening in South Africa,

many cases are probably missed. Newborn screening will enable us to provide appropriate acute and long term care of patients with classic galactosaemia.

PP 83

Phenotypic Consequences of FANCA Founder Mutations in Afrikaans Individuals With Fanconi Anaemia

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Fanconi anaemia (FA) is a rare autosomal recessive genetic condition characterized cytogenetically by chromosome instability and breakage. Clinically, affected individuals variably present with growth and somatic anomalies and many progress to haematological complications of the disease, principally bone marrow aplasia. In South Africa, affected individuals of Afrikaans ancestry have been shown to carry pathogenic founder mutations in the Fanconi A gene (FANCA).

The aim of this study was to describe the physical phenotypic consequences of FANCA founder mutations in a cohort of Afrikaans FA patients. Affected individuals were recruited from paediatric haematology/oncology centres in Pretoria, Polokwane and Bloemfontein. In the pilot phase of the study, eight patients underwent a comprehensive clinical examination to document growth measurements, facial dysmorphology, skin pigmentary anomalies and congenital abnormalities of the upper limb, gastrointestinal, cardiovascular and renal systems. A review of the hospital files augmented the data collected.

Although the sample size was small, a high frequency of skin pigmentary (75%), renal (50%), radial ray (100%) and ulnar ray (62.5%) anomalies was observed as well as a high incidence of hearing loss (37.5%). A detailed case report is presented for each affected individual.

The observations of the pilot study indicate a need for further research into the phenotypic consequences of FANCA mutations in affected Afrikaans individuals with the aim of guiding appropriate care.

PP 84

CYP450 Pharmacogenetic Profiling for Analgesic Pain Management in an African-American Pediatric Sickle Cell Disease Patient Cohort

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Allelic variability in the *CYP2D6, CYP2C9* and *CYP2C19* enzymes is an important cause of interindividual variability in analgesic drugs prescribed for sickle cell disease (SCD) pain.

We determine the *CYP2C9, CYP2Cl9* and *CYP2D6* allelic and genotypic frequencies in a pediatric SCD patient cohort.

Eight variant *CYP2C9* alleles, eleven *CYP2CI9* alleles, and nineteen *CYP2D6* alleles were genotyped across thirty patients using the Tag-It™ Mutation Detection Kit and the eSensor 2C 19 Test.

A total of 11 *CYP2D6* alleles were detected in the cohort. The most common alleles identified were **1, *2,*10*, and **17* and their frequencies were 0.339, 0.210, 0.065 and 0.161 respectively. The *CYP2C19*1* frequency was 0.533. Three different *CYP2C19* alleles were identified with the following frequencies:**17* (0.300), **2* (0.150) and **13* (0.0I7) respectively. For the *CYP2C9* enzyme, the **1* allele frequency was 0.823.The combined frequency for all variants: **2, *3, *5, *6, *8*, and **11* was 0.177. Phenotypically, 67.7% of the cohort was extensive metabolizers: 29% and 3.2 % respectively were intermediate and poor metabolizers. For the *CYP2D6*, phenotypes were distributed into ultra-rapid (6.5%), extensive (77.4%) and intermediate (12.9 %) metabolizers. For the *CYP2C19*, the metabolic phenotypes were distributed as ultra-rapid (46.7%), extensive (20%) and intermediate (33.3%) metabolizers.

Some of the *CYP2C9, 2C19,* and *2D6* variant alleles identified in our study are being reported for the first time among pediatric SCD patients in the United States. Our data underscore the need for pharmacokinetics studies on substrate-specific effects of variant alleles common in racial populations with high prevalence of SCD.

PP 85

Congenital adrenal hyperplasia - the need for individualised genetic diagnostics in South Africa

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Congenital adrenal hyperplasia is an autosomal recessively inherited disorder characterized by impaired cortisol secretion from the adrenal cortex. The majority of cases stem from mutations in the *CYP21A2* gene encoding steroid 21-hydroxylase.

Molecular diagnosis of 21-hydroxylase deficiency (21-OHD) is complicated by the presence of an inactive pseudogene (*CYP21P*) which shows 98% homology to *CYP21A2*. This is mainly overcome by using primers specific to the active gene. However, as the close proximity and high homology between pseudogene and active gene results in a high rate of gene conversion, a standardized approach to mutation identification is very difficult, necessitating individualized genetic diagnostics.

We present a summary of the cases seen at the Inherited Metabolic Diseases (IMD) laboratory since introducing this individualised approach in 2008, and the strategies used to genotype them. Eight patients (from seven families) were screened for mutations in the *CYP21A2* gene. Two siblings were homozygous for the common i2G mutation; while three patients carried three different compound heterozygous mutation sets: the common exon 6 cluster conversion and the Q318* mutation; the -126>T, -113G>A, -110T>C, -103A>G, P30L cluster conversion and a full gene deletion/conversion; and a

conversion involving exon 3 and one involving exon 7 and 8 (both resulting in frame shifts). The final patient was heterozygous for the R479L mutation, which only slightly reduces activity (no second mutation found). No mutations were identified in the two remaining patients.

As illustrated by our results, no common mutations have been identified in our population group to date.

PP 86

Ashkenazi Jewish Genetic Testing: Utilization of Services, Genetic Knowledge and Perceptions of Stigma

K Stoler (National Health Laboratory Service), A Krause (University of the Witwatersrand)

Genetic carrier testing programmes establish whether individuals carry an inherited autosomal recessive genetic mutation that could cause a couple's offspring to have a genetic condition if both are carriers. This study aimed to investigate the utilisation of Ashkenazi Jewish carrier testing, while also assessing genetic risk knowledge and evaluating the perception of stigma associated with being a carrier.

The study sample included Ashkenazi Jewish men and women between 18 and 40 years in Johannesburg, South Africa. The study was advertised through several Jewish community resources. Data were collected through an online structured questionnaire over a one-month period.

Two hundred and ninety eight individuals participated in this study with 32.6% (97/298) male and 67.4% (201/298) female. From the total number of participants, 44% (130/298) had genetic carrier testing when they were either single, dating or engaged. The timing has implications as individuals would have different reproductive options available at different stages. This study found that genetic knowledge for the common AJ genetic conditions was poor. Participants seemed to underestimate the frequency of the genetic condition but misunderstood the implications of being a carrier. Overall, no personal nor social stigma was found in the study indicating that little stigma appears to exist regarding carrier status in the Jewish community in Johannesburg, South Africa.

This study highlights the need for genetic services to provide educational programmes in the community so that individuals are encouraged to test for the 9 common Ashkenazi Jewish conditions.

PP 87

Pharmacogenetics: Investigating the genetic basis of cisplatin-induced ototoxicity in adult South African cancer patients

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Cisplatin is administered as first-line treatment of soft-tissue cancers but is limited by the high incidence of associated ototoxicity. Cisplatin-induced ototoxicity (CIO) is characterised by irreversible bilateral hearing loss. A retrospective study, conducted at Groote Schuur Hospital (GSH), indicated 63% of patients were affected. We aimed to determine the role of variation in genes involved in cisplatin metabolism and disposition contributing to CIO.

To date, 102 adult patients on cisplatin treatment with complete baseline auditory and clinical data were recruited from GSH and genotyped for 10 SNPs in *COMT*, *TPMT*, *LRP2*, *XPC* and *GSTP1*. Copy number variation of *GSTP1*, *GSTT1* and *GSTM1* was determined using MLPA and the *OTOS* gene was sequenced to identify novel variation.

Fifty-five patients (54%) presented with significant hearing loss following cisplatin administration. Age of patients and higher cumulative cisplatin dosage was associated with increased ototoxic risk. Further, preliminary genotyping results suggest a trend toward the development of ototoxicity in patients carrying the *COMT* c.615+310C allele (p=0.07). Novel variation/s observed within *OTOS* will be correlated with CIO as well as *in silico* prediction of variant function.

Genotypic data of indigenous Africans and derived populations, and their phenotypic association, is vital to ensure the safety and efficacy of cisplatin administration in South Africa. This study identifies genetic markers associated with CIO susceptibility, focusing on the unique Cape Mixed Ancestry population. An improved understanding of the inter-individual differences in cisplatin pharmacokinetics and response will contribute towards the potential treatment personalization to improve quality of life in cancer survivors.

PP 88

The mutation spectrum of Rett syndrome in South Africa

E Vorster (National Health Laboratory Service), F Essop (National Health Laboratory Service), A Krause (University of the Witwatersrand)

Rett syndrome (RTT) is a heterogeneous, progressive, neuro-developmental disorder primarily affecting females, caused by mutations (of which more than 95% are de novo) within the *MECP2* gene. Males with a *MECP2* mutation have diverse phenotypes varying from severe congenital encephalopathy to intellectual disability. The *MECP2* gene consists of four exons and encodes for the MeCP2 protein, which acts as a transcriptional modifier. The aim of the study was to perform an audit of patients, referred with a suspected clinical diagnosis of RTT for sequence analysis of *MECP2* to NHLS, Johannesburg, Division of Human Genetics, from March 2002 to June 2013.

Mutation analysis of *MECP2* was performed using a combination of RFLP, DHPLC and sequencing analysis.

A total of 314 unrelated patients (290 females, 24 males) were referred for diagnostic testing, 5 females for carrier testing and 2 prenatal tests were performed. Sixty-two females (62/314, 19.7%) tested positive for a pathogenic mutation. Eight recurrent mutations accounted for 53.2% (33/62) of positive RTT patients with p.R168X being the most common mutation identified

(16.1%, 10/62). Four patients (3 Black, 1 White) were found to have novel mutations (6.5%, 4/62). Patients referred for carrier and prenatal testing were found to test negative.

This study will assist in elucidating the mutation spectrum and clinical presentation of RTT in South African populations.

PP 89

Patient's perceptions of their cancer risk prior to genetic counselling for inherited breast/ovarian cancer.

T Haw (National Health Laboratory Service), A Krause (University of the Witwatersrand)

Patients attending genetic counselling for hereditary breast/ovarian cancer (HBOC) have been shown to experience anxiety and often have an inaccurate perception of their risk of developing cancer. These factors may undermine the effectiveness of the genetic counselling consultation.

Patients seen at our clinic for HBOC complete a questionnaire prior to genetic counselling. The questionnaire asks about family history and about the patient's perception of their cancer risk and the impact this has on them. Questionnaires from 11 unaffected female patients have been analysed and we will analyse at least another 20.

We aim to determine whether the patient's perception of risk prior to counselling correlates with that calculated using the programme, Tyrer Cuzick (IBIS). We also aim to determine whether an increased perceived risk is associated with the amount of time the patient reports thinking about cancer and with self-reported altering of their mood. We will also record the impact that having a first-degree relative die of breast/ovarian cancer has on patients, in terms of altering their risk perception and effect on mood.

The data so far show that the majority of patients overestimate their risk of developing cancer prior to genetic counselling. Women who had a first-degree relative die of cancer seem to be more likely to report an altering of their mood when thinking about cancer.

An understanding of the factors placing women at risk of becoming pre-occupied with thoughts about cancer and/or over-estimating their cancer risks will enable the genetic counsellor to address their concerns better.

PP 90

Hearing loss in Sub-Saharan Africans is not linked to mutations in the Connexin 43 Gene (GJA1)

J Bosch (University of Cape Town), J Nziale (University of Yaoundé), C Dandara (University of Cape Town), N Makubalo (University of Cape Town), A Wonkam (University of Cape Town)

Mutations affecting connexin genes are a large cause of genetic hearing loss. We previously showed that mutations in connexin 26 gene (GJB2) do not account for autosomal non-syndromic hearing loss in Africans. We aim to

investigate the involvement of GJA1 (connexin 43 gene) in a selected group of Cameroonian and South African blacks with non-syndromic congenital hearing loss.

The complete coding region of the GJA1 gene is being amplified and sequenced in 100 patients with recessive non-syndromic sensorineural deafness and 64 ethnically matched controls. Patients are both from the Xhosa population in South Africa and from various regions in Cameroon, to approximate African diversity. Allele, genotype and haplotype frequencies will be compared using SHEsis. The 1000 Genomes data will be used to create a phylogeny and to perform principal components analysis to both complement the phylogenetic grouping and to determine which SNPs serve to differentiate populations.

Sequencing data on 80 patients and 23 controls is currently available. Four heterozygous variants have been found; three synonymous changes (rs139688042, rs57946868 and novel p.N122=) and one non-synonymous change (rs17653265). These alone could not explain the hearing loss and only the synonymous SNP rs57946868 occurs in more than one patient. Provisional comparison of South African cases and controls shows no significant differences.

Preliminary results show that mutations in GJA1 do not account for non-syndromic congenital hearing loss in African patients.

PP 91

Fragile X-associated tremor/ataxia syndrome (FXTAS) in patients with a spinocerebelar ataxia (SCA) phenotype

K Kuhn (National Health Laboratory Service), F Essop (National Health Laboratory Service), A Krause (University of the Witwatersrand)

FMR1-related disorders are X-linked and include Fragile X-associated tremor/ataxia syndrome (FXTAS), Fragile X syndrome (FXS) and *FMR1*-related primary ovarian insufficiency (POI). FXTAS and POI are caused by an increased number of CGG trinucleotide repeats in the *FMR1* gene premutation range (55-200). FXTAS is characterized by late-onset, progressive cerebellar ataxia and intention tremor in males and females. Spinocerebellar ataxia (SCA) patients presents with progressive cerebellar ataxia. In this study patients that tested negative for the common SCA's were tested for FXTAS due to the overlapping phenotype of cerebellar ataxia.

To determine the presence of an *FMR1* premutation in patients that tested negative for SCA.

A total of 289 (185 males and 104 females) patients referred for SCA and who tested negative were selected. PCR analysis for the *FMR1* CGG repeat was performed. Of the 289 SCA negative patients, 132 (89 males and 43 females) have been tested. Thus far 120 (88 males, 32 females) patients tested negative for a premutation, 3 patients (2 females and 1 male) had an intermediate allele (45-54) and 9 female patients had an equivocal result (1 allele).

Premutations have not been identified in the cohort tested, suggesting that it occurs at low frequency in the SCA negative patients. The significance of the

intermediate alleles is uncertain. Testing is in progress of the remainder of the cohort.

PP 92

Inadequacies of Genetic Risk Assessment Tools in Black South African Women with Breast Cancer

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Black women in South Africa are diagnosed with breast cancer younger, have more aggressive disease manifestations and poorer prognoses compared to an equivalent white population. Little research has been undertaken regarding inherited forms of breast cancer in the black South African population.

This study aimed to investigate family histories and efficacy of internationally utilised genetic counselling risk assessment tools providing information regarding lifetime breast cancer and *BRCA* mutation risks in black South African women with breast cancer.

A retrospective, file-based analysis of black women diagnosed with breast cancer before the age of 50 years was undertaken. Probands (N=45) were ascertained from the Genetic Counselling Clinic at the Breast Clinic, Chris Hani Baragwanath Hospital. Personal details and cancer and family histories were collected from their files and analysed. Three risk assessment tools (Claus Model, Tyrer-Cuzick model and Manchester Scoring System) were evaluated.

Results indicated four women (4/45; 9%) had significant family histories of cancer. Unlike international trends, age at diagnosis may be a poor predictor of inherited breast cancer risk in this population. All of the assessment tools showed increased lifetime risk and mutations risks compared to the general population, but with marked inconsistencies amongst the programs.

Existing risk assessment methods should be used with caution in the South African black population and molecular genetic diagnostic testing is essential to establish efficacy. Information obtained from this study should direct future research regarding genetic counselling and testing for inherited breast cancers in black South African women.

PP 93

Investigating the Genetic Aetiology of PTEN Hamartoma Tumour Syndrome (PHTS) in a Black South African Cohort

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PTEN hamartoma tumour syndrome (PHTS) is an autosomal dominant condition caused by germline mutations in the *PTEN* gene. PHTS includes both Cowden syndrome (CS) and Bannayan-Riley Ruvalcaba syndrome (BRRS). These syndromes are characterised by benign and malignant tumours (especially of the breast, thyroid and endometrium) as well as congenital abnormalities. This study aims to investigate the spectrum of *PTEN* mutations in black South Africans.

Three black South African women with personal and family histories suggestive of CS and three black individuals with clinical features suggestive of BRRS were selected for this investigation. The Cleveland Clinic risk assessment tool was used to estimate the likelihood of identifying a *PTEN* mutation in each individual. Sequencing analysis of the coding regions and exonic flanking regions of the *PTEN* gene is being performed.

Preliminary risk analysis indicated that the three possible CS patients were unlikely to harbour *PTEN* mutations however the tool is based on the individual's personal history only, therefore making this a potentially inaccurate assessment. The three individuals suspected of having BRRS all satisfied clinical criteria for *PTEN* mutation testing according to the tool.

This study aims to provide insight into the prevalence of *PTEN* mutations in patients clinically suspected of having CS or BRRS in South Africa. Additionally, this work will contribute to the broader knowledge of inherited cancer syndromes in black South Africans.

PP 94

Genetic profiling of CYP2D6 variants known to alter enzyme activity in a black South African population

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CYP2D6 is responsible for the metabolism of 20-25% of currently prescribed drugs and thus the study of its polymorphisms are of particular importance to pharmacogenomics, as the various combinations of these polymorphisms result in a diverse set of metabolic profiles. However, little is currently known about the frequency of functionally important *CYP2D6* SNPs in black South Africans. Therefore, this study aims to genotype four functionally important SNPs across the coding region of *CYP2D6,* in order to determine the allelic frequencies and their resulting impacts on drug metabolism in black South Africans. 94 unrelated black Bantu-speaking participants will be genotyped, using the tetra ARMS-PCR method for each of the four SNPs: rs16947, rs1135840, rs1065852 and rs28371706. These frequencies will then be compared with those from other populations, using International HapMap and 1000 Genomes data. Furthermore, these four SNPs will be combined with existing genotype data in order to augment the SNP coverage across *CYP2D6.* This increased coverage will allow us to gain a more comprehensive picture of the various drug metabolic classes that exist in the black South African population. Preliminary analysis using 1000 Genome

data, shows that Africans possess different allelic frequencies for all four SNPs when compared to other populations. In particular, the derived allele of rs28371706 is almost exclusively found in African populations. Therefore, knowledge of the frequencies of these polymorphisms and their resulting metabolic classes will prove important for reducing both adverse drug reactions and improving dosage calculations, with respect to the black South African population.

PP 95

Prevalence of GJB6 Mutations (Connexion 30) in African patients with Non-Syndromic Hearing Loss

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Background: Mutations affecting connexins genes are a large cause of genetic hearing loss. We previously showed that mutations in connexin 26 gene (*GJB2*) do not account for autosomal non-syndromic hearing loss in Africans. The second highest prevalent gene is *GJB6 (*connexin 30 gene), with deletion mutations del13s1830 and del13s1854, the most common mutation. Mutations in GJB6 gene have been rarely reported in African patients.

Objectives: to investigate the involvement of *GJB6* (connexin 30 gene) is a selected group of Cameroonian and South African Blacks with Non syndromic congenital hearing loss.

Patients and Methods: *Gap PCR to detect* del13s1830 and del13s1854 deletion mutations and the complete sequence of the coding region of the *GJB6* gene in 100 patients with recessive non-syndromic sensorineural deafness and 64 ethnically matched controls. Patients were both from the Xhosa ethnic group and from various regions in Cameroon, approximating African diversity.

Results: preliminary results showed that none of the South African patients had *GJB6*.del13S1830 (0/23).

Conclusion: Ongoing completion of the study will allow capturing of the implication this *GJB6* this group of Africans patients

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PP 96

Living with X-linked Agammaglobulinemia (XLA) in South Africa: challenges and the role of genetic counselling

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X-linked Agammaglobulinemia (XLA) is a primary immunodeficiency disorder caused by mutations in the Bruton tyrosine kinase (Btk) gene. In the South African context, XLA has a variable prognosis. In our experience, poorer outcome relates to low socioeconomic status, delayed diagnosis, limited

access to treatment, poor transitioning to adult care and high rates of infection exposure. Genetic counselling is an important part of comprehensive care for affected individuals and their families in our setting. We describe a mixed ancestry family from the Western Cape, affected with XLA, with a known disease-causing mutation in the Btk gene. A detailed pedigree is provided and used to highlight some important genetic counselling considerations in XLA. Genetic counselling includes obtaining a detailed family history, allowing for identification of at-risk female relatives who can be offered carrier testing, as well as prenatal diagnosis. Genetic counselling provides an opportunity for exploring understanding, experiences and the impact of the condition, to assess important social issues and to reinforce management principles. Specific challenges in this family included: low socioeconomic status, early death of affected individuals, denial of the diagnosis and burden of disease. This resulted in fear of the condition and difficulties in communicating and adjusting to the diagnosis. Primary immunodeficiency disorders, such as XLA, can dramatically impact on the lives of the affected individuals and their families, with particular challenges being faced in a developing world setting. Genetic counselling has a crucial role to play in these families and should be offered as part of standard care.

PP 97

The effect of preconception paternal alcohol intake on candidate gene expression in mouse embryos

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Background: Epigenetic mechanisms regulate gene expression, which are particularly vital during foetal development. These mechanisms have been shown to be sensitive to the presence of ethanol, which mediates its effects through epigenetic alterations. *Chromodomain helicase DNA binding protein 6* (*Chd6*) is essential in motor-coordination with the highest levels of expression in the brain.

Aim: It was hypothesised that chronic exposure of male mice to ethanol prior to conception would alter epigenetic regulatory mechanisms and consequently dysregulate gene expression. This study quantified *Chd6* expression in day 16.5 brains of embryos sired by ethanol-treated and sucrose-treated (control) male mice.

Methodology: RNA was extracted from whole embryo brain tissue and converted to cDNA. *Chd6* expression levels were quantified using hydrolysis probe multiplex real-time PCR and the results were analysed using the ΔΔCt method of relative quantification, using *beta actin* (*ACTB*) as an endogenous control.

Results: Preliminary data shows a trend for reduced *Chd6* expression levels in the brains of embryos sired by ethanol-treated males in comparison to the embryos of sucrose-treated males. Altered *Chd6* expression levels were associated with a reduction in brain weight of embryos sired by ethanol-treated males.

Conclusion: Chronic preconception paternal alcohol exposure appears to be associated with decreased *Chd6* expression and a concomitant decrease in brain weight of day 16.5 embryos.

PP 98

Prenatal diagnosis and outcome of congenital Lower Urinary Tract Obstruction (LUTO) at Tygerberg Hospital Fetal Medicine Clinic: an audit of the past 10 years.

H Bezuidenhout (Tygerberg Hospital), M Urban (Tygerberg Hospital), L Geerts (Tygerberg Hospital)

Lower Urinary Tract Obstruction (LUTO) represents a group of heterogeneous congenital renal tract anomalies causing bladder outlet obstruction. It is either an isolated anomaly with male predominance, or is associated with other anomalies, termed complex LUTO. Chromosomal anomalies associated with LUTO are reported as 5.6 % internationally. LUTO can be diagnosed antenatally and determine the management of that pregnancy. There are limited published statistics on congenital anomalies in South Africa and therefore inadequate information to develop guidelines on antenatal diagnosis and genetic counselling.

A retrospective study reviewed the presentation and outcome of fetuses with ultrasound LUTO features. Of a total of 59 LUTO diagnoses, 31 (64%) were isolated and 21 (36%) complex, with a female to male ratio of 1:10. Chromosomal anomalies were detected in 7 cases (12%), all associated with complex LUTO. Genetic diagnoses included Down syndrome (4 cases), Trisomy 13 (1) and Trisomy 18 (2). The median gestation age of detection was 23 weeks. The range of maternal age was 15 to 44 years, with 18 % of women over 35years. Termination of pregnancy (TOP) was performed in 18 cases.

Diagnosis of LUTO at an advanced gestation limits investigations and procedures and raises ethical dilemmas concerning late termination of pregnancy and feticide. The study findings support two recommendations - invasive prenatal testing for chromosome analysis in complex LUTO especially in males, but not in isolated LUTO and prompt evaluation for markers of Down syndrome in the presence of LUTO as Down syndrome is an under-recognised cause of LUTO.

PP 99

Identification of BANK1 polymorphisms by High Resolution Melting and their association with Systemic Lupus Erythematosus in Black South Africans

S Malindisa (University of the Witwatersrand)

Systemic Lupus Erythematosus (SLE) is a complex autoimmune disease, with more than 30 susceptibility loci being identified through GWAS studies. One such candidate region is the *BANK1* gene. Three functional single nucleotide polymorphisms (SNPs) within *BANK1*; rs10516487, rs3733197 and

rs17266594 have been shown to be associated with SLE in Caucasian and Han Chinese populations. The aim of this study is to determine whether *BANK1* polymorphisms are associated with SLE in black South Africans and to test the accuracy of High Resolution Melt (HRM) analysis.

Genotyping of the *BANK1* polymorphisms in 54 black South Africans with SLE who attended the Lupus Clinic at Chris Hani Baragwanath Hospital and 100 geographically and ethnically matched controls was carried out using HRM analysis. Allele and genotype frequencies of the SNPs were calculated and contrasted to those of African populations genotyped in the HapMap project.

The frequency of rs10516487 C allele was substantially increased in SLE patients compared to controls, 97.2% and 64.5% respectively. Also the frequency of rs17266594 C allele was moderately increased in SLE patients compared to controls, 63.0% and 46.0% respectively. Data analysis for the rs3733197 is still being completed.

The study shows that rs10516487 and rs17266594 polymorphisms are most likely to be implicated in susceptibility to SLE in black South Africans. Genotyping with HRM is simple, fast, accurate and cost-effective, however validation of results via sequencing is recommended.

PP 100

ALUGEN: A registry for systemic lupus erythematosus (SLE/lupus) in African patients

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Systemic lupus erythematosus (SLE) is a severe autoimmune disease affecting skin, joints, haematopoietic system, kidneys, lungs and central nervous system; and arising from a complex interplay between environmental and genetic susceptibility factors. SLE incidence has been studied in groups of various ethnicities, with evidence that SLE most frequently affects non-Caucasians; although the epidemiology of SLE in much of the developing world is still undetermined, particularly in Africa. Over the last century, incidence of SLE in Black Africans was thought to be negligible; with a perception of a general rarity of SLE (and other autoimmune diseases) in tropical malarial areas; as well as a popular theory of increasing prevalence of SLE in African people living outside Africa. Low measured prevalence could also be due to poor access to health care, low disease recognition and poor access to diagnostic tools. More recently, African researchers have clearly demonstrated occurrence of SLE in Black Africans in Africa. At Groote Schuur hospital, we have established a registry for SLE patients visiting our clinics. We are developing a research program to investigate SLE causes in these patients; and for outreach to other African lupus researchers and clinicians. We have initiated the African Lupus Genetics Network (ALUGEN),an informal network of African clinicians and researchers with an interest in SLE. We have conducted an African-wide questionnaire to begin to unravel the myths of non-occurrence of lupus in Africa, and we have begun to investigate some of the factors that may underlie SLE occurrence in African patient populations.

PP 101

Peroxidasin expression in human carcinoma cells

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Peroxidasin (PXDN), a newly-identified heme-containing peroxidase, has both an enzymatic peroxidase domain and protein-binding domains characteristic of extracellular matrix (ECM) proteins. PXDN mRNA expression is found in many human tissues although protein has only been detected in vascular tissue. One function of PXDN is in ECM stabilization by catalyzing sulfilimine crosslinks in collagen IV. As such, PXDN may be involved in cancer cell adhesion and metastasis, although this role has yet to be elucidated.

The aim of this study was to determine PXDN expression in a panel of previously uncharacterized human carcinoma cell lines (HT29, MCF7, A431, HeLa, Huh7, and MOLT4). PXDN mRNA expression was investigated by PCR, and protein expression by western blot of cell lysates and culture medium (following ammonium sulphate precipitation). The effect of transforming growth factor beta-1 (TGF-β1), which can promote metastasis through epithelial-to-mesenchymal transition, was determined by western blot after cell treatments.

We found a differential in PXDN mRNA and protein expression; high mRNA-expressing cells (MCF7, A431 and HeLa) showed no protein, whilst Huh7 and HT29 cells showed low/no mRNA but expressed high protein, only MOLT4 cells exhibited both, which may suggest a self-regulatory mechanism. Interestingly, Huh7 cells treated with TGF-β1 showed a dose-dependent up-regulation of PXDN. This study highlights the lack of correlation between mRNA and protein expression of PXDN in cancer cells, but more importantly, determining the role PXDN expression in cancer cell adhesion and metastasis (through our current investigations), may identify a new target for cancer therapy.

PP 102

Genetic variation in the CHRNA5-A3-B4 gene cluster in some southern African populations

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The binding of nicotine to nicotinic acetylcholine receptors (nAChRs) is known to influence smoking behaviour and thus nicotine dependence. Two single nucleotide polymorphisms (SNPs), rs1051730 and rs16969968 within the nAChR gene cluster CHRNA5-A3-B4, situated on the long arm of chromosome 15 (15q24-25.1), has been associated with an increase in nicotine dependence. The rs1051730 mutation is characterized by a synonymous C to T nucleotide change in the *CHRNA3* gene whereas SNP

rs16969968 is associated with a G to A mutation that changes the amino acid aspartate to asparagine (D398N). This synonymous mutation affects the function of the receptor.

In the present study we have developed a Taqman® assay to screen for these two SNPs and propose to examine approximately 400 individuals consisting of Bantu-speaking (southwestern and southeastern) as well as Khoe-San groups from southern Africa. Thus far we have obtained results for rs1051730 in a small sample of Herero [N = 25] and Nama [N=28] groups. The T-allele was found at frequencies of 4% in the Herero and 8.93% in Nama. The genotyping is ongoing, and when completed, we propose to compare the southern African data with published data to assess whether southern African populations have the mutations linked with nicotine dependency.

PP 103

Analytical validation of HER2/neu overexpression using formalin-fixed paraffin embedded tissue (FFPE) for microarray analysis in early stage breast cancer patients

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Breast cancer is a heterogeneous disease characterised by genetically distinct subtypes that differ in their response to treatment. More accurate determination of the risk of distant recurrence is now possible with use of genetic tests such as the 70-gene MammaPrint profile shown to reduce healthcare costs. It uses a highly versatile microarray platform with quantitative gene expression levels of ER, PR and HER2/neu (TargetPrint) provided in a separate readout as additional confirmation of immunohistochemistry (IHC) results. In this study the results for Her2/neu obtained with microarray analysis was compared with other testing platforms.

The study population consisted of 104 South African women with early-stage breast carcinoma referred for MammaPrint, using RNA extracted from 60 fresh tumours (58 patients) and 46 FFPE tissue samples. Her2 status using TargetPrint was performed in 88 samples for comparison with IHC and fluorescence hybridisation (FISH).

Sixty percent of the tumour specimens analysed were classified as low risk and 40% as high risk for breast cancer recurrence. Similar distribution patterns for MammaPrint low versus high-risk profiles were obtained irrespective of whether fresh tumour biopsies or FFPE tissue were used. The mean histopathological tumour size was 14.6 mm. Comparison of microarray analysis and IHC/FISH showed 100% agreement for Her2 status.

The cost-saving implications of gene profiling support incorporation of the comprehensive microarray platform into treatment planning to1) select chemotherapy in relevant early-stage breast cancer,2) confirm receptor status by providing quantitative gene expression assessment ,3) as well as molecular subtyping of Luminal A and B.

PP 104

Metformin Negatively Alters the response of Oesophageal Squamous Cell Carcinoma Cells to Cisplatin by Reducing DNA Adduct Formation

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OSCC is among the highest causes of cancer-related death in South Africa. Cisplatin, commonly prescribed for OSCC, is a DNA binding agent, which displays both high incidence of chemoresistance and chemotoxicity. Combination therapies can however be used to circumvent these effects. Metformin, an anti-diabetic drug, has anti-proliferative effects on cancer cells. This study investigates the effects of metformin on South African-derived OSCC cell lines and its combination with cisplatin.

OSCC cell proliferation was assessed by haemocytometer counts. Stage of the cell cycle was assessed by flow cytometry. Cytotoxicity of metformin-cisplatin combination therapy was evaluated by MTT assay. The effect of metformin on cisplatin-DNA adduct formation was quantified by inductively coupled plasma mass spectrometry.

Metformin significantly reduced OSCC cell proliferation (WHCO1 50%, WHCO5 32%, and SNO 39%) and increased the ratio of cells in G0/G1:G2/M (WHCO1 74%, WHCO5 62%, and SNO 74%) relative to untreated controls. However, metformin-treated cells displayed higher EC50 values versus cisplatin treatment alone (WHCO1 78%, WHCO5 140%, and SNO 156%), which our findings show is due to reduced cisplatin-DNA adduct formation (WHCO1 19.3%, WHCO5 14.3% and SNO 18.7%). Resistance to cisplatin may result from up-regulation of genes that may repair DNA or facilitate cisplatin export in response to metformin.

This study demonstrates that metformin can halt proliferation of OSCC cells. However it abrogates the effects of cisplatin, warranting caution against combined use. Further investigations may reveal suitable anti-cancer agents that work in combination with the anti-proliferative effects of metformin for less a harmful chemotherapeutic option.

PP 105

A clinical and molecular investigation of two families with Simpson-Golabi-Behmel syndrome

C Spencer (University of Cape Town)

Introduction

Simpson-Golabi-Behmel syndrome (SGBS) is an X-linked overgrowth syndrome characterised by macrosomia, distinctive facial features, and multiple congenital abnormalities. Two genes,glypican 3 (*GPC3*) and glypican 4 (*GPC4*), have been found to be associated with SGBS. Mutations in *GPC3* are detected in 37-70% of affected males.

This research aimed to describe the phenotype of two unrelated boys and to attempt to make a molecular diagnosis by investigating *GPC3*.

Methods

Two male probands were identified, proband B and S. Their clinical records were reviewed and their physical manifestations documented.

DNA was extracted from both boys as well as their mothers. All eight exons of *GPC3* were amplified by polymerase chain reaction (PCR). The products were first analysed for large gene deletions and thereafter for point mutations.

Results

The clinical phenotype of proband B and S was found to be consistent with that reported in the literature.

DNA analysis of proband B revealed a mutation in exon 4 of *GPC3*. It can be labelled as p.358Arg-PheFSX373 (NM_004484.3).

No *GPC3* mutations were identified in proband S.

Discussion

The phenotype of the boys included in this research is similar to that previously reported in the literature. The main clinical manifestations, which prompted a diagnosis of SGBS in the two boys, were macrosomia, coarse facial features, macroglossia and a grooved tongue.

The importance of regular tumour surveillance is reinforced in this research by virtue of the Wilms tumour that proband B developed.

The mutation found in proband B represents a novel, and likely disease-causing mutation.

PP 106

The significance of CYP2D6 pharmacogenetic testing in oestrogen receptor-positive breast cancer patients

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Introduction

Variation in the CYP2D6 gene may increase recurrence risk in estrogen-positive breast cancer breast cancer patients treated with Tamoxifen (TAM), especially with concomitant use of certain antidepressants.

Aim

The aim of this study was twofold: 1) to determine the frequency of the relatively common inactivating CYP2D6*4 allele in South African breast cancer patients and 2) to determine the prevalence of depression/use of antidepressants in breast cancer patients referred for genetic testing.

Subjects and Methods

A total of 114 South African breast cancer patients, including 52 Caucasian and 62 Coloured (Mixed ancestry) individuals, and 63 Caucasian control individuals were genotyped for the most common inactivating allele (CYP2D6*4, rs3892097) in the CYP2D6 gene.

Results

In the initial validation data set consisting of 25 Caucasian and 62 Coloured patients, the CYP2D6*4 allele frequency was significantly higher in Caucasian compared to Coloured patients (24% vs. 3%, $p<0.001$). Extended CYP2D6 genotyping was subsequently performed in an implementation data set of 27 Caucasian breast cancer patients, to determine the prevalence of depression and use of antidepressants in a clinical setting. A medical history of depression and/or use of antidepressants were reported in 37% (10/27) of these breast cancer patients.

Conclusion

The weight of current scientific evidence in relation to risk-benefit assessment supports CYP2D6 genotyping in breast cancer patients who (1) are receiving TAM and (2) are at high risk for tumour recurrence (e.g. family history, BRCA1/2 mutation-positive) or (3) are required to take potential competing antidepressants.

PP 107

Website for "What is Genetic Counselling?"

S Erasmus (GC Network), N Kinsley (GC Network)

In 2012 the first readily available resource for genetic counselling was launched in South Africa. Up until then information was mainly restricted to healthcare and academic networks.

The provision of genetic counselling has recently shifted focus from the provincial-academic setting to include the private practice.

This has been driven by changes in healthcare structures and funding.

The decision to brand genetic counselling in South Africa was birthed from the knowledge that less than 10% of the local genetic needs are being fulfilled. Highlighting the necessity for increased awareness of the service on a national scale.

The website www.geneticcounselling.co.za was developed to create a clinical genetics community in South Africa centered on genetic counselling.

This is achieved by supplying information on genetic counselling services and access, types of genetic counselling, support groups, helpful resources, news and events, and assistance with queries.

The three focal communities are; the curious public, potential clients and support groups; healthcare professionals; marketing services for genetic counselling including media awareness, social networking and information pamphlets for display and distribution.

The website is a workable solution for information on genetics and genetic counselling. In 18 months over 6,000 visitors have accessed the site with 43% searching for information on genetic counselling services alone. Other successes include; nationwide referral for services, individualised support to healthcare professionals, corporates, and the media with a steadily increasing number of online queries. The website has been commended by the Genetic Counsellors of South Africa for innovation and value to the profession.

PP 108

Effect of genetic variation on iron status and age of first presentation in South African patients with multiple sclerosis.

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Introduction

Multiple sclerosis is a chronic inflammatory disease of the central nervous system. Iron deficiency was previously demonstrated in 30% of patients with MS. Structural variations in matriptase-2, one of the major regulatory proteins of the iron absorption pathway may result in functional iron deficiency. This study investigated a common SNP in matriptase-2 TMPRSS26, c.2207C>T (RS855791) on serum iron status and disease onset/diagnosis in South African patients with MS.

Study population: 126 patients diagnosed with MS and 169 controls without neurological disease. TMPRSS6 was genotyped using high throughput real-time polymerase chain reaction (PCR) with the Applied Biosystems (ABI™) TaqMan® assay, ABI™ 7900HT, following analytical validation against direct sequencing as the gold standard.

Results: Homozygosity for the iron-deficiency risk allele c.2207T was associated with significantly lower serum iron levels (13.45 ± 1.84 µmol/L) compared to the CC homozygotes (19.93 ± 1.33 µmol/L) in the whole study population ($p = 0.018$) and with significantly lower transferrin saturation values (22 % ± 2.33 vs 29 % ± 1.95; $p = 0.047$). There was a trend towards earlier age of MS diagnosis (TT homozygotes 36 ± 2.66 years and CC 40 ± 2.17 years) and presentation of first symptoms (TT 28 ± 2.89 years and CC 36 ± 2.31 years; $p = 0.05$ (Fisher least significant difference LSD post hoc test.)

Conclusion: The c.2207C>T (V736A) variant of matriptase-2 could contribute to iron deficiency in MS, assisting in clinical management for improved disease outcomes in a subset of MS patients with low iron.

PP 109

Ethical issues surrounding the development of the NHLS Stellenbosch H3Africa Biorepository

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Background: The H3Africa Initiative was launched in October 2012 with first round NIH and Wellcome Trust awards given to several research projects, including funding for the development of two biorepositories. As one of these will be located in South Africa, the national legislation and regulations on biorepositories need to be explored. Currently national ethics in South Africa is governed by The National Health Act, Act no 61 of 2003, although chapter 8 and 9 addresses research surrounding biological material and the ethics involved, biobanking in South Africa is a new phenomenon and not clearly discussed.

Methods: Intricate mechanisms of pioneering governance through the formation of external and internal governance bodies will be required to oversee the evolution and development of legislature surrounding biobanking in South Africa. All the existing regulations and legislature of biobanking in South Africa will be investigated and compared to other African countries and existing international biobanks. The project, through the H3Africa PIs and their Ethics collaborators, will be closely involved in adapting country guidelines and regulations to facilitate this initiative.

Anticipated Results: Important ethical issues such as informed consent, benefit sharing, return of results and rights to privacy will be debated to reach consensus amongst African collaborators during the establishment of the biorepositories in the H3Africa Consortium.

Conclusion: Improved understanding of ethical issues surrounding biobanking in South Africa and on the African continent will facilitate the development of biobanking legislature as well as the harmonization of policies and regulations on biospecimen sharing between African countries.

PP 110

Development of the H3Africa Cape Town Biorepository as an Integral Part of the H3Africa Consortium to Facilitate Studies on biodiversity, Disease &Pharmacogenomics of African Populations

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Background: The H3Africa Initiative was launched in October 2012 with first round NIH and Wellcome Trust awards given to several research projects, including funding for the development of two biorepositories and a bioinformatics network. The Cape Town pilot biorepository will be directed by Professor Akin Abayomi, from NHLS/Stellenbosch University, in collaboration with SANBI, RUCDR, and The Scripps Research Institute, Centre for Regenerative Medicine.

Methods: During Phase I, the biorepository team will set up governance, operations and protocols for human tissues. Room temperature storage, cell line creation using conventional methodology and iPSC technology will also be evaluated. Commercial and open resource LIMS/BIMS platforms will be compared and a business model of sustainability developed. Progress will be reviewed at the end of phase I, with sufficient progress leading to expansion over a further five years.

Anticipated Results: Pilot studies will be conducted to receive, store, and distribute biological research samples obtained in the H3Africa Initiative, with the aim to store over 100,000 samples per year at the end of Phase I. The goals for the Phase II scale-up include providing a fully functioning biorepository capable of receiving and distributing samples from and to African countries, utilizing international standards.

Conclusion: Creation of a full scale biorepository will facilitate scientific capacity building on the African continent and will serve to preserve human samples, harmonize sample collection efforts, be a centralized point for sample dissemination for coordinated genomic analysis efforts, a training centre for African scientists and a community outreach portal for public education.

PP 111

The Value of External Technical Support and Benchmarking to Evolving Centralised Biobanks in Africa

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Background: The development of centralised biobanks in Africa forms an important part of the H3Africa initiative as a resource to facilitate research development and sustainability in both SA and the African continent. As most biobanks are in the developed world, a benchmarking exercise of two international biobanks was performed to identify common practices that can be implemented at the NHLS-Cape Town-H3Africa Biobank (NCB-H3A).

Methods: An SOP on how to perform a benchmark was developed to collect information on best practices relating to biobanking operations, using an online interview tool compiled from the report "Case Studies of Existing Human Tissue Repositories: Best Practices for a Biospecimen Resource for the Genomic and Proteomic Era". Questions covered aspects related to specimen collection, transportation, storage, processing and annotation, consumer/user needs, bioinformatics and data management, business plan and operations, privacy, ethical concerns, consent issues, intellectual properties and other legal issues as well as public relations, marketing and education. Benchmarking was also performed via site visits, interviewing directors and technical staff, and e-mails.

Anticipated results: This exercise will allow us to determine our existing biobanking capacity, develop the envisioned scale up plans, and assist us in addressing unfamiliar issues and challenges that are unique to the African continent.

Conclusion: Performing such an exercise and utilising the external technical support on ground will aid in capacity building, technology exchange and will also assist in creating a benchmarking paper as a valuable resource to evolving biobanks on the African continent.

PP 112

PALB2 variants in black South African women with breast cancer

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PALB2 (partner and localizer of *BRCA2*) is a breast cancer susceptibility gene, when mutated, confers a doubling of breast cancer risk with moderate penetrance. Studies in different population groups (mostly of European ancestry), have shown that deleterious mutations in *PALB2* account for approximately 1% of familial breast cancer cases. We have previously

investigated *PALB2* mutations in a cohort of white South African women (dx ≤45years), unselected for family history. A 2% carrier frequency was established for this group. There have been no *PALB2* studies of black South African women with breast cancer.

We aimed to determine the involvement of *PALB2* in 238 black women with breast cancer, (dx 18-65years, unselected for family history, negative for *BRCA1* and *BRCA2* mutations).

All exons and intron/exon boundaries of *PALB2* were Sanger sequenced and 27 variants (5 novel) were identified. One pathogenic splice acceptor site mutation was found in a 40-year-old woman with no family history of cancer. Combinations of one or more of the additional 26 variants, (including nine non-synonymous, eight synonymous, six intronic, and three variants in the 5'- and 3'-UTRs), were detected in 174/238 patients. *In silico* methods predicted that two missense mutations, both located in the RAD51 binding domain of PALB2, may be deleterious.

Consistent with previous population studies, we have confirmed that pathogenic mutations in *PALB2* are rare. Compared with our previous study, we report a carrier frequency of ~0.9% (1/110) in black South African women with breast cancer diagnosed at a relatively early age (≤45years).

PP 113

Occult hepatitis B and carcinogenesis markers in Chronic Hepatitis C infection

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The presence of HBV-DNA in the patient serum without detectable HBsAg is called occult infection. Coinfection with occult HBV in chronic HCV patients Detection of occult HBV infection in Chronic hepatitis C virus by using quantitative PCR and detection of CD45- and CD90+ in HCC by flow cytometry

Methods: Serum HBV was detected in 30 patients with histologically verified HCV related chronic liver disease. In addition to 10 healthy control subjects

Results: of 30 patients, the sera of 9(30%)were positive for HBV DNA by different PCR assays documenting HBV infection. It was found that 5 are positive for HBV DNA (surface gene)(16.6%), and only one positive for (core gene)(3.3%).Only two from the nine were positive for both surface and x gene. Identification of CD45- and CD90+ in HCC suggests that it can be used as a marker for human liver cancer

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PP 114

Bioinformatics Knowledge Transfer Programme (KTP) for Human and Capital Development

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The multidisciplinary nature of the bioinformatics field, coupled with rare and depleted expertise, is a critical problem in the advancement of bioinformatics in Africa. Further impediments include limited internet connectivity, lack of computational resources and sub-standard research facilities. Individuals who receive international training in highly resourced environments usually struggle to function or implement their expertise and knowledge when they return to their resource-stricken native environments. Short-term training programs have proved to be unsustainable and costly since knowledge and skills are not retained over time and limit the impact of such training.

The KTP is a research and education model conceptualised to address these problems in a sustainable manner to stimulate, enhance and strengthen African bioinformatics capacity. Its aim is to cost effectively facilitate the transfer of knowledge and skills from experienced, internationally recognised experts to local scientists. The CPGR, with its partners as hosts, facilitate the process by identifying experts, training requirements and potential research associates. Instead of several associates travelling to well established labs for short periods of time, experts are brought in to interact and conduct high-quality research agendas locally. Experts and associates are brought together to work on relevant projects through which transfer of knowledge and skills is achieved naturally. The advantage of the approach is that several associates benefit from one expert, while minimising travel and accommodation expenditure. The programme is assessed by project outputs such as number and quality of publications, conference participation, patents or intellectual properties secured and the number of successfully trained associates.

PP 115

Whole Genome Sequencing: '*Local is lekker*'

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The South African Whole Genome Sequencing (WGS) capacity does not yet reflect the field's impressive global advancements and utility. Next generation WGS service providers have continually endeavoured to reduce costs, while improving data quality and turnaround time. Experimental platform innovations now enable one to sequence their genome at 40X coverage in as little as 27 hours. Read length has continued to extend in the company of improved base-calling, facilitating downstream bioinformatics analyses and improving accuracy. Globally, we are nearing the $1000 genome and personalised

medicine milestones. *But how feasible is WGS in South Africa?* In the near-term, challenges are many, including costly sequencing (approximately R65,000 per genome) and extensive computational requirements for data transfer, analysis, and storage. Supposing these challenges are overcome, confidence in the obtained results is often lacking or indeterminable. Finally, and perhaps most importantly, the translation of confident results into clinical utility is sparse. We use a pilot study of WGS on malignant oesophageal tissue from four South African individuals infected with human papilloma virus (HPV) to explore these challenges. We employ a network of local and international resources to achieve collaborative synergism and highlight several key aspects in maximising value from data generated on a limited budget. Using this knowledge, we aim to bolster local capacity and help shift South African research towards a world standard of WGS data provenance, clinical compliance and innovative utility.