THEME 4: MOLECULAR AND COMPARATIVE BIOSCIENCES

ABSTRACTS FOR ORAL AND POSTER PRESENTATIONS

ORAL PRESENTATIONS

MCB-O-01

Micro-architecture of the human postnatal maxilla across the stages of dentition

Xolisiwe Mhlathi, Erin Hutchinson, Candice Small

University of the Witwatersrand, Faculty of Health Sciences, School of Anatomical Sciences

Abstract: The growth, modeling and remodeling of the maxilla are shaped by the biomechanical forces exerted on it. As functional demands of the masticatory system become complex due to dental development and eruption, the intensity and complexity of the biomechanical forces increases and influences associated micro-architecture. There is paucity of literature on the changes of maxillary micro-architecture during these important stages of growth. Thus, this study aimed to assess these changes in relation to dental development and eruption. The study sample included seventy-nine individuals (0-18 years), subdivided into three dentition groups: deciduous dentition (0-5 years; n = 28), mixed dentition (6-12 years; n = 9), and permanent dentition (13-18 years; n = 42). Maxillae were scanned using micro-computed tomography, reconstructed using Nikon CTPro software and analyzed using VGStudio Max. Seven regions were selected in each dental crypt for evaluation of micro-architecture, including bone volume ratio (BV/TV), bone surface to volume ratio (BS/BV), trabecular thickness (TbTh), trabecular number (TbN), trabecular spacing (TbSp). The data was analyzed using multivariate analysis of variance and Tukey's post hoc test. The micro-architecture of the maxilla changed from a more porous to a less porous nature, following patterns of bone remodeling. These changes included an increase in bone volume ratio while bone surface to volume ratio and trabecular number both significantly decreased. The observed micro-architecture reflects changes in the state of the dentition as well as adjustments to the complex functional environment of the oral cavity complex.

Keywords: Micro-architecture, maxilla, dentition, micro-CT

MCB-O-02

Are anatomical variations relevant for South African clinicians?

Ozyl Marufu, Nicholas Bacci, Joshua Davimes

School of Anatomical Science, Faculty of Health Sciences, University of Witwatersrand

Abstract: Anatomical variation research has been conducted extensively by anatomists for many years to improve clinical practice. Comprehensive knowledge of variants is essential for understanding and preventing diagnostic and surgical complications, failure of which may lead to malpractice, morbidity, and/or mortality. However, there is no research on South African clinicians' preparedness, exposure, or consideration of anatomical variations. Hence, this study investigated the relevance of anatomical variants to clinicians through a validated, self-administered, online questionnaire disseminated to surgeons and radiologists from public and private South African health institutions. The perceived value of variant research and the exposure to variants across different clinical sectors were statistically tested. A total of 52 (public: 51.9%, private: 28.8%, both: 19.2%) clinicians participated. Most clinicians (71.2%) were taught about anatomical variations in undergraduate and postgraduate medical training and 55.8% encountered variants regularly in clinical practice (radiologists: 93.8%, surgeons: 45.2%). Public sector clinicians observed significantly less variations than private sector clinicians (U=427.00; p-value=0.001). Clinicians encountering variants more frequently, were significantly more likely to interact with variant research (ρ=0.375; p-value=0.009). However, overall, most clinicians (65.4%) seldomly engaged with anatomical variant research, 40.4% preferred scientific manuscripts over other sources such as textbooks, scientific magazines, clinical guidelines, congresses/conferences, clinical trial studies, documentaries/media and CPD training for variant information. This indicates that while South African clinicians are generally well-trained on anatomical variations, there is an overall lack of engagement with ongoing research intended to inform them. As such, scientific reporting of variations should be better optimized between anatomists and clinicians.

Keywords: Anatomical knowledge, Variations, Clinicians

MCB-O-03

MicroRNA expression and arterial function in type II diabetes mellitus

Batsheva Goldfein

School of Physiology

Abstract: Type II diabetes mellitus (T2DM) is a major health concern, contributing significantly to global cardiovascular disease (CVD) mortality. Arterial dysfunction is an early predictor of CVD and the role of microRNAs (miR), particularly miR-146a-5p, in the development of arterial dysfunction in diabetic populations has yielded contradictory results. Therefore, the aim of this study was to determine the role of miR-146a-5p in the development of arterial dysfunction in patients with T2DM. This cross-sectional, case-control study (n=118) included 67 diabetic and 51 control participants. CVD risk was evaluated using the Framingham risk score. Serum levels of inflammatory and fibrotic markers and miR-146a-5p were measured using ELISAs and real time quantitative PCR, respectively. Arterial function was measured using applanation tonometry. MiR-146a-5p expression was increased in diabetic compared to control participants (p=0.02) and

was associated with tumour necrosis factor α (TNFα) concentration (partial r=0.23, p=0.02) and markers of obesity and dyslipidaemia (all p<0.05). In multivariate analysis, miR-146a-5p expression was not associated with arterial function (all p>0.05). However, when stratifying participants based on CVD risk, in high-risk participants, miR-146a-5p was inversely associated with arterial pressure pulsatility measures, including central pulse pressure (Std β=-0.76, p=0.01) and central systolic blood pressure (Std β=-0.38, p=0.02). When including the arterial remodelling marker, matrix metalloproteinase 1 (MMP1), as a confounder, these associations were no longer significant. These results suggest that miR-146a-5p may have a regulatory role in the development of arterial dysfunction that is mediated through arterial remodelling in persons at high risk for CVD.

Keywords: miR-146a, Cardiovascular disease, Arterial function, Type II diabetes

MCB-O-04

Simvastatin significantly reduced Alcohol-induced brain damage in adolescent mice

Robin du Preez

School of Anatomical Sciences, Faculty of Health Sciences

Abstract: Alcohol abuse in South Africa, especially among adolescents in struggling communities, is concerning due to regulatory oversights. Misuse of alcohol harms the developing brain and causes neurodevelopmental problems. Alcohol negatively affects the neurogenic process important for generating new neurons from progenitor cells, leading to cognitive and memory impairments. The financial burden on health systems due to alcohol-related problems is substantial in South Africa, emphasizing the necessity for interventions to prevent alcohol-induced hippocampal damage. Simvastatin, a blood cholesterol-lowering drug, demonstrates potential in treating brain injuries due to its neuroprotective and anti-inflammatory properties, although its effectiveness in treating alcohol-induced brain damage remains unknown. Four-week-old C57BL/6J mice were administered 20% alcohol (intraperitoneal, i.p.), 5 or 15 mg/kg Simvastatin orally followed by 20% alcohol (i.p.) or the controls (i.e. 5 mg/kg Simvastatin only or non-treated). After 28 days, the left cerebral hemisphere was sectioned sagittally and processed for Nissl, proliferating cell nuclear antigen (PcNA) and doublecortin (DCX) immunolabelling. Positively immunolabelled PcNA or DCX cells were counted along the suprapyramidal blade of the dentate gyrus using QuPath software and cell distribution was quantified. Results showed that alcohol reduced PcNA- and DCX-positive cell distribution. In PcNA-positive cells, 5 mg Simvastatin suppressed the effects of alcohol in both sexes, although 15 mg was more effective in females. In DCX+ cells, 5 mg Simvastatin suppressed alcohol effects in both sexes, but no effect was seen at 15 mg. Ultimately, Simvastatin protects against alcohol effects on neurogenic capability and may potentially aid in the treatment of alcohol-related brain diseases.

Keywords: Alcohol, Neurogenesis, Adolescence, Simvastatin

MCB-O-05

The relationship between the morphology of the incisura fibularis tibiae and the pattern of posterior malleolus fractures

Papa Kyei, Collen Nkosi, Richard Paterson, Abdirashid Aden

Orthopaedic Surgery

Abstract: The approach to fractures of posterior malleolus (PM) has evolved in recent years and an area of importance at the distal tibia is called the Incisura fibularis tibiae. Studies have shown that the anatomy of the incisura is variable and that there are different patterns of PM fractures. This study aimed at evaluating the relationship between the anatomy of the incisura and pattern of PM fractures. A retrospective review was done of patients who sustained PM fractures at an academic hospital over a three year period. The demographics, Incisura and PM characteristics of preoperative bilateral ankle computed tomography (CT) scans were analysed. The relationship of the anatomy of the incisura on the unaffected ankle and pattern of PM fracture on the injured ankle was evaluated. Incisura morphologies were measured using axial CT images from 5mm proximal to the tibial plafond. The PM fractures were categorized according to the PM classification systems. 145 cases who sustained PM fractures were included. Females were the majority, accounting for 61% while the right ankle was the most commonly injured. We analysed the incisura depth, incisura rotation, incisura width, and incisura shape, and the data revealed statistical significance ($p < 0.001$). There was a statistical difference with the types of PM fractures and incisura pattern (p-value= 0.0131). A statistically significant relationship was observed between the fibula engagement and PM fractures ($p = 0.049$). In conclusion certain fractures of the posterior malleolus were more prevalent in individuals with particular anatomical configurations of the tibia incisura and fibula.

Keywords: incisura, posterior malleolus fracture

MCB-O-06

PAM50 intrinsic subtypes in black South African women with breast cancer

Therese Dix-Peek, Boitumelo P. Phakathi , Eunice J. van den Berg, Caroline Dickens, Tanya N. Augustine, Herbert Cubasch, Alfred I. Neugut, Judith S. Jacobson , Maureen Joffe, Paul Ruff, Raquel A.B. Duarte

Department of Medicine, University of the Witwatersrand, Faculty of Health Sciences, South Africa; Charlotte Maxeke Surgical Breast Unit, Charlotte Maxeke Johannesburg Academic Hospital, South Africa; Department of Surgery, University of the Witwatersrand, Faculty of Health Sciences, South Africa; Department of Histopathology, National Health Laboratory

Service, Chris Hani Baragwanath Hospital, South Afric;a Department of Anatomical Pathology, University of the Witwatersrand, South Africa; Batho Pele Breast Unit, Chris Hani Baragwanath Academic Hospital, , South Africa; Non-Communicable Diseases Research Division, Wits Health Consortium, , South Africa; Herbert Irving Comprehensive Cancer Centre, Vagelos College of Physicians and Surgeons, Columbia University, United States of America; Department of Epidemiology, Mailman School of Public Health, Columbia University, United States of America; MRC Developmental Pathways to Health Research Unit; Department of Paediatrics, University of the Witwatersrand, Faculty of Health Sciences, South Africa

Abstract: Breast cancer is a heterogeneous disease characterized by varying gene expression profiles, treatment options, and outcomes. In South Africa, tumours are classified using immunohistochemistry (IHC). In high-income countries, multiparameter genomic assays are employed, which influence tumour classification and treatment decisions. We examined the concordance between tumour classifications by IHC and the PAM50™ gene assay in a cohort of 378 breast cancer patients from the SABCHO study. Using immunohistochemistry, patients were classified as ER-positive (77.45%), PR-positive (70.56%), and HER2-positive (32.28%). These results, along with Ki67, were used as surrogates for intrinsic subtyping, resulting in 7% IHC-A-clinical, 73% IHC-B-clinical, 5% IHC-HER2-clinical, and 15% triple negative (TNC). The PAM50 classification identified 19% luminal-A, 32% luminal-B, 24% HER2-enriched, and 25% basal-like subtypes. The highest concordance was observed between the basal-like and TNC groups, while the luminal-A and IHC-A groups had the lowest concordance. By adjusting the Ki67 cutoff and reclassifying HER2/ER/PR-positive patients as IHC-HER2, we improved the concordance with intrinsic subtypes. We recommend adjusting the Ki67 cutoff to 20-25% in our population to better align with luminal subtype classifications. This change would improve treatment guidance for breast cancer patients in settings where genomic assays are unaffordable.

Keywords: African, breast cancer, microarray

MCB-O-07

Developing Monoclonal Antibodies Specific for Snake Venom Neurotoxins

Carla Hattingh

Department of Haematology and Molecular Medicine; Wits, Vaccines and Infectious Diseases Analytics (VIDA) Research Unit

Abstract: Snakebite is recognised as the most neglected tropical disease by the World Health Organisation. Current antivenom is effective but rudimentary, yielding a batch variable, unsafe, and unaffordable product. Mamba and non-spitting cobra venoms are amongst the deadliest due to their neurotoxicity. They are primarily composed of α-neurotoxins which bind post synaptically at the neuromuscular junction inducing paralysis and respiratory failure. These α neurotoxins are non-enzymatic three finger-fold toxins (3FTx) with a common tertiary structure

consisting of three β-stranded loops (fingers) extending from a hydrophobic, disulphide-rich core. 3FTx neurotoxins have evolved diverse functions that include calciseptine-like calcium channel inhibitors and long/short chain α-neurotoxin n-acetylcholine receptor inhibitors . The aim of this study was to assess the breadth of five monoclonal antibodies (mAbs) previously identified in the Wibmer laboratory that recognize calciseptine-like (mAbs 2D, 2H, and 4E) or α-neurotoxin-like (mAbs 4E, 3A, 3D) 3FTxs. Recombinant toxin genes were amplified by PCR, cloned into mammalian expression vectors, and confirmed by Sanger sequencing. Toxins and antibodies were expressed in expiCHO suspension cultures and purified by nickel or protein A affinity chromatography respectively, and then size exclusion chromatography. Binding breadth was evaluated by ELISA. The mAbs 3A, 3D, and 4E had breadth against α neurotoxins, while mAbs 2D and 2H had comparatively narrower binding breadth and recognised only calciseptine-related toxins. Somatically related mAbs 3A and 3D also displayed remarkably broad binding of both long and short chain α-neurotoxin subclasses, recognising antigens with only 29% sequence conservation. These monoclonal antibodies represent exciting lead candidates for modernizing this essential medicine.

Keywords: Monoclonal Antibodies Neurotoxins

MCB-O-08

Broadly neutralizing antibodies targeting SARS-CoV-2 and HIV expressed in an efficient fungal protein production system, retain potency and function.

Rebecca T van Dorsten, Sashkia Balla, Nelia P. Manamela, Nonkululeko Mzindle, Tandile Hermanus, Simone Richardson, Previn Naicker, Penny L Moore, Patrick B Arbuthnot, Kubendran Naidoo

Antiviral Gene Therapy Research Unit; National Institute for Communicable Diseases, South Africa; South African Medical Research Council Antibody Immunity Research Unit; Infectious Diseases and Oncology Research Institute, Faculty of Health Sciences, University of the Witwatersrand, South Africa; Council for Scientific and Industrial Research, South Africa; Rubic One Health, Johannesburg; South African Medical Research Council Antiviral Gene Therapy Research Unit; Wits Research Institute for Malaria, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, National Health Laboratory Service, South Africa

Abstract: Thermothelomyces heterothallica (C1) is a fungus that has attracted interest as a recombinant protein expression platform, which can produce impressive amounts of protein at a significantly lower cost when compared to mammalian cells. C1 can also be modified to allow for appropriate mammalian post-translational modifications (PTMs). Broadly neutralizing antibodies (AIRU946-A6 and -E4) targeting SARS-CoV-2 variants and HIV (CAP256.25) have been isolated from patients. However, their use as a therapy is dependent on cost of production. The aim of this study is to produce antibodies against important pathogens using C1 and evaluate

their potency and function. Antibody gene sequences for AIRU-A6, -E4 and CAP256.25 were synthesized and used to transform C1 protoplasts to create stable cell lines that express each antibody. Cell lines were verified using PCR and small-scale recombinant protein expression. Antibodies were purified using affinity and ion-exchange chromatography and confirmed using SDS-PAGE and immunoblot analysis. Fc effector assays, ELISA and TZM-bl neutralization assays were performed to determine antibody function. Analysis showed that SARS-CoV-2 antibodies had similar binding, potency and breadth, as well as levels of antibody-dependent cellular cytotoxicity, when compared to those produced in mammalian cells. CAP256.25 produced in C1 however, showed reduced potency due to the absence of a tyrosylprotein sulfotransferase. This enzyme enables a PTM to allow for electrostatic interactions between the antibody and HIV epitope. Future studies will include complementing C1 with the enzyme. We were able produce effective antibodies using C1. This alternative platform has the potential to express antibodies for therapeutic applications.

Keywords: therapeutic antibodies, protein expression platform, Thermothelomyces heterothallica (C1) Prof Matthew E. Hurles Prof Prosper Lukusa Prof Koenraad de Vriendt, **Prof Aimé Lumak Prof Amanda**

MCB-O-09

CNV detection from exome sequencing data: Outcomes from the DDD-Africa study

Nadja Louw, Phelelani T. Mpangase, Petr Danecek, Prince Makay, Zané Lombard

Department of Human Genetics, Sydney Brenner Institute for Molecular Bioscience, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa, Human Genetics Programme, Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton CB10 1RQ, UK., Center for Human Genetics, Faculty of Medicine, University of Kinshasa, Kinshasa, DR Congo Prof Helen V. Firth, Human Genetics Programme, Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton CB10 1RQ, UK AND East Anglian Medical Genetics Service, Cambridge University Hospitals NHS Foundation Trust, Cambridge CB2 0QQ, UK; Human Genetics Programme, Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton CB10 1RQ, UK; Center for Human Genetics, Faculty of Medicine, University of Kinshasa, Kinshasa, DR Congo; Center for Human Genetics, University Hospitals, University of Leuven, Leuven, Belgium ; Krause, Division of Human Genetics, National Health Laboratory Service & School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa Dr Nadia Carstens, Genomics Centre, South African Medical Research Council, Tygerberg, Cape Town, South Africa; Division of Human Genetics, National Health Laboratory Service & School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa AND Department of Internal Medicine, School

of Clinical Medicine, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

Abstract: Developmental disorders are rare conditions, causing mental or physical impairment. Although individually rare, it is estimated that up to 6% of the population is affected. These disorders are typically heterogeneous, posing great diagnostic challenges, often leading to multiple genetic testing and ultimately to a diagnostic odyssey. Copy number variants (CNVs) play a major role in the pathogenesis of developmental disorders and exome sequencing (ES) has allowed detection of CNVs and single nucleotide variants (SNVs) exome-wide with a single test. Research shows that combined SNV and CNV analysis may increase the diagnostic yield by 18% from the SNV only pipeline. In this study, 500 African patients with an undiagnosed developmental disorder were recruited, detailed clinical phenotyping and ES performed. Three bioinformatics CNV calling tools (CANOES, CLAMMS and XHMM) were implemented on data of the first 117 affected individuals and their parents (total n=287). Nine pathogenic/likely pathogenic CNVs were detected, including six de novo, representing an additional diagnostic yield of ~8%. The detected CNVs comprised eight deletions and one duplication with an average size of ~5.7Mb, spanning several OMIM morbid genes including ARID1B, SOX5 and NOG. These tools have been implemented on the remaining samples, and outcomes will be discussed as analyses continue at present. Integrating CNV detection into standard ES variant analysis can improve diagnostic yield and lead to an improved cost benefit for ES. These results may end the diagnostic odyssey for patients and lead to better care and management for families with developmental disorders in Africa.

Keywords: copy number variation, exome sequencing, bioinformatics

MCB-O-10

Immunogenicity of self-amplifying RNA vaccines against Hepatitis B virus

Nazia Samudh, Dylan Kairuz, Kristie Bloom, Kubendran Naidoo, Abdullah Ely, Patrick Arbuthnot

Antiviral Gene Therapy Research Unit (AGTRU); Wits/SAMRC; IDORI; Molecular medicine and Haematology, Pathology

Abstract: Vaccination against Hepatitis B virus (HBV) remains the most effective means of preventing infection. However, some individuals fail to develop neutralising antibodies, and currently available subunit vaccines are ineffective at eliciting Th1 cytotoxic immune responses which are required for eradication of intracellular pathogens. The alphavirus-derived self-amplifying messenger RNA (saRNA) vaccine platform enables the in-situ synthesis of antigens which trigger the innate immune system (interferon response) and promote a Th1-biased immune response. The aim of this project was to develop and investigate the immunogenicity of saRNA-based vaccines against HBV. saRNAs encoding the reporter protein luciferase, or HBV surface antigens, were synthesised by in vitro transcription and formulated with ionisable lipid

nanoparticles for in vivo delivery. Balb/c mice received either 1 or 5 micrograms of saRNAs administered intramuscularly as a 4 week prime-boost regimen. Expression of luciferase was examined by bioluminescence imaging. Humoral immune responses were examined by HBV neutralization assay. Expression of cytokines involved in the innate immune system response, and antigen-specific T-cell responses were measured using a bead-based multiplex assay and intracellular cytokine staining of HBV-peptide stimulated splenocytes respectively. saRNA vaccines triggered the interferon response in a dose-dependent manner. However, this did not hamper antigen expression, which was observed for an extended period, and was limited to the site of injection. saRNA vaccines elicited neutralising antibodies, and HBV-specific cytotoxic T-cell responses which were not observed in mice that received subunit-based HBV vaccines. saRNA-based HBV vaccines provide enhanced humoral and cellular immunity against infection, with possible therapeutic applications.

Keywords: HBV, saRNA, vaccine, immunogenicity

MCB-O-11

Impact of urinary uromodulin concentration on renal and haemodynamic parameters in a community of African ancestry with a high prevalence of hypertension.

Aaliah Charles, Manishka Reddy, Tanisha Naidoo, Monica Gomes, Angela J Woodiwiss, and Vernice R Peterson

Cardiovascular Pathophysiology and Genomics Research Unit

Abstract: Populations of African ancestry have a high prevalence of primary hypertension and its comorbidities, primarily exhibiting a volume-dependent form of hypertension. The role of nephron components in this context needs exploration. Uromodulin is a potential biomarker for renal function and tubular reserve, but its relationship with renal function, haemodynamic parameters, and hypertension in African ancestry populations is unknown. This study explored the relationship between urinary uromodulin (uUMOD) concentration, renal, and hemodynamic parameters in an African community with a high prevalence of volume-dependent hypertension. Haemodynamics (central pressures [SphygmoCor], echocardiographic aortic velocity, and diameter in the outflow tract), uUMOD concentrations (ELISA), and renal function (creatinine clearance from 24-hour urine [n = 370]) were determined in a community of African ancestry (n = 397). No relationships between uUMOD concentrations and renal function, age, BMI, BP, or hypertension were noted. However, uUMOD concentrations were higher in females than males, even after adjusting for confounders (P = 0.0007). An inverse relationship was observed between stroke volume (SV) and uUMOD (P = 0.0023), independent of confounders, and present in hypertensives (P = 0.007) but not normotensives (P = 0.43). Hypertensives had a higher SV than normotensives (P = 0.047). In a community sample with a high prevalence of volume-dependent primary hypertension, uUMOD was inversely related to SV, particularly in hypertensives.

Although uUMOD is not a biomarker for renal function in this population, these data suggest the need to investigate mechanisms linking uUMOD to SV to assist in identifying novel pathways for better treatment of volume-dependent hypertension.

Keywords: Uromodulin, Hypertension, renal, Haemodynamic

MCB-O-12

Neurophysiological effects of citalopram in a rodent model of ACTH induced HPA axis dysregulation

Andrea Lubbe, Aletta Millen

School of Physiology

Abstract: Despite the multifaceted theories surrounding the pathophysiology of depression, the monoaminergic theory remains the focus of pharmacological treatments, with selective serotonin reuptake inhibitors (SSRIs) being commonly prescribed. Despite this, approximately 30% of depressed patients are treatment-resistant, necessitating alternative therapeutic strategies. This study aimed to validate a rodent model of depressive-like symptoms via chronic ACTH administration and examine citalopram's effects on HPA axis dysfunction using neurobehavioral tests (open field test (OFT) and sucrose preference test (SPT) and regional brain mRNA expression of depression associated molecular markers such as BDNF, CREB and TrkB. Sprague-Dawley rats were divided into control and depressed groups, receiving saline or ACTH, followed by citalopram co-treatment. Neurobehavioral assessments revealed increased OFT immobility after 6 weeks in both sexes in all groups. Males that received ACTH exhibited a lower sucrose preference ratio. BDNF mRNA expression significantly differed between sexes: females treated with ACTH and citalopram showed higher striatal expression, while males had increased midbrain expression under the same treatment. Sex and treatment influenced CREB mRNA expression in the prefrontal cortex and midbrain, with males generally exhibiting higher levels. Females treated with ACTH + citalopram displayed elevated TrkB mRNA expression in the prefrontal cortex, and the striatum, ACTH administration led to increased TrkB mRNA expression in females. These findings suggest males are more susceptible to ACTH's neurobehavioral effects, potentially due to its impact on dopamine, while oestrogen in females may provide protection.

Keywords: Depression, SSRI, HPA-axis, ACTH

MCB-O-13

Empirical Design of Novel Ionisable Lipids Derived from Cashew Nutshell Liquid for Delivery of RNA Vaccines

Dylan Kairuz, Robin Klintworth, Connor Stockly, Aneesa Gani, Abdullah Ely, Charles de Koning, Patrick Arbuthnot, Kristie Bloom

Department of Molecular Medicine and Heamatology, Antiviral Gene Therapy Research Unit; Molecular Sciences Institute, School of Chemistry, University of the Witwatersrand; Molecular Sciences Institute, School of Chemistry, University of the Witwatersrand; Wits/SAMRC Antiviral Gene Therapy Research Unit, Faculty of Health Sciences, Infectious Diseases and Oncology Research Institute (IDORI), University of the Witwatersrand; Wits/SAMRC Antiviral Gene Therapy Research Unit, Faculty of Health Sciences, Infectious Diseases and Oncology Research Institute (IDORI), University of the Witwatersrand

Abstract: Sustainable manufacture is critical for equitable clinical translation of mRNA-lipid nanoparticle (LNP) vaccines within lower-middle income countries (LMICs). These versatile RNA vaccine platforms promote strong cellular and humoral immune responses and are beneficial for rapid response to combat current pathogens and future pandemics. The LNP delivery systems are essential to vaccine success by bypassing barriers to mRNA cytosolic delivery. Traditionally, ionisable lipids are derived from petrochemical sources, and require multiple purification steps, making them costly to synthesise. Identifying novel lipids and lipid sources for more sustainable mRNA-LNP vaccine production remains an important development milestone. We have identified cashew nutshell liquid (CNSL), a biorenewable waste product, as an inexpensive source of building blocks to synthesise novel ionisable lipids. High quality LNPs with diameters <150 nm were successfully formulated using these CNSL-derived ionisable lipids. mRNA was encapsulated within LNPs with high efficiency (>90%) and strong reporter gene expression was measured with no cellular toxicity in vitro. Selected candidates successfully delivered RNA encoding a reporter gene in vivo after intramuscular and intradermal injection, showing localised expression. This delivery efficiency was strongly dependant on the ionisable head group, and hydrophobic tail structures of the ionisable lipid. A SARS-CoV-2 vaccine delivered using CNSL-derived LNPs has shown strong anti-spike T-cell based immunogenicity with increases in IFN-γ, TNF-α, and IL-6 pro-inflammatory cytokines, comparable to a commercially available LNP control. This study has laid the groundwork for evaluation of novel sustainable LNP formulations for applications in LMICs, using empirical design of lipid chemical structures for improved vaccine delivery.

Keywords: mRNA, Vaccines, lipid nanoparticles, biorenewable resources

MCB-O-14

Surveillance of Candida auris from South African Wastewater Treatment Plants

Silondiwe Nzimande, Sabelle Jallow, Nelesh Govender

National Institute for Communicable Diseases (NICD), National Health Laboratory Service, South Africa. School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, South Africa. Division of Medical Microbiology, Faculty of Health Sciences, University of Cape Town, South Africa. Mycology Division (WHC).

Abstract: Introduction: Wastewater surveillance may serve as a complementary tool for estimating the burden of pathogens of public health importance such as Candida auris, a multidrug resistant healthcare-associated fungal pathogen with a reported high mortality rate among infected individuals. The aim of this study was to identify and characterize Candida auris isolates in wastewater samples collected from urban centres across South Africa. Method: A total of 477 wastewater samples were collected over a period of 6 months from 15 wastewater treatment plants across South African provinces. The CDC enrichment assay for the identification of C. auris from colonization swabs was adapted to identify C. auris from pelleted wastewater solids. Further identification was done on Chrom Agar Candida plus media and C. auris suspected colonies were identified to species-level using MALDI-TOF. Antifungal susceptibility testing was performed using Y10 broth microdilution method and whole genome sequencing was done on some Candida auris confirmed isolates (Illumina Next Seq platform). Results: and Conclusion We identified a total of 421 C. auris isolates from 53 wastewater samples and a majority of these were identified from hospital effluents from Gauteng province. This finding is consistent with clinical data from a national survey where C. auris candidemia was the highest in this province. Of 27 C. auris tested, 10 were resistant to azoles and echinocandins. Upon phylogenetic analysis all wastewater C. auris clustered with Clade III African clade. The findings of this study will contribute in efforts to integrate wastewater surveillance to the clinical surveillance.

Keywords: Candida auris, Wastewater, Invasive infections

MCB-O-15

Association of let-7g expression and the histopathological features of melanoma

Kwezi Cele, Musa Marimani, Henry Adeola, Adam Botha, Mulalo Molaudzi, Pumza Magangane

Department of Anatomical Pathology, University of the Witwatersrand; Hair and Skin Research Lab, Department of Medicine, University of Cape Town; Oral Biological Sciences, School of Oral Health, University of the Witwatersrand

Abstract: Melanoma is a relatively rare and serious type of skin cancer that can arise due to multiple different risk factors including sunlight exposure, reproductive factors, and age. Melanoma consists of various clinicopathologic subtypes including superficial spreading melanoma (SSM), nodular melanoma (NM), acral lentiginous melanoma (ALM), and lentigo maligna melanoma (LMM). The let-7 family contributes to the regulation and suppression of tumors. This study aims to determine the association between let-7g expression and the histopathological features of melanoma. We analyzed 25 samples made up of 9 ALM, 3 SSM, 3 LMM, 6 NM and 4 NM in acral sites samples. Total RNA was extracted using the Invitrogen PureLink FFPE RNA Isolation Kit. The extracted miRNA underwent reverse transcriptase and real-time PCR, where Qiagen MiRCURY LNA miRNA PCR Assays were used for quantification. The study showed a significant association between the melanoma subtypes and ulceration, with the ALM

subtype having the highest number of tumors showing ulceration, followed by the acral NM subtype. There was no significant difference observed in let-7g expression in the melanoma subtypes. However, there was a significant association between let-7g and neurotropism. There was an association between the let-7g expression and tumor infiltrating lymphocytes (TILs). Let-7g is not differentially expressed between melanoma subtypes, but may be involved in melanoma progression and metastasis.

Keywords: Melanoma, Let-7g expression

MCB-O-16

Investigating the anti-depressive mechanism of action of ketamine in a rodent model of depressive-like symptoms

Farhanah Sallie, Leandrie Pienaar, Andrea Lubbe, Ms Sanelisiwe Xhakaza, William MU Daniels, Aletta ME Millen, Sooraj Baijnath

Wits Integrated Molecular Physiology; School of Physiology Research Initiative; Wits Health Consortium; School of Physiology, Faculty of Health Sciences, University of the Witwatersrand

Abstract: Clinical outcomes show that patients with major depressive disorder often present with HPA axis dysfunction, with over one third of MDD patients showing resistance or partial response to common anti-depressant agents. In rodents, chronic ACTH administration induces HPA axis dysfunction and depressive-like symptoms. Ketamine has emerged as a potential 'silver bullet' in the treatment and management of depression, even in patients with treatment resistant depression. Ketamine is proposed to exert its anti-depressive effects by promoting neurogenesis and correcting alterations in neurogenic markers such as brain-derived neurotrophic factor (BDNF) and cAMP-response element binding protein (CREB). However, ketamine's effect on neurogenic pathways in HPA axis associated depressive-like symptoms in rodents remains unclear. Forty male Sprague-Dawley rats were randomly assigned to the control (n = 20; saline 0.1ml, sc) or ACTH group (n = 20; 100 µg/day, sc) that were treated for two weeks. Thereafter, in addition to receiving ACTH or saline, rats in the control and ACTH groups were further divided to receive saline (n = 20; 0.2 ml, ip) or ketamine (n = 20; 15 mg/kg, ip) for four weeks. mRNA expression of neurogenic biomarkers was measured in the prefrontal cortex, striatum, hippocampus and midbrain using RT-PCR. Chronic ketamine administration reversed ACTH-induced increases in hippocampal BDNF mRNA expression and CREB mRNA expression in the striatum, hippocampus and midbrain. These results demonstrate ketamine's ability to reverse ACTH-induced alterations in neurogenic biomarkers in key brain regions highlighting ketamine's potential therapeutic mechanism in MDD associated with HPA axis dysfunction.

Keywords: HPA axis dysfunction, Depressive-like symptoms, Ketamine, Neurogenesis

MCB-O-17

Acute exposure to LPS induces cardiac dysfunction via the activation of the NLRP3 Inflammasome

Tiiso Maluleke, Ashmeetha Manilall, Nandi Shezi, Aletta Millen, Sooraj Baijnath

WITS Initiative, Integrated Molecular Physiology Research Initiative

Abstract: Systemic inflammation contributes to left ventricular (LV) dysfunction. The role of the NLRP3 inflammasome in LV dysfunction in acute high-grade inflammatory conditions is unclear. This study investigated the role of the NLRP3 inflammasome in the development of acute cardiac structural and functional changes in vivo and in vitro in lipopolysaccharide (LPS)-induced inflammation. Sprague-Dawley rats were injected with either LPS or saline and terminated after 24 hours. Echocardiography, blood and tissue samples were collected at termination for analysis using matrix-assisted laser desorption ionisation mass spectrometry imaging (MALDI-MSI), enzyme-linked immunosorbent assay (ELISA), histology and PCR. AC16 cardiomyocytes were incubated in LPS or plain media for 24 hours. Cells were harvested for determining gene expression using PCR. MALDI-MSI showed increased LPS metabolite levels in LV tissues of rats exposed to LPS. In rats exposed to LPS, LV internal diameter was decreased, with no change in wall thickness or collagen volume. Additionally, rats exposed to LPS showed impaired relaxation, which likely contributed to decreased stroke volume. While global systolic function was preserved, LPS exposure resulted in impaired myocardial deformation, measured using speckle-tracking echocardiography. Exposure to LPS resulted in upregulation of NLRP3 inflammasome components, increased gene expression of downstream cytokines IL-1β and IL-18, and antioxidant SOD2, and elevated markers of pyroptosis (GSDMD) and apoptosis (BAX/Bcl2) in vivo and in vitro. These findings suggest that inflammation-induced adverse cardiac structural and functional changes may be mediated by the NLRP3 inflammasome in acute, high-grade inflammatory states.

Keywords: Lipopolysaccharide, NLRP3 inflammasome, left ventricular dysfunction, AP-MALDI mass spectrometry imaging

MCB-O-18

The effects of dolutegravir and folic acid on the development of the neural tube: implications on neural tube defects.

Thabiso Tshabalala

Anatomical Sciences

Abstract: Dolutegravir (DTG) is the preferred antiretroviral therapy for many patients living with HIV, including women of childbearing age. However, studies have reported an increased risk of neural tube defects (NTDs) in children born to women who were administered DTG. These NTDs have been associated with lack of folic acid (FA) food fortification. However, it is not clear whether FA impacts any association between periconceptional DTG exposure and NTDs. Hence this study aimed to investigate whether FA has the propensity to rescue DTG developmental toxicity in the developing neural tube. Avian cranial neural tubes containing neural crest cells were cultured in the presence of peak plasma levels of DTG and FA individually, and in combination. As controls, the neural tubes were cultured in plain culture medium and DMSO reconstituted in the culture medium. The developing neurites were stained with rhodamine phalloidin and DCX in order to evaluate the actin cytoskeleton and neurite thickness. Quantitative PCR was used to determine the expression levels of Rac and Rho genes which are associated with NTDs. The DTG-treated neurites were thicker and shorter, while the DTG/FA-treated and control cultures appeared longer and thinner. The actin filaments in the control and FA-treated cultures were distributed throughout the entire perimeter of the neurite, while a disarray of the actin cytoskeleton was observed in the DTG-treated neurites. Rac and Rho genes were upregulated in the FA-treated and DTG/FA-treated neurons, while downregulated in the DTG-treated cultures. These findings suggest that FA may have protective properties against DTG cytotoxicity.

Keywords: Dolutegravir Neural tube defects

POSTER PRESENTATIONS

MCB-P-01

Postprandial glucose variability and clusters of sex hormones, liver enzymes, and cardiometabolic factors in a Black African ancestry population

Ms. Bontle Masango, Julia H. Goedecke, Michèle Ramsay, Karl-Heinz Storbeck, Lisa K. Micklesfield, Tinashe Chikowore

South African Medical Research Council/University of the Witwatersrand, Developmental Pathways for Health Research Unit (DPHRU), Department of Paediatrics, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa; Sydney Brenner Institute for Molecular Bioscience, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa. Department of Biochemistry, Stellenbosch University, Stellenbosch, South Africa. South African Medical Research Council/University of the Witwatersrand, Developmental Pathways for Health Research Unit (DPHRU), Department of Paediatrics, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.

Abstract: Introduction: This study aimed to, firstly, determine the clusters of sex hormones, liver enzymes and cardiometabolic factors associated with postprandial glucose (PPG) and, secondly to evaluate the variation these clusters account for jointly and independently with polygenic risk scores in Black African ancestry men and women. Methods: PPG was calculated as the integrated area under the curve (iAUC) for glucose during the oral glucose tolerance test (OGTT) using the trapezoidal rule in 794 participants from the Middle-aged Soweto Cohort (MASC) T2D. Principal component analysis was used to cluster sex hormones, liver enzymes and cardiometabolic factors, stratified by sex. Multivariable linear regression was used to assess the proportion of variance in PPG accounted for by principal components and type 2 diabetes (T2D) PRS while adjusting for selected covariates in men and women. Results: The T2D PRS did not contribute to the PPG variability in men or women. In men, the principal components cluster of sex hormones, liver enzymes, and cardiometabolic explained 10.6% of the variance in PPG, with principal component 1 (PC1) (peripheral fat), PC2 (liver enzymes and steroid hormones), and PC3 (lipids and peripheral fat) contributing significantly to PPG. In women; principal component factors of sex hormones, cardiometabolic factors, and liver enzymes explained a similar amount of the variance in PPG (10.8%), with PC1 (central fat) and PC2 (lipids and liver enzymes) contributing significantly to PPG. Conclusion: We demonstrated that inter-individual differences in PPG responses to an OGTT may be differentially explained by body fat distribution, serum lipids, liver enzymes, and steroid hormones in men and women.

Keywords: Type 2 diabetes, Postprandial glucose, Polygenic risk score, Variability

MCB-P-02

Investigation of ectrodactyly in a patient with distal 1q21.1 microdeletion: an atypical phenotype or dual pathology?

Didintle Kgaugelo Tsitsi, Maria Mudau , Kgisane Mophiring , Fiona Baine-Savanhu , Bianca Rossouw, Michael Urban

Division of Human Genetics

Abstract: The distal 1q21.1 syndrome is a recurrent microdeletion of contiguous genes. Typical clinical features include developmental delay, microcephaly and facial dysmorphism. We present a case of a 9-month-old female with chromosomal microarray findings indicating distal 1q21.1 syndrome. Clinical findings included mild dysmorphism and ectrodactyly, however, ectrodactyly has not been previously described as a feature of the syndrome. A 9-month-old female was referred for medical genetic assessment of ectrodactyly and minor dysmorphism. The family and pregnancy history were non-contributory. Facial dysmorphic features included depressed nasal bridge, anteverted nostrils and mild micrognathia. Growth parameters were in the normal range, the head circumference plotted on the 50th centile, and neurodevelopment was normal. Ectrodactyly was present on three limbs: ectrodactyly of the 3rd metacarpal with 4th and 5th digit syndactyly on the right hand, ectrodactyly of 2nd and 3rd digits of the right foot, and partial ectrodactyly of the 2nd metacarpal with vestigial 2nd digit on the left foot. Chromosomal

microarray analysis on the Agilent platform reported a heterozygous interstitial loss of chromosome 1q21.1q21.2, including nine genes and measuring approximately 1.3 Mb from position 147,099,720 bp to 148,352,079 bp. The microarray finding suggests a distal 1q21.1 syndrome, with the infant presenting with non-specific features of the syndrome. Ectrodactyly may be a previously undescribed feature of distal 1q21.1 syndrome, or this may present a case of dual pathology. Whole exome sequencing is under way using a virtual panel analysis approach for genes associated with ectrodactyly, as well as additional analysis of morbid genes.

Keywords: 1q21.1 microdeletion, ectrodactyly, whole exome

MCB-P-03

An audit of genetic testing performed for intersex patients

Ms. Balebogiye Nonyane, Nakedi Lekgate, Monica Araujo

Division of Human Genetics, National Health Laboratory Services and School of Pathology, Faculty of Health Sciences, University of the Witwatersrand

Abstract: Intersex variations, also known as differences of sex differentiation (DSD) are congenital medical conditions characterised by a discrepancy between an individual's external and internal genitalia, chromosomes, and/or gonads. The aim of this study was to perform an audit on the genetic testing conducted for patients with intersex variations through the Division of Human Genetics, NHLS and Wits. This study reviewed records of patients with clinical features suggestive of intersex variations who were referred to the division for genetic testing between 2020 and 2023. The records analysed were sourced from the in-house Excel and Access databases, with additional patient information obtained from TrakCare. A total of 621 patients were identified, and data were collected on the age at referral, reasons for referral, referral site, type and outcome of testing, and sex assigned at the time of referral. The data were recorded and analysed using Microsoft Excel. Preliminary analysis indicates that the mean age at referral is 8.2 years (± 11.4 standard deviation). The primary clinical feature reported was ambiguous genitalia. The primary testing method utilized was QF-PCR, and among the samples tested with this technique, the diagnostic yield was 15%, revealing Turner syndrome, XXX syndrome, Klinefelter syndrome, and abnormalities in X chromosome gene dosage. The majority of referrals originated from the Gauteng province. This study underscores the significance of genetic testing in DSD cases, as it provides crucial genetic sex determination for patients and a necessity for genetics clinics in other provinces.

Keywords: Intersex DSD

MCB-P-04

The effects of CBD on markers of inflammation

Sanelisiwe Xhakaza

School of Physiology

Abstract: Cannabidiol (CBD), a pharmacologically active constituent of the Cannabis sp., has shown to have anti-inflammatory properties, however, the empirical evidence that it crosses the blood-brain barrier (BBB) and have anti-inflammatory effects in the brain is uncertain. Therefore, the aim of this study was to evaluate which brain regions are targeted by CBD should it cross the BBB using mass spectrometry imaging (MSI) technique and evaluate the anti-inflammatory properties of the drug by investigating changes in inflammatory markers across different brain regions including the hippocampus, cerebellum, striatum, midbrain, prefrontal cortex, hypothalamus, and cerebral cortex. 40 male Sprague-Dawley (SD) rats were randomly divided receiving saline (0.1 mL) or CBD (10 mg/kg, 0.1 mL). The acute group received a single dose of CBD, via intraperitoneal injection, whereas the chronic group received the same dose was repeated for a period of 4 weeks. Brain tissue samples were collected, and different brain regions dissected. We measured the penetration of CBD across the BBB and the presence of its metabolites in the different brain compartments using MSI. We showed region specific presence of CBD in the brain. To determine the anti-inflammatory effects, we measured several markers of inflammation and components of the NLRP3 inflammasome using PCR. CBD administration impacted the activation of the NLRP3 inflammasome and inflammatory cytokines in different brain regions. These results suggest that CBD may have anti-inflammatory properties in the brain, however the effects may be region specific.

Keywords: CBD, Inflammation, Inflammasome

MCB-P-05

Targeting snake venom phospholipases with next generation antivenoms

Frances Ayres

Virology: Pathology

Abstract: Snake envenomation, a neglected tropical disease disproportionately affecting peri-urban and rural communities, leads to ~150 000 deaths, and ~500 000 cases of morbidity annually. Commercial antivenom is made from the pooled plasma of hyperimmune horses. It is expensive, non-specific, and can cause severe anaphylaxis. Monoclonal antibody therapeutics could provide a safer, more targeted, and potentially cost-effective modern medicine alternative, but these will need to neutralize diverse antigens from several distinct toxin classes. One major toxin class found in both elapids and vipers, snake venom phospholipase A2 (svPLA), induces myotoxic,

neurotoxic, or anticoagulant effects despite conserved structure, representing an attractive antivenom target. A panel of 25 svPLA toxins representing snakes from diverse geographies were cloned into pcDNA3.4, recombinantly expressed in expiCHO, purified by affinity/size-exclusion chromatography, and evaluated for enzymatic activity. Peripheral blood mononuclear cells from hyperimmune horses were stained with representative svPLAs to obtain toxin binding memory B-cells, from which immunoglobulin V-genes were amplified by RT-PCR and cloned for expression in expiCHO to assess binding by ELISA and structure by x-ray crystallography. Almost all svPLA2s were successfully expressed and purified, with several displaying potent enzymatic activities. Twenty-four V-gene pairs were cloned from toxin binding memory B-cells for high-throughput recombinant expression to assess antibody cross-reactivity and in vitro neutralization via the inhibition of enzymatic activity when compared to Varespladib, a small molecule inhibitor of svPLAs used as a control. Isolation of a novel, cross-reactive mAb to svPLA2 may form a key component of next-generation antivenoms comprised entirely of recombinant monoclonal antibody therapeutics.

Keywords: antibody discovery, antigen-specific B-cell sorting, snakebite envenomation, X-ray crystallography

MCB-P-06

The impact of binge alcohol consumption on the cytoarchitecture and remodeling of the mandible in adolescent Sprague Dawley (SD) rats

Akaashni Bhika, Amadi Ogonda Ihunwo, Diana Pillay

School of Anatomical Sciences, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa. Department of Human Anatomy and Histology, School of Medicine, Sefako Makgatho Health Sciences University, Ga-Rankuwa, South Africa.

Abstract: Underage binge drinking raises concerns since it negatively impacts the growth and development of the adolescent skeleton, a problem that affects many people globally as well as in South Africa. Improving knowledge on the effects of long-term binge drinking on the adolescent mandible can help discover possible intervention measures and increase public awareness of the dangers of alcohol use. Therefore, this study aimed to determine how prolonged binge drinking affects the cytoarchitecture and remodeling of the mandible in adolescent Sprague Dawley (SD) rats. The study comprised of SD rats (n=24), aged 7 weeks, placed into either the alcohol-exposed [n=12 (6 males and 6 females)] or pair-fed control group [n=12 (6 males and 6 females)]. The treatment of the groups was as follows; the alcohol-exposed group and the pair-fed control were administered a single daily dose of 3 g/kg of 20 % alcohol 3 days a week (alternate days) for 28 days and a caloric equivalent dose of maltose dextrin via oral gavage, respectively. The animals were terminated on day 28 via pentobarbital injection. Subsequently, the mandibles were harvested and processed for histology and

immunohistochemistry (IHC) staining using H&E and the anti-ki67 and anti-alkaline phosphatase (ALP) antibodies respectively. Images were taken using a light microscope fitted with an axiocam HRC digital camera (Zeiss Axioscope 2 plus), and cells were quantified using Fiji Image J software. Results indicated changes in cytoarchitecture and a decrease in cell numbers in the male and female alcohol-exposed groups, indicating that chronic binge alcohol exposure has

Keywords: Adolescent, Chronic binge, Cytoarchitecture, Immunohistochemistry

MCB-P-07

Investigating the genetic cause of oculocutaneous albinism type 2 in individuals of African descent through exome sequencing

Phophi Mmbi, Thandiswa Ngcungcu,Phelelani Mpangase Robyn Kerr

Division of Human Genetics, National Health Laboratory Service and School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg. Sydney Brenner Institute for Molecular Bioscience, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg. Division of Human Genetics, National Health Laboratory Service and School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg

Abstract: Oculocutaneous albinism type 2 (OCA2) is a hypopigmentation disorder resulting from mutations in the OCA2 gene. In individuals of African descent, the most common mutation is a 2.7kb deletion (including exon 7), found in 78% of mutated OCA2 chromosomes in Southern Africa. While testing for this deletion is available through the Division of Human Genetics in Johannesburg, no further testing is provided for those who test negative or heterozygous for this deletion. This study aimed to identify other pathogenic variants in the OCA2 gene among individuals of African descent who tested heterozygous for the deletion. We performed whole exome sequencing using the Ion Torrent S5, analyzing the OCA2 gene and other pigment-related genes. Variants were prioritized based on the gene of interest, predicted impact on the protein, and a minor allele frequency of <5%. The prioritized variants were classified using ACMG guidelines, and significant variants were validated using Sanger sequencing. The study identified a pathogenic splice variant in the OCA2 gene (NM_000275.3: c.1503+5G>A) in 3/7 (~43%) individuals. This variant was previously considered benign and has now been reclassified as disease-causing based on functional evidence of the variant's disruptive nature and current variant interpretation tools. Despite the small sample size, this finding suggests that the splice site variant could be a second common causative variant of OCA2 in the Southern African population. The study highlights the importance of using advanced techniques and tools for variant identification and interpretation.

Keywords: OCA2, African, Exome sequencing, Oculocutaneous Albinism Type 2,

MCB-P-08

Copper complexes induce apoptotic cell death in SF-268 glioblastoma cells

Niyanta Jade Rooplal, Carla Martins-Furness, Leonie Harmse, Orde Munro

Department of Pharmacy and Pharmacology, School of Therapeutic Sciences

Abstract: A glioblastoma is a malignant brain tumour with a poor prognosis. Drawbacks to current chemotherapy include poor blood-brain barrier penetration, limited efficacy and severe drug adverse reactions. Alternative and superior chemotherapeutic agents are needed. Copper complexes have induced apoptotic cell death at low concentrations in a variety of cancer models. In this study SF-268 cells were maintained according to standard cell culture protocols. The MTT assay was used to determine the IC50 values and fluorescence microscopy to evaluate cellular morphological changes caused by the complexes. The extent of cellular apoptosis was evaluated by annexin-V binding, caspase-3/7 and caspase-8 activity and mitochondrial membrane potential. The formation of reactive oxygen species (ROS) was measured with the CellRox® Assay and p21 expression determined using immunofluorescence microscopy. Changes in the expression levels of apoptotic proteins was determined with a proteome array. Copper-imidazo[1,2-a]pyridines (JD88, JD47), a phenanthroline-theophylline copper complex (AD3) and an 8-aminoquinolone based copper complex (OM) were active against SF-268 cells with pertinent IC50 values between 2.25 and 4.41 ?M. Cell morphology indicated apoptosis, confirmed by annexin-V binding and increased caspase-3/7 activity. The complexes caused a loss of mitochondrial membrane potential but no caspase-8 activation, indicating intrinsic apoptosis. JD88 and AD3 induced ROS and DNA damage as indicated by increased expression of p21. JD88 decreased the expression of inhibitors of apoptosis proteins (cIAP1, cIAP2 and XIAP), anti-apoptotic proteins Bcl-x, PON2, and increased expression of HO-1/HMOX-1/HSP32 and HSP60. This indicated ROS-mediated apoptosis. These copper complexes warrant further preclinical investigations using an animal model.

Keywords: Glioblastoma, Cancer, apoptosis, copper

MCB-P-09

Characterization of respiratory syncytial virus genomes from South African infants hospitalized with severe respiratory illness, during 2016 to 2018

Riona Mohanlal Kathleen Subramooney Hloniphile Mthyane Florette Treurnicht

Centre for Vaccines and Immunology National Institute for Communicable Diseases, Division of National Health Laboratory Service; Department of Virology , University of the Witwatersrand; Virology National Health Laboratory Service Charlotte Maxeke Johannesburg Academic Hospital and the University of the Witwatersrand

Abstract: Respiratory syncytial virus (RSV) is one of the leading causes of severe respiratory illness in infants and elderly individuals. With the use of Next-generation sequencing, we can provide information such as genomic strain variability and strain surveillance. This can provide better treatment and further development/ enhancement of vaccines. Vaccinations for RSV are new and under trial, better treatment and information for vaccine development can lead to improved outcomes for these at-risk populations. This study aims to characterize the RSV genomes from infants hospitalized with severe respiratory illness from 2016 to 2018 Nucleic acid extraction usingthe DaAn Gene RNA/DNA Purification Kit (spin column-based) (Da An Gene Co., Ltd; Sun Yat-sen University), followed by a nested polymerase chain reaction (PCR), optimized to amplify the whole RSV-B genome. Phylogenetic analysis was conducted using Nextclade tree-building software to build maximum-likelihood trees. Seventeen samples from PCR were sent for next-generation sequencing, with genome coverage breadth up to 80% and coverage depth at 4619.7x. Thirteen of these were further analysed for sequence segment coverage and depth for the NS1, NS2 P, F.Sixty-two percent (8/13) of samples displayed 100% coverage for the NS1 sequence segment. G clades were used to cluster the sequences provided and determine the phylogeny of the sequence segments selected for analysis (NS1, NS2 P, F and G). The common G clades that the analysed samples sequence segments clustered with the GB2 and GB5 clades, further diverging into the GB5 subgroups as the majority being of the GB5.0.5a and GB5.0.4a clades. This study showed dominant circulation of GB5.0.5a. and GB5.0.4a clade viruses over the study period from 2016 to 2018. However, it should be noted that majority of samples were from 2018. For some strains discordant G gene clade indicate potential recombination in GB5 strains.

Keywords: RSV , charcterization

MCB-P-10

Evaluation of the Standard Q TB MPT64 Antigen test for the detection of Mycobacterium tuberculosis complex from liquid and solid cultures

Blake Dove

Tuberculosis (TB) is an infectious disease that typically infects the lungs, and it is caused by Mycobacterium tuberculosis (MTB). The disease remains a significant public health challenge globally and in South Africa, necessitating reliable and rapid diagnostic methods. Therefore, this study aims to evaluate the performance of the Standard Q TB MPT64 Antigen test in comparison to the current rapid antigen tests, Capilia TB-Neo and MGIT TBc, for the detection of Mycobacterium tuberculosis complex from cultured isolates. A minimum of 64 characterized, stored isolates, will be sub-cultured into liquid and solid media, the samples will be processed according to the manufacturer's instructions for the Standard Q TB MPT64 Antigen test. The results will first be used to determine the sensitivity and specificity of the Standard Q TB MPT64 Antigen test, these values will then be compared to those from the Capilia TB-Neo and MGIT TBc tests, and finally agreement will be determined with the use of the kappa statistic. We expect to

identify the level of concordance between the tests, providing insights into the reliability and potential advantages of the Standard Q TB MPT64 Antigen test. The findings could significantly enhance TB diagnostics, particularly in high-prevalence regions like South Africa, thereby contributing to better disease management and control. The results from this study will address the knowledge gap regarding the sensitivity and specificity of the Standard Q TB MPT64 Antigen test as well as determine the agreement between the three antigen tests.

Keywords: TB, Antigen test, Diagnostics

MCB-P-11

Sleep disturbance in a valproic acid rat model of autism

Mokgatjane Gaffane

School of Anatomical Sciences

Sleep disturbances are the most prevalent comorbidities in autistic individuals and have been implicated in the disorder's etiology, however, their exact role in autism pathology is not fully understood. Therefore, the aim of this study was to evaluate the sex-specific effects of sleep disturbance on autism symptomology. A reversed 8hr light/ 16hr dark cycle was used to induce sleep disturbance in a valproic acid rat model of autism. Autism-like behaviours were assessed using the marble burying (repetitive behaviours), balance beam (motor balance and co-ordination), y-maze (spatial working memory), novel object recognition (working memory) and three-chambered (sociability) assays. For each assayed behaviour, the interactions between sleep condition, autism symptomology and sex were assessed using the three-way factorial ANOVA. No significant three-way interactions were found, however, main effects for sleep condition and autism symptomology were found for the NOR and BB assays respectively. Additionally, the only significant two-way interaction was found between sleep condition and sex in the NOR assay. Despite failing to show significance for majority of the interactions, trends indicated the role of sex in mediating the relationship between sleep condition and autism symptomology across behavioural domains related to repetitive behaviours, motor co-ordination and balance, cognitive flexibility as well sociability. Additionally, results support the growing understanding of a sex-specific presentation of autism, further highlighting the necessity for greater inclusivity in diagnostic criteria which are currently biased towards male presentations of the disorder.

Keywords: autism; comorbidity; sleep; valproic acid

MCB-P-12

Analysis of Whole Exome Sequence Data from African Patients with HD-Like Features and No Known HD Phenocopy

Racilya Naicker Fiona Baine-Savanhu Amanda Krause,

Division of Human Genetics, National Health Laboratory Services and School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

Abstract: Huntington disease (HD), a rare progressive neurodegenerative disorder, results from a CAG repeat expansion within the huntingtin gene (HTT). Several syndromes, termed HD phenocopies, present with HD-like features without the HTT expansion. Huntington disease-like 2 (HDL2), a known phenocopy, is most commonly observed in individuals with African ancestry. Moreover, previous diagnostic testing in the Division of Human Genetics, National Health Laboratory Service (Johannesburg, South Africa) screened for both HD and HDL2 in patients with HD-like phenotypes and African ancestry. Patients with negative results for both syndromes remain undiagnosed, highlighting the need for further testing strategies. This study therefore aimed to identify variants implicated in the HD-like phenotype of these patients. In a group of nine undiagnosed patients with Black African ancestry, a virtual gene panel was analysed through WES. The data was filtered, and candidate variants were prioritised. A total of 20 candidate variants in 15 genes were shortlisted and classified according to ACMG/AMP guidelines. Notably, variants in the mitochondrial DNA polymerase subunit gamma (POLG; c.2246T>C; p.Phe749Ser) and the glutaryl-CoA dehydrogenase (GCDH; c.877G>A; p.Ala293Thr) genes were classified as likely pathogenic and pathogenic, respectively. Genotype-phenotype correlation indicated a potential diagnosis of autosomal dominant progressive external ophthalmoplegia in the patient carrying the POLG variant, whereas the GCDH variant was considered an incidental finding due to a lack of correlation with glutaric aciduria type 1 characteristics. These findings highlight the diagnostic challenges in the African context for patients with HD-like clinical features and call for extended analyses for patients without putative pathogenic variants.

Keyworsds: Huntington Disease; Huntington Disease Phenocopies; Whole Exome Sequencing; Virtual Gene Panel

MCB-P-13

Loeys-Dietz Syndrome - Developing an Assay for a Familial TGFB3 Variant in the first reported case in an Afrikaner patient

Daughné Jeffries Clarice Smal Anneline Lochan Fahmida Essop

Division of Human Genetics, National Health Laboratory Service and School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

Abstract: Heritable connective tissue disorders are characterized by cardiovascular and musculoskeletal features. Loeys-Dietz Syndrome (LDS) is an autosomal dominant connective tissue disorder which primarily affects the arterial walls and increases the risk of aggressive vascular disease. LDS is caused by variants in genes associated with the TGF-β signaling pathway, including TGFBR1, TGFBR2, SMAD3, SMAD2, TGFB2 and TGFB3. There are no known reported LDS cases in South Africa. Here we present a 28-year old Afrikaner female, who presented with features suggestive of a connective tissue disorder and has a family history of similar features. Following genetic counselling and testing using next generation sequencing on an overseas connective tissue disorder gene panel, a novel pathogenic variant, c.170del (p.Pro57LeufsTer5) in exon 1 of TGFB3 was identified in the patient, her mother and brother. The aim of this project was to develop a Sanger sequencing assay for this familial variant for the proband's maternal cousin who also presented with features suggestive of LDS. Primers were designed using primer 3, followed by PCR optimization and Sanger sequencing. The data was analyzed using Qiagen CLC Genomics Workbench v23.0.4. The c.170del TGFB3 variant was confirmed in the proband. The proband's maternal cousin tested negative for the TGFB3 variant. This assay could further be used in the future to test other at-risk family members to direct clinical management. Early diagnosis is crucial for LDS patients as it allows for implementation of an appropriate surveillance plan which can reduce the risk of aggressive vascular disease.

Keywords: Loeys-Dietz Syndrome (LDS), transforming Growth Factor Beta (TGFB))TGFB

MCB-P-14

Molecular detection of Histoplasma Capsulatum in environmental samples collected from South African caves

Tsidiso Maphanga and Rutendo Mapengo

School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, South Africa

Abstract: Histoplasma capsulatum can cause disseminated histoplasmosis in people living with HIV. South African caves have been documented as probable sources of exposure for cavers since 1977. However, detection from the environment has not been confirmed. We used molecular assays to detect and confirm the presence of H. capsulatum in regularly-explored caves. Environmental samples were collected from seven South African caves from Gauteng, Western Cape and Eastern Cape provinces. DNA was extracted directly from the samples using DNeasy PowerSoil Pro Kit. In-house internal transcribed spacer (ITS) panfungal PCR, pan-dimorphic reverse transcriptase-quantitative (RT-q) PCR and nested Hc100 PCR assays were used to detect H. capsulatum. Identity was confirmed using BLAST tool following Sanger sequencing of the Hc100 nested-PCR product. H. capsulatum was detected in five of the seven caves. Of 56 samples tested, 18 were positive from three caves in Gauteng [cave 1 (3/10); cave 2 (7/10); cave 3 (5/10)] and one cave in the Western Cape [cave 4 (2/10) and one in the Eastern Cape [cave (1/5)]. These

samples were positive either by RT-qPCR or Hc100 PCR assays. The test positivity was 21% (12/56) with both RT-qPCR and Hc100 PCR assays. Seven percent (4/56) of samples were only RT-qPCR assay-positive and 4% (2/56) only Hc100 PCR-positive. None of the 56 samples tested positive with the ITS PCR assay. H. capsulatum is probably present in several regularly-explored caves with bat populations. This finding should be confirmed by culture. The RT-qPCR and Hc100 PCR assays could be useful tools for wider environmental surveillance.

Keywords: Molecular, Histoplasma, Environment, Caves

MCB-P-15

Re-evaluating Next Generation Sequencing data: Assessing the utility of re-analysis in patients tested using an Inherited Disease Panel

Debora Jackson, Clarice Smal, Fahmida Essop

Division of Human Genetics, National Health Laboratory Service, and School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

Abstract: Next Generation Sequencing (NGS) has revolutionized genetic testing by enabling rapid, cost-effective genomic analysis, providing insights into inherited diseases. However, challenges in interpreting genetic data, especially in understudied populations like those of African ancestry, highlight the need for periodic re-evaluation of NGS data. Re-analysis in diagnostic laboratories is becoming more common and can lead to new diagnoses where initial analysis failed to identify any variants. This study aimed to evaluate the utility of re-analysing NGS data for patients referred to the Division of Human Genetics, NHLS, Johannesburg, before 2022 for diagnostic molecular testing using an Inherited Disease Panel (IDP) covering about 500 genes associated with various inherited disorders. NGS data from six patients who were referred for haemophilia A (HPA), haemophilia B (HPB), Rett, and Rett-like (RTT) syndromes, who previously tested negative for variants or had variants of uncertain significance (VUS), were re-analysed for SNVs using updated information. Variant annotation and prioritization were conducted with the Ensembl Variant Effect Predictor (VEP) and confirmed with Ion ReporterTM v.5.16.02, followed by classification using ACMG-AMP guidelines. No new variants were identified for HPA (F8), HPB (F9), and RTT (MECP2, ATRX, CDKL5, MEF2C, SLC9A6, UBE3A, ZEB2, NTNG1) on re-analysis. This study recommends CNV analysis and reviewing QC data and parameters. Whole exome sequencing is also recommended for patients with highly suggestive clinical phenotypes. The re-evaluation of IDP-generated data showed limited utility for the selected disorders, possibly due to the small number of patients, limited genes on the panel, or the short interval between analyses.

Keywords: IDP, NGS, re-analysis

MCB-P-16

Snapshot of the gut microbial diversity of an urban South African population: Impact of diet, lifestyle and socioeconomic status

Mrs Claudine Nkera-Gutabara Robyn Kerr, Jerolen Naidoo

NHLS, University of the Witwatersrand; Bioengineering and Integrated Genomics Research Group, CSIR; Human Genetics: Pathology, Sydney Brenner Institute for Molecular Biosciences (SBIMB)

Abstract: The human gut microbiome, comprising symbiotic, pathogenic, and commensal organisms, generally maintains a stable composition in adults but is influenced by diet, lifestyle, and environment. South Africa's (SA) shift toward urbanization, with associated increases in sedentary lifestyles and dietary changes, may profoundly impact health, yet our understanding of these effects on SA's gut microbiome remains limited. Our study represents one of the first to characterize the gut microbiota of an urban professional cohort within SA. Recruitment at the CSIR campus (Pretoria) and at Wits University (Johannesburg) included a comprehensive survey (n = 123 data points) and a stool collection for microbiome profiling. We surveyed n = 220 participants, collected n = 242 stool samples (n = 22 second collections), and performed PacBio 16S long-read sequencing, with a subset undergoing additional Illumina shotgun metagenomics. A rarefaction normalization analysis revealed a sampling depth of n = 90 retains 74% of samples and 80% (n = 1628) amplicon sequence variants for diversity analyses. Univariate analyses highlight statistically significant differences: Shannon (Alpha) diversity analysis showed increased diversity in individuals who use public transport or consume dairy, and decreased diversity in those using gas heating or multivitamins (Wilcoxon-rank sum test $P < 0.05$); Weighted Unifrac Distances (Beta) showed increased diversity for participants who consume herbal supplements or indicate the absence of food allergies (PERMANOVA $P < 0.05$). We present results from a tailored QIIME2 and MAG bioinformatics workflow with comparisons to seminal cohorts, providing insights into baseline microbial profiles and the effects of exogenous factors.

Keywords: Gut microbiome, long-read sequencing, bioinformatics, 16S rRNA

MCB-P-17

PHENOTYPIC AND MOLECULAR CHARACTERISATION OF EMERGOMYCES AFRICANUS CAUSING DISSEMINATED DISEASE AMONG SOUTH AFRICANS LIVING WITH HIV

Silondiwe Nzimande, Nelesh P. Govender, Sabelle Jallow, Tsidiso Maphanga

National Institute for Communicable Diseases (NICD), a Division of the National Health Laboratory Service, South Africa, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand and Division of Medical Microbiology, Faculty of Health Sciences, University of Cape Town, South Africa, Mycology Division (WHC)

Abstract: Introduction: Emergomyces africanus causes disseminated emergomycosis in people with advanced HIV disease. Multi-gene sequencing revealed that phylogenetic groupings may exist within E. africanus. We aimed to characterize E. africanus clinical isolates through whole genome sequencing as well as to describe the relationship between phenotypic features and phylogeny. Methods: We re-examined 90 South African E. africanus clinical isolates archived over 14 years. Dimorphism was confirmed through the conversion of the mould phase to the yeast phase. Isolates were characterized using culture/microscopy/urease testing and whole genome sequencing. Minimum inhibitory concentrations of azoles, flucytosine, echinocandins, flucytosine and amphotericin B were obtained from the yeast phase. Results: All isolates converted to the yeast phase at 370C and were urease-positive within 24 hours. Two colony types were observed: 90% white, raised with powdery segments; 10% flat with matte periphery/ centrally wrinkled; however, these phenotypes were indistinguishable by light microscopy. Phylogenetic analysis revealed 3 major groups. Sequences clustered together despite colony differences. Group 1 consisted of the first cases reported from 2009-2013 in the Western Cape (n=8) and Free State (n=1) provinces. Group 2 consisted only of cases from the WC Province (n=9). Group 3 consisted of 5 subgroups with cases from WC (n=17), Gauteng (n=17), Mpumalanga (n=1), EC (n=6), KZN (n=8). Amphotericin B and azoles had potent activity against all isolates (MIC range, 0.008-0.25 µg/mL). Fluconazole, echinocandins and flucytosine were least potent. Conclusion: Phylogenetic analysis confirmed clade-like groupings within E. africanus, with no phenotype association. These findings need to be linked to epidemiological and clinical case

Keywords: Emergomyces africanus, Advanced HIV

MCB-P-18

Immunogenicity of self-amplifying RNA vaccines against Hepatitis B virus

Mrs Nazia Samudh, Dylan Kairuz, Kristie Bloom, Kubendran Naidoo, Abdullah Ely, Patrick Arbuthnot Wits/SAMRC; AGTRU; IDORI;

Molecular Medicine and Haematology: Pathology, Antiviral Gene Therapy Research Unit

Abstract: Vaccination against Hepatitis B virus (HBV) remains the most effective means of preventing infection. However, some individuals fail to develop neutralising antibodies, and currently available subunit vaccines are ineffective at eliciting Th1 cytotoxic immune responses which are required for eradication of intracellular pathogens. The alphavirus-derived self-amplifying messenger RNA (saRNA) vaccine platform enables the in-situ synthesis of antigens which trigger the innate immune system (interferon response) and promote a Th1-biased immune response. The aim of this project was to develop and investigate the immunogenicity of saRNA-based vaccines against HBV. saRNAs encoding the reporter protein luciferase, or HBV surface antigens, were synthesised by in vitro transcription and formulated with ionisable lipid nanoparticles for in vivo delivery. Balb/c mice received either 1 or 5 micrograms of saRNAs

administered intramuscularly as a 4 week prime-boost regimen. Expression of luciferase was examined by bioluminescence imaging. Humoral immune responses were examined by HBV neutralization assay. Expression of cytokines involved in the innate immune system response, and antigen-specific T-cell responses were measured using a bead-based multiplex assay and intracellular cytokine staining of HBV-peptide stimulated splenocytes respectively. saRNA vaccines triggered the interferon response in a dose-dependent manner. However, this did not hamper antigen expression, which was observed for an extended period, and was limited to the site of injection. saRNA vaccines elicited neutralising antibodies, and HBV-specific cytotoxic T-cell responses which were not observed in mice that received subunit-based HBV vaccines. saRNA-based HBV vaccines provide enhanced humoral and cellular immunity against infection, with possible therapeutic applications.

Keywords: HBV, saRNA, vaccine, immunogenicity

MCB-P-19

HPV16 and 18 prevalence in OSCC patients as determined by the HPV E6 gene

Mr Phuti Mphaki, Mulalo Molaudzi, Pumza Magangane, Julitha Molepo

Department of Oral Biological Science, Faculty of Health Science, University of the Witwatersrand; Department of Anatomical Pathology, Faculty of Health Science, University of the Witwatersrand; Department of Oral Biological Science: Oral Health Sciences

Oral squamous cell carcinoma (OSCC) is the most common type of tumour in the oral cavity, accounting for more than 90% of all oral cancers. The human papilloma virus (HPV) has been identified as a risk factor for developing OSCC. The E6 protein is an oncoprotein that is responsible for HPV oncogenesis in infected squamous epithelia and is used as a biomarker for HPV genotyping in infected individuals. The aim of the study was to assess the prevalence of HPV16 and 18 in patients with OSCC using the E6 gene. Histopathology and demographic data for 62 OSCC cases was collected from pathology reports and summarised. Formalin-fixed paraffin embedded tissue samples were assessed for the presence of the HPV16 and HPV18 E6 gene using SYBR green qPCR. The average age of patients with OSCC was 61 ± 8.89 years, with a male to female ratio of 3.7:1. Furthermore, the most commonly affected oral site was the tongue (40.3%) followed by the floor of the mouth (27.4%). Following HPV analysis, 96.8% (60/62) of cases were HPV 16 E6 positive. Only 32.2% (20/62) of the cases were positive for HPV18 E6 gene, and they were also positive for HPV16 E6. Furthermore, tongue (40%) and floor of the mouth (23.5%) tumours were co-infected with HPV16 and 18. Infection with HPV16 is more prevalent than HPV18 in OSCC patients. Co-infection with HPV 16 and 18 was detected in approximately one third of our cohort and is associated with certain tumour sites.

Keywords: carcinoma, oncoprotein, oral, papilloma

MCB-P-20

In vitro characterisation of mRNA-mediated delivery of multispeific bNAbs for HIV-1 immunoprophylaxis.

Caitlyn O'Connor, Abdullah Ely, Mark Killick

Antiviral Gene Therapy Research Unit, Faculty of Health Sciences, University of the Witwatersrand Prof Maria Papathanasopoulos, HIV Pathogenesis Research Unit, Faculty of Health Sciences, University of the Witwatersrand. HIV Pathogenesis Research Unit, Faculty of Health Sciences, University of the Witwatersrand. Molecular Medicine and Haematology: Pathology, HIV Pathogenesis Research Unit

Abstract: HIV-1 bNAbs show potential at both reducing viraemia and preventing HIV-1 infection, however, their application may be limited due to high manufacturing costs and the requirement for combination-based therapies. This project describes the development of in vitro transcribed (IVT)-mRNA delivery of multispecific antibodies as a potential cost-effective, passive immunisation strategy for HIV-1 prevention. Previously described bispecific (Bi-scFv and Bi-NAb) and trispecific (Tri-NAb) antibodies combining VRC01/PGT121 and VRC01/PGT121/10e8 paratopes, respectively, were selected. Antibody-encoding DNA plasmid constructs, including a novel single open reading frame (sORF) polycistronic Tri-NAb construct for comparison to the Bi-NAb/10e8 co-transfection (Co-T) strategy, engineered with T7-IVT mRNA transcription compatibility were generated. Parental and multispecific antibodies were expressed from DNA constructs, purified, and biochemically characterised. Multispecific antibody-encoding mRNA transcripts were IVT from linearised DNA, purified of dsRNA, enzymatically capped (5' Cap1 structure), and transfected into 293F cells. Functionality of the purified antibodies and mRNA-transfected cell culture supernatants were assessed in vitro against a panel of 17 HIV-1 tier 2 pseudoviruses with appropriate VRC01/PGT121 sensitivity/resistance profiles. Purified multispecific antibodies demonstrated improved neutralisation potency and coverage compared to the parental monoclonal antibodies, as expected. Unpurified, mRNA-expressed multispecific antibodies matched the neutralisation coverage of purified antibodies (94%), with sufficient multispecific antibody titres to generate inhibitory dilution factors conferring 80% neutralisation (median ID80) >150: Bi-scFv (1 427), Bi-NAb (655), Tri-NAb Co-T (327), and Tri-NAb sORF (166). These data provide proof-of concept and suggest smaller, less complicated tandem scFv multispecific conformations (Bi-scFv and Bi-NAb) may be preferred for further development and preclinical evaluation.

Keywords: HIV-1 prophylaxis, multispecific antibodies, IVT-mRNA

MCB-P-21

The effects of acute and chronic inflammation on aortic function in Sprague-Dawley rats

Adalayne Ramsamy

Faculty of Health Sciences, School of Physiology

Abstract: Arterial remodelling contributes to the development of cardiovascular disease. Inflammation is a major driving factor of arterial remodelling; however, the mechanisms involved in inflammation-induced arterial remodelling remain uncertain. This study investigated the molecular pathways involved in aortic remodelling in rat models of acute (single lipopolysaccharide (LPS) administration) and chronic (four weekly LPS administrations) inflammation. Aortic function was determined using echo-tracking ultrasound. Aortic tissue gene expression of pro-inflammatory and arterial remodelling markers were measured using RT-PCR. In rats terminated 24 hours after single LPS administration (n=12), there were no changes in aortic stiffness (all $p>0.05$), despite increased markers of inflammation (TNF-α $p=0.01$, IL-6 $p=0.04$) and endothelial activation (VCAM-1 $p=0.005$) compared to controls. In rats terminated one week after single LPS administration (n=18), echo-tracking results showed increased flow velocity (Vmax $p=0.04$, VTI $p=0.02$) and decreased compliance ($p=0.04$) compared to controls. Despite no group differences in pro-inflammatory markers, LPS upregulated the gene expression of matrix metalloproteinase-9 ($p=0.03$) and downregulated that of vascular endothelial growth factor ($p=0.02$) compared to controls. In rats terminated after repeated LPS administrations (n=19), measures of flow velocity and stiffness were increased (all $p<0.05$), while distensibility was decreased ($p<0.0001$) compared to controls. Upregulated gene expression of IL-6 ($p=0.0001$) and bradykinin receptor B1 ($p=0.004$), a novel marker associated with chronic inflammation, was not accompanied by increases in the expression of arterial remodelling markers. These results suggest that arterial remodelling may be initiated in the early stages of inflammation and could result in substantial functional changes within the aorta.

Keywords: Inflammation, Arterial remodelling, Aortic function

MCB-P-22

The expected utility of whole-exome sequencing results and the psychosocial impacts of receiving a diagnosis

Samantha Schnell

Division of Human Genetics: Pathology

Abstract: Developmental disorders (DDs) are a heterogenous group of conditions that alter the trajectory of childhood development. These disorders are characterised by intellectual disability and/or delays in a variety of developmental domains. Due to the clinical and genetic heterogeneity of these disorders, identifying a diagnosis remains challenging. While genetic causes account for up to 40% of cases, a large proportion of individuals affected with DDs remain

undiagnosed and their families subsequently experience a prolonged diagnostic journey. Whole-exome sequencing (WES) greatly increases the diagnostic yield of DDs in comparison to the recommended first-tier genetic testing, chromosomal microarray. However, around 60% of individuals affected with DDs remain without an identified cause. The Deciphering Developmental Disorders in Africa (DDD-Africa) study recruited patient-parent trios to undergo WES to identify the possible genetic aetiologies of DDs in affected individuals. This study aims to analyse participant demographic data, the pre-result feedback questionnaire responses exploring caregivers' expected utility of WES testing results and the caregiver's experience of diagnostic odyssey. Additionally, genetic counselling consultation summary letters that encompass the psychosocial impact of receiving a diagnosis of a rare disease will be analysed. The impact of receiving diagnoses of rare DDs following WES remains relatively understudied in the South African context. Ultimately, this study aims to explore caregivers' expected clinical and personal utility of WES results received through the DDD-Africa study. Data analysis will begin in July with an anticipated 70 families included in the study cohort. Preliminary results will be available for presentation at the Faculty Research Day 2024.

Keywords: Developmental delay, caregivers' perspective, WES, utility

MCB-P-23

Occurrence and characteristics of Klebsiella species isolated from selected South African wastewater treatment Plants

Murendeni Manavhela, Taku Ongeziwe, Mokgokotleng Ruth, Perovic Olga, Jallow Sabelle

NICD-CHARM

Abstract: In recent years, Klebsiella pneumonia has been recognized as an important opportunistic pathogen that colonizes the skin, gastrointestinal tract and respiratory tract and causes a range of serious nosocomial and community-acquired infections such as pneumonia, meningitis, urinary tract and bloodstream infections in immunocompromised individuals. Patients with Klebsiella species infections can transmit the bacteria and other drug-resistance bacteria via direct personal contact, medical devices, contaminated environments as well as daily excretion. In hospitals, Klebsiella pneumonia is considered as one of the most common infectious disease pathogens and a significant public health threat due to high rates of antimicrobial resistance. Hospital wastewater thus represents the ideal pool for the exchange of resistance genes between clinical and environmental bacteria. Resistant bacteria and genes that survive wastewater treatment processes can spread and persist in the environment, representing a steady reservoir of antimicrobial resistance and a constant health risk to humans and animals. There is limited data on Klebsiella species from the environment, therefore, this study aims to detect and describe resistance mechanisms of Klebsiella species isolated from wastewater

treatment plants. Wastewater influent samples are collected from 5 provinces in various wastewater treatment plants. Samples are then directly inoculated on Klebsiella Blue Agar. Klebsiella species are then identified using MALDI-TOF. Isolates identified as Klebsiella species will be subjected to antibiotic susceptibility test and polymerase chain reaction to identify genes responsible the resistance. The optimization protocol and characteristics of the isolated species will be shared. During optimization we have successfully isolated different Klebsiella species from wastewater.

Keywords: Klebsiella spp, Wastewater, Carbapenemases, Antimicrobial Resistance

MCB-P-24

An 8-aminoquinolone-naphthyl copper complex causes apoptotic cell death by modulating the expression of apoptotic regulatory proteins in breast cancer cells.

Nonzuzo Myeza, Cathy Slabber, Orde Munro, Carla Martins-Furness, Dr Leonie Harmse

Molecular Sciences Institute, School of Chemistry, University of the Witwatersrand and School of Chemistry, University of Leeds UK. Department of Pharmacy and Pharmacology, Therapeutic Sciences, Faculty of Health Sciences, University of the Witwatersrand

Abstract: Breast cancer is a leading cause of cancer-related deaths among women. Despite the combination of chemotherapy with targeted therapy, like monoclonal antibodies and kinase inhibitors, drug resistance and treatment failure remain a common occurrence in triple negative breast cancer and metastatic disease. Copper, complexed to various organic ligands, has gained attention as potential chemotherapeutic agents due to their lower toxicity to normal cells. The cytotoxic efficacy and the mechanism of cell death of an 8-aminoquinoline-naphthyl copper complex (Cu8AqN) in MCF-7 and MDA-MB-231 breast cancer cell lines was investigated. Cu8AqN inhibited the growth of MCF-7 and MDA-MB-231 cells with IC50 values of 2.54 ± 0.69 µM and 3.31 ± 0.06 µM, respectively. The complex induced apoptotic cell death, evidenced by nuclear fragmentation, increased annexin V binding, and caspase-3/7 activity. The loss of mitochondrial membrane potential, increased caspase-9 activity, absence of active caspase-8, and decreased tumor necrosis factor receptor-1 expression indicated activation of the intrinsic apoptotic pathway. Additionally, increased ROS formation and haem oxygenase-1 expression suggested activation of cellular stress pathways. Expression of p21 protein in the nuclei was increased providing additional evidence for apoptosis, whilst the expression of inhibitor of apoptosis proteins like cellular inhibitor of apoptosis protein 1, X-linked inhibitory apoptosis protein and survivin were decreased. Phosphorylated p53 species; phospho-p53(S15), phospho-p53(S46), and phospho-p53(S392) accumulated in MCF-7 cells indicating the potential of Cu8AqN to restore p53 function in the cells. In combination, the data indicates that Cu8AqN is a useful lead molecule worthy of further exploration as a potential anti-cancer drug.

Keywords: 8-aminoquinolone-naphthyl-copper complex, apoptosis, inhibitor of apoptosis proteins, haem oxygenase-1

MCB-P-25

Behavioral and musculoskeletal correlates in a rat model of autism spectrum disorders: A comparative study using X-ray tomography

Patience Mamali, Busisiwe Maseko and Christine Dignon

School of Anatomical Sciences

Autism spectrum disorders (ASD) are diagnosed based on two main behavioral features: (i) persistent deficits in social communication and interaction, and (ii) excessive and persistent restricted, repetitive behaviors, interests, or activities. Approximately 0.6 - 2% of children worldwide are affected by ASD, with prevalence increasing. This study included both male and female rats due to the 1:4 male-to-female ratio in autism prevalence. We paired behavioral tests with bone morphometry to assess the link between behavior and the musculoskeletal system. Motor ability assessments showed that the number of errors made and the time taken to cross the balance beam were similar in all groups. While some hypotheses suggest that the musculoskeletal system is affected in people with autism, our motor behavioral analysis did not show any significant differences. However, bone morphometry did reveal differences that support some hypotheses while contradicting others. X-ray tomography was used to assess the morphometries of all rat bones, with microCT used to measure bone volumetrics. Morphometric measurements (body, tail, and paw lengths; brain and body weights) were decreased in the autism model compared to controls, and also decreased in females versus males. Notably, a higher prevalence of autistic-like characteristics was observed in valproic acid-induced autism model males compared to females, despite identical environments. Furthermore, the heterogeneity of autism was widely noted, underscoring that autism is a spectrum and not all behaviors will be extreme in any study.

Keywords: Autism, Bone, Motor skills, Model

MCB-P-26

Copper complexes cause apoptosis in pancreatic cancer cell lines while haem oxygenase-1 inhibition sensitized the cells to copper complex-induced apoptosis

Zakeeya Jhetam Orde Munro Catherine, Slabbert Marietha Nel, Leonie Harmse

School of Chemistry, Leeds University, UK. School of Chemistry, Wits University; Department of Surgery, Wits University; Department of Pharmacy and Pharmacology, Pharmacology division: School of Therapeutic Sciences

Abstract: Pancreatic cancer (PC) has a poor prognosis with a 5-year survival rate of 1% when diagnosed at stage IV. Current chemotherapy treatments have poor efficacy against PC. Novel copper complexes have previously shown promising cytotoxic activity against colorectal, breast, lung, glioblastoma and leukaemic cell lines. In this study, we evaluated copper complexes for their cytotoxic activity and their effect on haem oxygenase-1 (HO-1) against the AsPC-1 and MIA PaCa-2 cell lines. IC50 values of complexes were obtained using the MTT cell proliferation assay. Annexin-V, JC-1, caspase-3/7 and caspase-9 assays were used to confirm apoptotic cell death. ROS formation was measured with the CellRox® DeepRed reagent. Immunofluorescence and western blots were used to determine the effect of complexes on HO-1 expression. Four copper complexes were active against both cell lines at low micromolar concentrations, ranging between 1.08 □M and 3.15 □M. The complexes increased binding of annexin-V to both cell lines, indicative of apoptosis. The presence of JC-1 monomers in all treated cells was indicative of mitochondrial outer membrane depolarization. Caspase-9 and caspase-3/7 was activated by all the complexes, confirming apoptotic cell death. All the complexes increased ROS formation and induced the stress-response protein, HO-1. Inhibition of HO-1 decreased the IC50 values of the copper complexes in both cell lines. The copper complexes were effective inhibitors of two pancreatic cancer cell lines at low micromolar concentrations and induced intrinsic apoptotic cell death. The induction and implication of increased HO-1 requires further investigation.

Keywords: Copper complexes; haem oxygenase-1; pancreatic cancer; apoptosis

MCB-P-27

The Effects of Acute Binge Alcohol Consumption on the Trabecular Morphometry and Tensile Strength of Adolescent Sprague Dawley Rat Femora

Ndabezinhle Ronald Mngoma Akashni Bhika Diana Pillay

School of Anatomical Sciences, Faculty of Health Sciences, University of the Witwatersrand, South Africa. Department of Anatomy, Faculty of Health Sciences, Sefako Makgatho Health Sciences University, South Africa

Abstract: Excessive alcohol consumption adversely affects bone metabolism, thus resulting in reduced bone length, density, and strength. Moreover, these deficits in bone density and strength are likely to increase the risk of fragility fractures and the early onset of osteoporosis. While excessive alcohol consumption is an established risk factor for osteoporotic fractures, there remains a dearth of information in the literature about bone effects of binge alcohol

consumption in adolescents. Therefore, our study aimed to examine the effects of acute binge alcohol consumption on the adolescent bone micro-architecture and tensile strength. Twelve male Sprague Dawley rats aged 7 weeks were randomly placed in 2 groups: alcohol (n = 6), receiving alcohol (3g/kg), and pair-fed control (n = 6), receiving an isocaloric equivalent of maltose dextrin via oral gavage for 3 days in one week (on alternative days). The femora were dissected and scanned using a Micro-Focus X-ray Computed Tomography (3D-mCT). Following reconstruction, trabecular morphometry was assessed in both the proximal and distal epiphysis, using a Volume Graphics Studio® software. A three-point bending test was employed to examine the effect of alcohol on the tensile strength of the bone. Results showed trabeculae parameters to be affected in the distal epiphysis of the femur, while in the proximal epiphysis, it remained unaffected. Tensile strength parameters were also not affected by the consumption of alcohol. These findings may suggest that acute binge alcohol consumption has detrimental effects on the bone micro-architecture specific to the distal epiphysis.

Keywords: Adolescent; Alcohol; Trabeculae; Femur.

MCB-P-28

Evaluating the neuroprotective effects of Obestatin in ethanol-induced neurotoxicity in C8D1A and SHSY5Y cell lines

Gizelle Govender

Faculty Health Sciences, School of Anatomical Sciences

Background: Alcohol is a known neurotoxin that damages brain cells through the upregulation of oxidative stress, neuroinflammation, impairment of mitochondrial function, and excitotoxicity. Given the effects of ethanol-induced neurotoxicity, investigations into possible ameliorative agents are needed. Obestatin, a naturally occurring gastric hormone, has been shown to promote cell proliferation and survival and hinder inflammation and apoptosis in different cells. Thus, this study explores the neuroprotective capabilities of Obestatin against ethanol-induced neurotoxicity in the SH-SY5Y neuroblastoma cells and C8D1A astrocytes. Methods: The effects of Obestatin and ethanol on cell viability were determined using MTT assays. The mechanism of neuroprotection of Obestatin following ethanol toxicity was determined by measuring the levels of reactive oxygen species (ROS) and adenosine triphosphate activity (ATP) in the C8D1A cells and the SH-SY5Y cells using cell-based assay kits. To further investigate the neuroprotective effects of obestatin on cells, DAPI, Acridine Orange and DCFDA-stained cells were used to assess changes in cellular morphology. Results: Results showed that treatment with obestatin protected the C8D1A and SH-SY5Y cells from ethanol-induced neurotoxicity by inhibiting ROS generation and ATP depletion. Obestatin also reduced acidic vesicular organelle formation, visible from acridine orange staining, and increased the number of nuclei from DAPI staining compared to the ethanol-treated cells alone. Conclusion:This study provides evidence of Obestatin's

neuroprotective potential against ethanol-induced neurotoxicity, and ongoing studies will look at the signalling pathways underpinning this effect.

Keywords: neuroprotective effects Obestatin neurotoxicity C8D1A and SHSY5Y cell lines