St George's University of London

St George's Research Day

30th November 2011

10.00 am – 6.00 pm

Programme and Abstracts

St George's Research Day 2011

This day includes contributions from all Divisions at St. George's as well as from our South West London Academic Network partners, Kingston University and Royal Holloway University of London. This year we would also like to welcome attendees from Roehampton University and the National Physical Laboratory.

This year's special symposium will again highlight the cutting edge research being carried by new appointees within the Research Divisions at SGUL, at our SWan partner institutions Kingston University and Royal Holloway, University of London and from the University of Roehampton. We have retained the extended poster session to accommodate all poster submissions that showcase the diversity and excellence of research activity at the three sites.

We would like to acknowledge the support provided by St. George's and the exhibitors. We would also like to thank the Principal, Prof. Peter Kopelman; Postgraduate Dean, Dr Tony Michael; The Dean of Enterprise and innovation, Prof M Patton; the Director of the South West London Academic Health and Social Care System, Laurence Benson; Prof. George Dickson (RHUL) and Prof. Declan Naughton (Kingston University) for their support and provision of prizes. Finally, we would particularly like to thank Joan Mitchell and Sheryl Pond for their help in organising the exhibitors; producing the abstract books and making the day run smoothly.

We hope you enjoy the day and thank you for your contribution.

Debbie Baines and Iain Greenwood

Full Programme

St George's Annual Research Day - November 30th 2011

Time	Speaker	Location
10.00 am	Andrew Snabaitis, Kingston University "Proximal regulation of type 2A protein phosphatase in cardiomyocytes"	Michel Heron Lecture Theatre
10.20 am	Colin Davidson, Division of Biomedical Sciences, SGUL "Neurochemical effects of "legal highs""	
10.40 am	Keith Foster, RHUL "Big is Beautiful - Myostatin Inhibition for Muscular Dystrophy and Muscular Atropy"	
11.00 am	Tariq Sadiq, Division of Clinical Sciences, SGUL "Modern Diagnostics for STIs"	
11.20 am	*Coffee*	*Board Rooms 1-4*
11.50 am	Cecilia A. Essau, University of Roehampton "Anxiety disorders in children and adolescents: From Epidemiology to prevention"	Michel Heron Lecture Theatre
12.10 pm	Gita Ramdharry, Joint Faculty, SGUL "Gait, posture and rehabilitation research in people with inherited neuroapthy"	
12.30 pm	*Lunch & Posters*	*Board Rooms 1-4*

Exhibitors 2011

Poster Abstracts

St Georges University of London
Divisions:
Biomedical Sciences
Cardiac & Vascular Sciences
Cellular & Molecular Medicine
Clinical Developmental Sciences
Community Health Sciences
Faculty of Health & Social Care Sciences
Mental Health

1. Lucy Addae

Characterisation of post-ovulatory wound repair and its contribution to ovarian carcinogenesis

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Division of Biomedical Sciences, St George's, University of London
Ovulation is a natural process that causes repeated damage to the ovary. It has been hypothesised that these successive bouts of injury and repair contribute to ovarian surface epithelial (OSE) cell damage and transformation, which could possibly lead to ovarian cancer. However, the main problem that arises when looking into this field is that ovulatory 'wound repair' has not been well characterised; without this information, how this process goes awry will remain unknown. We hypothesise that ovulation brings about a repair process similar to that observed following other acute wounds, including inflammation and scar formation. OSE consistently exposed to inflammation and/or in direct contact with scar-associated matrix are expected to adopt cancer-like behaviours. Adult female mice were hormonally stimulated to ovulate, and ovaries collected at time-points coinciding with ovulation/post-ovulatory repair were processed for histology/immunohistochemistry. Inflammatory cell numbers were quantified by immunohistochemistry, and the effects of inflammatory mediators on OSE cell morphology and gene expression were investigated in cell culture studies. Scarring within the ovary was analysed via histological staining. Analysis of the inflammatory response associated with post-ovulatory repair revealed that both neutrophil and macrophage numbers in the wound vicinity increase, and then partially resolve within 16h of ovulation. OSE treated with LPS-stimulated macrophages became smaller in size, were reluctant to form inter-cellular adhesions and typical tightly-packed colonies, and migrated at a faster rate compared to controls. This suggested the cells were possibly undergoing epithelial-to-mesenchymal transition (EMT). RT-qPCR and western blots confirmed that inflammatory mediators may indeed induce a partial and transient EMT, as an increase in vimentin expression was observed in the treated cells. Collagen staining within the ovary at post-ovulatory time-points showed alterations in the abundance and arrangement of the extra-cellular matrix that is perhaps reminiscent of scarring during skin repair. Ovulation-induced tissue damage has been found to elicit certain aspects of wound healing observed in other tissues, including inflammation and scar formation. These wound-associated alterations are anticipated to have significant effects on cell behaviour and in the long-term on tumour initiation and progression.

2. Rabia Ahmad

Changes in central serotonin turnover, 5HT1A and oxytocin receptor binding after chronic exposure to corticosterone in male Wistar rats

Rabia Ahmad[1,2]; Ella Hirani [2]; Jolanta Opacka-Juffry[1]
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It has been known that mood disorders such as depression associate with irregularities of the Hypothalamic Pituitary Adrenal Axis (HPA) and implicate increased levels of circulating glucocorticoids[1]. There are established views that depression as a stress-related condition is associated with reduced monoaminergic tone, however, it is now widely accepted that depression is a multifaceted disorder. There is evidence that the efficacy of the selective serotonin reuptake inhibitors (SSRI's) as antidepressants, is in part due to the fact that they induce the release of oxytocin[2]. Here, we investigate further the interactions between serotonergic and peptidergic responses to glucocorticoids. Male Wistar rats (220g) received

either corticosterone (CORT, 400μg/mL) or vehicle containing drinking water for 21 days[3]. All animal procedures were carried out according to the U.K. Animals (Scientific Procedures) Act 1986. Adrenal and thymus weights were decreased by approximately 35% ($P<0.001$) after CORT treatment. 5-HT1A and Oxytocin receptor binding was quantified using autoradiography with [3H]WAY100635 and [125I]OVTA, respectively. Serotonin turnover was calculated as the ratio of 5-hydroxyindole acetic acid (5-HIAA) to 5-HT; both were analysed by reverse phase HPLC with electrochemical detection. 5-HT turnover was decreased in frontal and prefrontal cortex by 11% and 20% respectively ($P<0.05$). This was coupled with an increase in 5-HT1A binding by 28% - 25% in the prelimbic, primary and secondary motor cortices ($p<0.001$). An increase in 5-HT1A receptor binding was also observed in dorsal hippocampus (CA1-rad, 13%; $p<0.05$). Binding at the oxytocin receptor increased by 57% and 50% in raphe and lateral septal nuclei respectively ($p<0.001$). A 25% reduction in oxytocin receptor binding was observed in basolateral amygdala ($p<0.05$). These data suggest that long-term exposure to glucocorticoids can affect the prefrontal cortical-amygdala pathways with an involvement of the 5-HT1A and oxytocin receptors. These findings are of relevance to the emotional dysfunction associated with stress-related mood disorders.

3. Samrah Ahmed

Profiles of connected speech in early stage Alzheimer's disease: An autopsy-confirmed case series

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Background: The results of detailed analyses of connected speech samples have been reported in patients with probable frontotemporal dementia (FTD), and the syndromes of primary progressive aphasia (PPA). Less attention has been paid to language production in clinically typical Alzheimer's disease (AD). This aim of this study was to describe profiles of connected speech in patients with pathologically confirmed AD, and compare them with the profiles associated with the sub-syndromes of PPA.
Methods: Data from 22 normal controls and 22 AD patients, all with neuropathological confirmation, were obtained from former participants in a longitudinal cohort study of ageing and dementia. Spoken language samples were obtained using the Cookie Theft picture description task, and analysed using the scoring system described by Wilson et al. (2010).
Results: Composite measures of speech production, syntactic complexity, lexical content, and fluency errors were obtained. Group comparisons between normal controls and AD patients showed no significant overall differences but a hierarchical cluster analysis revealed two distinct profiles of preservation and impairment across linguistic variables. Cluster 1 (n=13) was characterised by an isolated reduction in syntactic complexity. Cluster 2 (n=5) showed no impairment on any of the language variables, and performance was statistically comparable to controls.
Conclusion: In contrast to the syndromes of PPA (Wilson et al. 2010), the impairment found in AD was restricted to reduced syntactic complexity, and therefore did not corresponded to the linguistic profiles described in any of the sub-syndromes of PPA. Our findings suggest that the connected speech profile associated with the early stages of clinically typical AD is characterised by a reduction in syntactic complexity. The results suggest that further investigation is warranted into the diagnostic potential of language profiling in early stage AD.

4. Blerina Ahmetaj

Nitric oxide regulates macrophage motility and phagocytosis

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Nitric oxide (NO), a naturally occurring free radical, increases vasodilatation and promotes angiogenesis via cell motility in endothelial cells. Although macrophages are integral to the inflammatory response and phagocytosis, they can combine with ox-LDL to form atheroma in vessels thus leading to heart disease, stroke and heart attacks. The effect of NO on the motility of macrophages and therefore the possible prevention of atherosclerosis has not been studied to date. Human U937 differentiated macrophages were treated with the nitric oxide synthase (NOS) inhibitor ADMA, and its inactive isomer SDMA, at different concentrations (1, 10, and 100µM). Macrophage migration and distance travelled was tracked for 16 hours using real-time imaging and Image J analysis. Primary murine peritoneal macrophages were obtained from wild type (WT) and dimethylarginine dimethylaminohydrolase 2 knock out (DDAH2 -/-) mice, the enzyme that metabolises ADMA, and their cell motility tracked. Phagocytosis was measured by the addition of a fluorescent E-coli¬ bio-particle to cells and fluorescence measured at 450nm- 520nm. Nitrite, a stable end product of NO, was measured using the Griess method. Cell proliferation was measured by the MTT assay. Cell motility and distance travelled in U937 cells decreased with increased concentrations of ADMA; but not SDMA. Similarly, primary peritoneal macrophages obtained from WT mice and treated with ADMA (100µM) reduced motility from 0.38µm/min to 0.26µm/min after addition of an inflammatory cocktail containing LPS/TNFα/IFNγ. Interestingly macrophages from DDAH2-/- mice moved significantly less than WT when treated with the inflammatory cocktail or left untreated (0.25µm/min ± 0.018; 0.18µm/min ± 0.026; in WT and DDAH2-/- respectively). Addition of an NO donor, SNP (100µM), to DDAH2-/- macrophages significantly reversed this effect (0.22µm/min ± 0.023). NO production from macrophages in DDAH2-/- compared to WT mice was significantly lower at 48 hours (34.91µM ± 6.31; 11.89µM ± 1.57; at 48 hours in WT and DDAH2 respectively). Cell proliferation data from WT macrophages suggests that ADMA (10µM) reduces proliferation by 9% whereas SNP (100µM) significantly increases proliferation by 22% ($p<0.05$; 4 experiments). The data shows that DDAH2-/- reduces motility and phagocytosis compared to WT with and without inflammatory stimuli. Supplementing NO in these DDAH2-/- improves motility and proliferation to levels seen in WT. This infers the pivotal role of NO to the motility and phagocytotic capability of macrophages.

5. Aiman Alassar

Transcatheter Aortic Valve Implantation is associated with significant regression of left ventricular hypertrophy but not improvement in left ventricular function_ A one year follow-up Study

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Purpose: Despite the increase in Transcatheter Aortic Valve Implantation (TAVI) procedures over the last few years, little is known about the effect of TAVI on left ventricular (LV) function and remodeling. The aim of this study is to assess the effect of TAVI on echo parameters of LV size, wall thickness, systolic and diastolic function.

Methods: Between January 2008 and August 2010, we studied 41 patients [age 83 (81-87) year, 18 male] with severe aortic stenosis (AS) before and one year after TAVI procedure

using Transthoracic echocardiography. The median LV ejection fraction of the population was 65 (54-70). This retrospective study involved only one patient with poor LV ejection fraction (EF < 45%) and two patients with severe mitral regurgitation (MR) before TAVI. Left ventricular dimensions, left atrial size, LV ejection fraction, LV diastolic function and wall thickness were measured.
Results: One year all-cause mortality was 17%. The LV mass decreased from 242±72 g/m² at baseline to 193±62 g/m² at one year follow-up ($p < 0.05$). The diastolic interventricular septum thickness (IVSd) was significantly decreased one year post-TAVI compared with pre-TAVI values (1.29±0.26 vs 1.08±0.20, $p < 0.001$). At one year follow-up the maximum wall thickness (Max WT) decreased from 1.33±0.29 to 1.11±0.17 ($p < 0.001$). There was no significant change in the left ventricular ejection fraction, LV end systolic diameter, LV end diastolic diameter, left atrial size, fractional shortening and left ventricular diastolic function. There were no significant changes in MR.
Conclusion: A significant regression of left ventricular hypertrophy was found one year following TAVI. However, this regression was not associated with changes in LV systolic function, LV diastolic function, LV size or changes in MR.

6. Aiman Alassar

One Year Mortality Following Transcatheter Aortic Valve Implantation: Incidence, Predictive factors and causes of deaths
Aiman Alassar, Jonathan Davey, Marjan jahangiri
Cardiac & Vascular Sciences Department, St George's University of London
One Year Mortality Following Transcatheter Aortic Valve Implantation: Incidence, Predictive factors and causes of deaths Aiman Alassar, Jonathan Davey, Marjan jahangiri
Background: Large registries have shown that transcatheter aortic valve implantation (TAVI) can be performed in selected high-risk populations with a high procedural success rate of more than 90% and a 30-day mortality rate ≤10% with varying rate of complications. Little is known about one year mortality after TAVI. The aim of this study was to establish the incidence, predictive factors and actual causes of one year mortality following TAVI. Methods Between January 2008 and January 2011, a total of 82 patients with symptomatic severe aortic stenosis underwent TAVI with either the Medtronic CoreValve (Medtronic CoreValve, Irvine, CA) or the Edwards SAPIEN (Edwards Lifesciences, Inc, Irvine, CA). Baseline characteristics and procedural complications were recorded. Causes of deaths were collected via the bereavement office, GP surgeries and the coroner's office. **Results:** One month and one year mortality was 4.8% and 15.8%, respectively. Postoperatively, 16 patients (19.5%) required permanent pacemaker implantation (PPM). Para-valvular aortic regurgitation was mild or moderate in 38 patients (46.3%). Pre-operative irregular heart rhythm (OR 3.78) and post-operative para-valvular aortic regurgitation (OR 3.45) were independent factors of one year mortality after TAVI. At one year, the most frequent causes of death were bronchopneumonia (38.4%), cardiac failure (15.3%), acute myocardial infarction (15.3%), cardiac arrest (7.6%), hypoxic brain injury (7.6%), pulmonary embolism (7.6%) and acute pancreatitis (7.6%). **Conclusion:** One year mortality following TAVI was 15.8%. Pre-operative irregular heart rhythm and para-valvular aortic regurgitation were the most frequent predictive factors. 38.4% of the causes of death were cardiac in origin.

7. Aiman Alassar

Acute Kidney Injury Following Transcatheter Aortic Valve Implantation: Incidence And Predictive Factors

Aiman Alassar, Nada Abdulkareem, Oswaldo Valencia, Stephen Brecker, Marjan Jahangiri
Cardiac & Vascular Sciences Department, St George's University of London

Acute Kidney Injury Following Transcatheter Aortic Valve Implantation: Incidence And Predictive Factors Aiman Alassar, Nada Abdulkareem, Oswaldo Valencia, Stephen Brecker, Marjan Jahangiri **Background**: Acute Kidney injury (AKI) is a common complication following surgical aortic valve replacement and is associated with increased mortality. Little is known about the occurrence of AKI after transcatheter aortic valve implantation (TAVI). The aim of the study was to establish the incidence predictive factors and prognostic value of AKI following TAVI.
Methods: Between January 2008 and January 2011, a total of 81 patients with severe aortic stenosis (AS) who underwent TAVI with the Medtronic CoreValve System or Edwards SAPIEN heart valve were included. Baseline characteristics and procedural complications were recorded. AKI was defined as an increase in creatinine levels more than 26.4 mol/L or more than 50% from baseline or more than 1.5 fold from baseline occurring within 48 hours.
Results: Postoperatively, 10 patients (12.3%) developed acute kidney injury which was completely resolved in 9 prior to hospital discharge. Predictive factors of acute kidney injury were diabetes (OR 6.722) and pre-renal dysfunction (OR 1.024). 11 patients (13.5%) died within one year following TAVI. 3 of the nonsurvivors (3.7%) had developed AKI postoperatively. AKI was not a predictive factor of one year mortality following TAVI.
Conclusion: Acute Kidney Injury occurred in 12.3% of the patients following TAVI and persisted in only one patient prior to hospital discharge. Diabetes and pre-renal dysfunction were found to be the main predictive factors of AKI after TAVI. AKI was not associated with increased one year mortality.

8. Nader Al-Dewik

Fluctuating BCR-ABL levels and transient insertion of three nucleotides in ABL tyrosine kinase is associated with resistance to Glivec (a single case report from Qatar)

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Health and Social Care Sciences, Kingston University and St George's University of London

Introduction: More than 45% of CML patients in Qatar resist the first line of Glivec treatment (Al-Dewik N et al., 2010); Internationally, ABL mutations are the most common cause of Glivec resistance (Deininger et al. 2005 and Quintas-Cardama A et al., 2009).
Aim: To screen CML patients for BCR-ABL kinase point mutations, insertions and/or deletions and study if these abnormalities correlate with resistance to treatment. Materials and methods Peripheral Blood (PB) and Bone Marrow (BM) samples were collected from 25 patients; total RNA was extracted and cDNA was produced via RT-PCR with special precautions to avoid amplification of wild type ABL and cover the whole ABL kinase domain.
Results: Over a period of three years, 39 PB and 30 BM samples from 25 patients receiving Glivec were studied for ABL mutations prior to treatment and at time of resistance. For all 25 patients we noticed three nucleotide changes at A1258G, A1426G and A1739G of ABL (GenBank accession no. M14752). However, when we compared these changes with major SNP databases (NCBI, ENSEMBL), these changes were described by others as ancestral allele that does not convey any pathological changes. Although, we found no evidence of ABL point mutations in patients at time of resistance, in one patient, who had complex cytogenetic abnormalities, we noticed a transient insertion of three nucleotides (AAG) at position 1432 which added an amino acid Lysine356 to the catalytic domain at time of

resistance.
Conclusion: Due to high rate of resistance of CML to Glivec, we tested our patients for BCR-ABL point mutations and could not find any of the described ABL domain mutations that are known to be associated with resistance. In our cohort of patients nucleotide insertions do not explain more than 4 % of resistant cases. However, if the high resistance problem is related to a low level of mutations that could be missed by direct sequencing, then an alternative approach might be needed to detect and quantify low level mutations such as High Resolution Melting (HRM) technology accompanied with sequencing.

9. Sumaira Ali

Development of novel monoclonal antibodies against human colorectal tumour cells for use in cancer diagnosis and therapy

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Colorectal cancer is the third most common cancer in the UK and each year more than 38,000 people are diagnosed with this cancer. In spite of the significantly improved response and survival rate, the efficacy of cytotoxic drugs and other inhibitors is limited with the development of a drug-resistant phenotype .Therefore there is a need for the identification of novel and more specific therapeutic targets in patients with colorectal cancer. Monoclonal antibody technology is an excellent tool for the identification of novel and over expressed cell surface antigens in human malignancies. The aim of this PhD project is to identify other cell surface antigens of biological and clinical significance in colorectal cancer using hybridoma technology and to investigate their potential as targets for antibody-based immunotherapy. A group of mice were immunized with four human colorectal tumour cell lines established from patients with Dukes' A, Dukes' B, Dukes 'C or one for which Dukes stage is unknown. Three days following the 3rd immunisation, the lymphocytes isolated from the spleen of the immunised mice were fused with the mouse myeloma cell line SP2 and plated in HAT medium. Ten to fourteen days after the fusion, hybridoma culture supernatants were screened by ELISA for the presence of antibodies which were directed against the over expressed cell surface antigen on a panel of colorectal tumour cell lines. Following the initial screening, the individual hybridoma colonies from positive wells were picked up and cloned twice for the production of monoclonal antibodies. To date, sixteen fusions and more than 250 hybridoma colonies have been screened for the secretion of antibodies which are directed against cell surface antigens of several colorectal tumour cells. The binding efficacy of these hybridoma supernatants to the colorectal tumour cells was determined by ELISA, FACS analysis and immunofluorescence staining. In addition, the diagnostic potential of these mAbs for the identification of such antigens in formalin-fixed paraffin embedded tissues was determined by immunohistochemistry. To date, seven novel antibodies of IgG1 isotype have been raised against antigens which are overexpressed in different types of colorectal cancer cells. Some of the antibodies are against sequential determinants and can be used in immunohiostochemical detection of such antigens in formalin fixed tissues.

10. Jamie Al-Nasir

Characterisation of cyclic neuroprotective PDZ binding peptides

Jamie Al-Nasir M.Pharm Prof Brian Austen
Department of Pharmacy, Kingston University

Neurodegenerative diseases feature neurochemical and neuropathological changes which are intimately linked with excitotoxicity. PSD-95 a post-synaptic scaffold protein of the NMDA

receptor complex, containing PDZ domains coupling to a PMCa2B calcium channel, NMDAR2, and i-nos, has been found to mediate Glutamate induced excitotoxicity. Neuronal damage may be mediated by calcium intrusion and oxidative stress and PSD-95 is a potential therapeutic target. PDZ binding ligands have been designed based on the C-terminal sequence of the PMCa2b calcium channel sub-unit in order to disrupt its interaction with PSD95 via PDZ domain 1. Current PDZ binding ligands currently have Kd constants in the micromolar range and tighter binding is required (Kd constants in nanomolar range) for therapeutic use. Structural alterations have been proposed by various researchers to this end including cyclisation of PDZ peptide ligands. Putative PDZ binding ligands were modeled in Silico based on existing structures but with important refinements to improve binding affinity. Ligand-Protein docking simulations were performed in order to select a group of ligands to synthesise. Solid Phase peptide synthesis was employed following the Fmoc protocol. Cyclisation was via amide formation between orthogonally protected side-chains. The PDZ binding peptides produced were tested in vitro on SHSY-5Y neuroblastoma cell lines in the presence of 2mmol Glutamate, and insult and cell viability quantified via MTT assay. The results indicate that the modification of a linker residue and side-chain on the PDZ ligand improves efficacy as seen by protection against excitotoxic cell death. Immunohistochemical studies of biotinylated PDZ peptides and their PSD-95 target demonstrated co-localisation and cell protection for an asparaginyl bridged peptide compared to a glutamate -bridged peptide Our results also show that cyclisation aloonr does not confer improvement in PDZ binding affinity contrary to what has been postulated by other researchers.

11. Mohamed Al-Sayegh

Role of SRF co-activators MRTF-A and MRTF-B in adipocyte differentiation.
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Serum Response Factor (SRF) is an essential transcription factor that binds CArG boxes and regulates the expression of immediate early genes such as c-fos and Egr-1 via TCF cofactor recruitment and cytoskeletal and muscle specific genes via MRTF-A/B coactivator recruitment. Recently, it has been shown that SRF inhibits adipogenesis in vitro although the mechanisms responsible for this remain unknown. Here we show that MRTF-A protein expression levels rapidly decrease during the early stages of pre-adipocyte differentiation and that this downregulation is in part triggered by cell-cell contact inhibition. Moreover, lentiviral-mediated RNAi depletion of MRTF-A promotes the differentiation of pre-adipocytes into adipocytes in the absence of any adipogenic stimuli as measured by immunofluorescence staining of lipid droplets. Our results suggest that MRTF-A/SRF are required to maintain the undifferentiated state of pre-adipocytes.

12. Ricardo Antunes

Apoptosis pathways are altered in CD4+CD28null T cells from patients with myocardial infarction
Antunes RF, Kaski JC & Dumitriu IE
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Background: T lymphocytes, the main effectors of adaptive immunity, have key roles in the development and progression of atherosclerosis. The frequency of the CD4+CD28null T cell subset increases significantly in patients with myocardial infarction. These cells, which characteristically lack the CD28 costimulatory receptor, have been suggested to mediate plaque instability and recurrence of myocardial infarction.

Aim: Our aim was to investigate the mechanisms that lead to the accumulation of CD4+CD28null T cells in patients with myocardial infarction, with the main focus on apoptosis pathways in these cells. **Methods:** CD4+CD28null T cells from peripheral blood of myocardial infarction patients and controls were tested for the expression of death receptors (Fas) and ligands (FasL), as well as the levels of anti-apoptotic (Bcl-2, Bcl-xL, survivin) and pro-apoptotic (Bax, Bim) proteins, using flow cytometry. Results: We found that CD4+CD28null T cells express lower levels of death receptor Fas and pro apoptotic proteins Bax and Bim in comparison to conventional CD4+CD28+ T cells. No differences were found in the levels of anti-apoptotic proteins.
Conclusion: We found that CD4+CD28null T cells from patients with myocardial infarction have alterations in Fas and pro-apoptotic proteins, which suggest that they are resistant to apoptosis. These findings could open the way for novel therapies aimed at targeted induction of apoptosis in CD4+CD28null T cells to stabilise atherosclerotic lesions.

13. Gavin Arno

Mutations in ACTA2 in a British cohort of Thoracic Aortic Aneurysm and Dissection (TAAD) patients
Arno G(1), Aragon-Martin JA(1), Harris S(1), Saggar A(2), Jahangiri M(3), Child AH(1).
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Background: Thoracic aortic aneurysm and dissection (TAAD) is a feature of several genetic conditions, such as Marfan (MFS) and Loeys-Dietz (LDS) syndromes. However, there is a growing body of work suggesting that non-syndromic TAAD is also of genetic origin. Furthermore, up to 21 % of non-syndromic cases of TAAD have been found to be familial. Recently studies have implicated mutations in ACTA2 (MIM#102620) as a cause of 14-21% of familial and 2.5-3.8% of sporadic TAAD. It was the aim of this study to determine the frequency of ACTA2 mutation in a consecutive series of TAAD patients attending a British MFS cardiac genetics clinic. Diagnosis was based on pedigree, physical examination, echocardiogram, CT and MRI studies and operative reports.
Method: A total of 78 UK patients (62M:16F, mean age 48.16, ±13.43) with known TAAD who did not fulfil the revised Ghent criteria for MFS, and with no demonstrable mutations in FBN1 or TGFBR2 were recruited to this study. These patients were screened for mutations in all exons of ACTA2 including intron/exon boundaries.
Results: Novel non-synonymous missense mutations were identified in 3/78 (3.85%) probands. These mutations comprised: in exon 3, p.Arg64Lys (c.191G>A); in exon 6 p.Arg179Cys (c.535C>T) affecting the same amino acid as a previously reported TAAD mutation (p.Arg179His); and in exon 7 p.Lue244Phe (c.732G>T). None of the mutations were found in 100 control chromosomes or are reported in any SNP databases.
Conclusion: This study supports data from previous studies that link mutations in ACTA2 with TAAD. In our cohort of UK patients, the detection rate is similar to that found in previous studies of consecutive sporadic cases of TAAD and lower than that found for familial cases. This reflects the mixed familial and sporadic nature of the UK patient population studied.

14. Simon Attree

Multiplexed point of care diagnostics using SERS nanotags
Attree SL, Kumarswami N,Worsley G, Kouassi J, Noble J, Horgan A,Porter RA & Knight A
Biotechnology, Analytical Science Division, National Physical Laboratory

SERS nanotags have been developed for multiplexed detection of analytes (Mulvaney et al., (2003) Langmuir 19:4784-4790). These core-shell structures have a gold core to enhance Raman scattering; a Raman label for detection; and a functionalised silica shell for the attachment of ligands such as antibodies. We have explored the use of these particles in multiplex point-of-care assays for markers of cardiac infarction in serum samples. Lateral flow (immunochromatography) devices are a common format for point-of-care diagnostic devices. They are robust and inexpensive, but normally provide only qualitative, single-analyte detection with a visual readout. By combining this format with SERS nanotags and an instrument for readout, we have developed quantitative assays and multiplex detection for myoglobin, troponin I and C-reactive protein. However, we find that fine-tuning of the assay design is critical. Limited binding capacity at test lines means that lateral flow assays may show signal saturation. In a multiplex assay, where all the capture antibodies are printed on one test line, this can lead to competition between the different nanotag-antibody conjugates for binding sites and consequently, interference between the signals from different analytes. We suspect that this is a generic problem which will be encountered in other nanoparticle-based multiplex assays with a common test line. We show that adjusting the assay conditions can minimise this effect so that it does not interfere with assay performance, although there may be a trade-off with assay sensitivity.

15. Irbaz Badshah

The role of ERK5 in the diabetic kidney

Badshah II, Dockrell M & Baines DL.
Clinical Sciences, SWTIRR. Biomedical Sciences, SGUL

Introduction: Diabetic nephropathy is the complication of kidney damage arising from diabetes and it is the primary cause of end-stage renal disease in many countries. The only treatment for this otherwise fatal condition is dialysis or kidney transplantation. Podocytes are highly specialised cells that exist at the initial interface of glomerular capillaries and renal tubules, whose function is to provide a selective barrier to regulate the molecules that pass through to form urine. Podocyte injury is a key instigator in the pathogenesis of diabetic nephropathy where they are lost resulting in an impaired glomerular filtration barrier. The extracellular signal-regulated kinase 5 (ERK5) is an atypical mitogen-activated protein kinase and has been implicated in mediating cell survival, proliferation and apoptosis in cancer. The aim of this study was to investigate the role of ERK5 in podocytes and whether its activation is protective against podocyte loss.

Methods: Experiments were conducted in terminally differentiated podocytes using a human podocyte cell line subjected to various growth factors (EGF, TGF-β), diabetic stimuli (high glucose) and inhibition of the upstream MAPK/ERK kinase 5 (MEK5) to explore ERK5 expression and activation along with apoptosis (cleaved caspase-3).

Results: Podocytes were demonstrated to express ERK5 and in response to EGF ERK5 activation was observed. EGF produced increased expression of cleaved caspase-3 as did treatment with high glucose. Inhibition of MEK5 prevented ERK5 activation without affecting total ERK5 levels. MEK5 inhibition also resulted in increased cell death along with de-differentiation of podocytes. **Conclusion:** ERK5 appears to be involved in mediating podocyte cell survival and is protective against apoptosis. Manipulation of the ERK5 signalling cascade could ameliorate podocyte loss and the damage to the glomerular filtration barrier that is observed in diabetic nephropathy.

16. Emma Baple

A founder mutation in the SAMHD1 gene identified in the Amish is associated with laryngeal involvement

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The characteristic clinical picture associated with Aicardi Goutières syndrome (AGS) is one of early onset subacute severe encephalopathy with intermittent sterile pyrexias, developmental regression and acquired microcephaly. Since the initial description of the condition, substantial phenotypic variability has been recognised, with some individuals having a later onset of disease and mild neurological manifestations. Clinical overlap with systemic lupus erythematosus (SLE) is also recognised. We report here on the clinical phenotype associated with a founder mutation in the AGS 5 gene (SAMHD1) in an Old Order Amish family. The phenotypic features include: significant short stature, joint stiffness, chilblain lesions, intracerebral aneurysms, oral ulceration and hoarse voice. The severity of the oral ulceration correlates with the degree of hoarseness of voice and laryngeal oedema is reported. Laryngeal involvement has not been previously described in AGS but is an accepted feature of SLE. This finding extends the phenotype associated with mutations in SAMHD1 and provides additional evidence of an overlap with the clinical presentation of SLE. Short stature has been reported in AGS resulting from SAMHD1 mutations and these cases further demonstrate that this is an associated feature. The clinical presentation of individuals with mutations in SAMHD1 appears to be extremely variable and as a result many cases may remain without a molecular diagnosis.

17. Tihana Bicanic

Determinants of Acute Outcome and Long-term Survival in HIV-associated Cryptococcal Meningitis: Results from a Combined Cohort of 523 Patients

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Background: Cryptococcal meningitis (CM) is a leading cause of death in HIV-infected patients in Africa. Identifying factors associated with mortality may lead to strategies to improve outcomes. Methods: 523 patients with HIV-associated CM were prospectively followed-up for 10 weeks during clinical trials in Thailand, Uganda, Malawi and South Africa. A sub-group of 263 were followed for 1 year. Baseline data, rate of clearance of infection (calculated from quantitative CSF cultures) and outcome were recorded. Regression modeling identified variables associated with mortality, rate of clearance of infection and abnormal mental status. Cox proportional hazards analysis identified associations with long-term survival and CM-IRIS.

Results: Median age 34 years, 51% male, median CD4 count 25 cells/μL, 97% ART naive, 25% abnormal mental status, 80% amphotericin (AmB) based initial treatment (0.7-1mg/kg, median 14 days), 20% fluconazole (median dose 1200mg daily for 14 days). Mortality was 16% at 2-weeks and 33% at 10-weeks, and was significantly higher in fluconazole treated vs AmB treated patients (27% vs 14%, $p=0.002$ at 2 weeks and 53% vs 28%, $p<0.001$ at 10 weeks). Variables associated with mortality were older age, lower CD4 count, abnormal

mental status, lower weight and higher baseline fungal burden. In a multivariate model, baseline fungal burden (OR 1.48 per log CFU/ml increase, 95%CI 1.16-1.89, p=0.002), weight (OR 0.96 per Kg increase, 95%CI 0.94-0.99, p=0.007) and abnormal mental status (OR 2.7, 95%CI 1.5-4.9, p=0.001) remained associated with mortality. Rate of clearance of infection was independently associated with 2-week mortality after adjusting for other variables (p=0.002). In the long term sub-group, mortality was 13% at 2 weeks, 32% at 10 weeks, 41% at end of follow-up. 170 patients started ART median 31d (IQR 23-45) from CM diagnosis. IRIS occurred in 22(13%) median 29d from ART start, and 4(18%) died. IRIS was associated with 2-week fungal burden (p=0.007) but not time to ART (p=0.4) or long-term survival (p=0.3).
Conclusions: Baseline fungal burden, weight, abnormal mental status and rate of clearance of infection predict acute mortality. Long-term survival with AmB and ART is good providing patients survive the acute period. Earlier introduction of ART was not associated with increased rates of IRIS. Association of IRIS with 2-week fungal burden provides further support for rapidly fungicidal agents in induction treatment of CM.

18. Lucien Bonfante

Identification and characterization of hyperpolarization-activated cyclic nucleotide-gated cation channels (HCN1) in the enteric nervous system of chick embryo
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Hyperpolarization-activated cyclic nucleotide (HCN) channels are reported to regulate rhythmic electrical activity, contribute to the resting membrane potential, and shape the input-output curves in excitable cells1. An increasing number of studies suggest that HCN channels are significant contributors to the cellular excitability under normal and pathological conditions, and are suggested to be linked to various types of neuropathic pain2. In the enteric nervous system (ENS), non-specific cation currents (Ih) which are carried by HCN channels are known to be prominent in AH neurons. Four mammalian genes that encode HCN isoforms (HCN 1–4) have been identified3,1. An investigation by Xiao and colleagues has revealed the presence of HCN channels in the ENS of mouse, rat and guinea-pig gastrointestinal tract4. The study has revealed that AH/Dogiel type II neurons, which have a prominent Ih, express HCN2 and 4 in the ENS of the guinea-pig and HCN1 and 2 in mouse and rat. In the present investigation, the presence/absence of HCN1 channels in the ENS of 10 days old chick embryo was studied. The differential distribution of the channels in the myenteric and submucous plexuses as well as enteric neurones and glial cells was monitored. Intestine of 10 days old chick embryo was removed, and processed for immunohistochemical investigation as described previously5. Briefly, the segments of intestine were fixed in 4% paraformaldehyde for 2 h and washed in 30% sucrose in phosphate buffered saline (PBS) solution. 15µm frozen sections of the intestine were exposed to primary antibodies to HCN1 and the neuronal and glial markers PGP and GFAP. The structures that are immunoreactive to the specific markers were visualized using fluorophores conjugated secondary antibodies using fluorescence microscope. Our results revealed the presence of HCN1-immunoreactive neuronal elements in both myenteric and submucous plexuses. The HCN1-immunoreactivity was more prominent in the submucous compared to myenteric plexuses. These findings may be indicative of specific role of HCN during development and certainly denote that protein HCN1 is well conserved along the evolutionary line. References 1. Robinson R.B. and S.A. Siegelbaum (2003). Hyperpolarization-activated cation currents: from molecules to physiological function. Annu Rev Physiol, 65: 453-480. 2. Jiang Y.Q., Q. Sun, H.Y. Tu, Y. Wan, (2008) Characteristics of HCN channels and their participation in neuropathic pain.

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19. Daniel Brierley

Effect of Harmine on Electrically Evoked Dopamine Efflux in the Rat Nucleus Accumbens Core and Shell Brain Slice

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Introduction: Harmine is the major beta-carboline alkaloid in the Banisteriopsis caapi vine, a principal component of the Amazonian traditional medicine Ayahuasca. Anecdotal evidence suggests that Ayahuasca consumption reduces incidence of relapse in drug dependent individuals. We tested whether harmine had any effect on modulation of evoked dopamine efflux by cocaine, in the rat nucleus accumbens (NAc) brain slice.

Methods: NAc brain slices taken from 8wk male Wistar rats were maintained in artificial cerebrospinal fluid at 32±0.5°C. Dopamine efflux was evoked in either the NAc core or shell using local electrical stimulation (10x1 ms, 10mA pulses at 20Hz; 450ms stimulation train), mimicking midbrain dopamine cell burst firing. Dopamine was measured using fast cyclic voltammetry at carbon fibre electrodes (CFE). A triangular voltage waveform (-1 to +1.4 V) was applied to the CFE 8 times/s, dopamine oxidised at around 600mV and the increase in current was measured at this potential.

Results: In the NAc core cocaine (1µM) increased electrically evoked dopamine efflux and slowed reuptake as previously shown. Harmine (0.3µM) had no effect alone or in combination with cocaine either on peak dopamine efflux or dopamine reuptake. In the NAc shell cocaine again increased peak dopamine efflux and slowed reuptake. Harmine increased peak dopamine efflux (by 50%), and also increased peak dopamine efflux by a further 80% in combination with cocaine. The effect of harmine on dopamine efflux was attenuated by 60min pre-treatment with the 5-HT2A/2C receptor antagonist ketanserin (1µM). NAc core NAc shell Ketanserin (NAc shell) DMSO control 101±2.3 106±4 103±4.2 Cocaine 1µM 23±17* 179±20* Not tested Harmine 0.3µM 108±4 150± 8* 121±3.6 Harmine+cocaine 220±31* 260±36* Not tested Table 1. Effect of harmine on peak dopamine efflux in the NAc shell. Values are means±SEM expressed as a percentage of baseline values. n=5-7 for each group. *P<0.05 vs control (two-way ANOVA).

Conclusions: Harmine can increase peak dopamine efflux in the NAc shell, but not the NAc core. This effect is attenuated by pre-treatment with ketanserin showing that harmine acts via activation of 5-HT22A/2C receptors. This novel finding suggests that harmine may have therapeutic potential for drug dependence via an 'agonist therapy' mechanism.

20. Emma Budd

Lineages: Are they barriers in the road to antibiotic resistance acquisition by clinical MRSA?

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Multiple antibiotic resistances may be accumulated by acquisition of mobile genetic elements such as large conjugative plasmids. CC30 and CC22 are dominant lineages of MRSA in UK

hospitals. All gentamicin resistant CC30 isolates in our hospital hold a large conjugative plasmid carrying the aac(6')-aph(2'') gene encoding resistance to gentamicin. In contrast, gentamicin resistance is rare in CC22 isolates and not due to conjugative plasmids. Restriction-Modification systems in S. aureus prevent gene transfer from other bacterial species; the Sau1 R-M system also restricts gene transfer between lineages. We aimed to determine if antibiotic resistance genes could be transferred from a clinical MRSA donor into clinical MRSA recipients from various lineage backgrounds and so establish the efficiency of barriers to transfer between lineages. A large conjugative plasmid carrying the aac(6')-aph(2'') gene was conjugated from a clinical CC30 MRSA donor into clinical MRSA recipients from CC30, CC22, CC8 and CC5 lineages and into CC30 MSSA. Transfer into CC30 MRSA recipients occurred at 3.5 transconjugants per 10^5 donors, transfer into CC8 MRSA recipients was significantly lower (1.2 transconjugants per 10^6 donors). Conjugation into CC22 and CC5 MRSA recipients was <1 transconjugant per 10^7 donors. Conjugation into a CC30 MSSA recipient occurred at 2.3 transconjugants per 10^7 donors, a significantly lower rate than into CC30 MRSA recipients. We conclude that conjugation appears to be effectively decreased between lineages and this may be due to Sau1. Our data suggests there may be additional barriers to conjugative transfer between MRSA and MSSA isolates of the same lineage.

21. Preet Chadha

Kv7 channels contribute to endogenous dilator mechanisms in rat mesenteric artery
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Introduction: KCNQ-encoded voltage-dependent potassium channels (Kv7) are expressed in vascular smooth muscle cells and play an important role in regulating vascular tone1. This study examines the contribution of Kv7 channels to endogenous dilator mechanisms in the rat mesenteric artery. **Methods:** Isometric tension recordings were made in response to endothelium-dependent (acetylcholine) and independent (sodium nitroprusside and isoprenaline) vasodilators in the presence of potassium channel blockers. Kv7.4 channel expression and localization was determined using Western blotting and immunohistochemistry.
Results: In preconstricted mesenteric arteries, β-adrenoceptor-mediated vasodilation to isoprenaline was significantly attenuated in the presence of the Kv7 channel blocker linopirdine (pEC50, 6.5±0.3 vs 7.2±0.1 vehicle control; n=7; P<0.05). This inhibitory effect was not observed with the Kv channel blocker 4-aminopyridine (4-AP; 1 mM), in fact isoprenaline responses were considerably enhanced in the presence of 4-AP (pEC50, 8.7±0.2 vs 7.2±0.1 vehicle control; n=7). Isoprenaline-induced dilations were unaffected by the KATP blocker glibenclamide. Endothelium-dependent dilation in response to acetylcholine was markedly reduced in the presence of 10 μM linopirdine (pEC50, 5.7±0.1 vs 6.8±0.1 vehicle control; n=4; P<0.001). Dilation to the nitric oxide donor sodium nitroprusside was unaltered by linopirdine. Abundant Kv7.4 channel expression was confirmed by Western blotting and immunohistochemistry, the latter indicating Kv7.4 localization in both smooth muscle and endothelium.
Conclusion: The present data show that vascular Kv7 channels may play a physiological role in endogenous vasodilation mechanisms, including a potential role in the vascular endothelium. 1. Greenwood and Ohya, 2009 British Journal of Pharmacology, 156, 1196-1203.

22. Madhumanti Chakraborty

To elucidate the genetics of Goldenhar syndrome.
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Abstract Birth defects account for about 3.5% of babies being affected worldwide. A maxillofacial defect, coupled with systemic organ defects is probably the most serious cause of morbidity,and mortality,in new-born babies. Carl Von Arlt-a German Physician first came caross this condition in 1845. This was then later clinically defined--- as an association of preauricular tags(cartilageneous masses in front of the ear) and epibulbar dermoids(benign tumours of the ye)--- by the Austrian physicina Maurice B . Goldenhar in 1952. It is a rare, congenital defect with a prevalence of 1/3500 to 1/5600. Males are more affected than females. This syndrome arises as a result of defect in the branchial arches;located vetrolaterally.the hallmark feature is hemifacial microsomia or one-sided facial asymmetry. Another terminology used is the extended Goldenhar syndrome or the oculo-Auriculo-Vertebral syndrome(OAVS).OAVS was a term coined by Gorlin in 1952. The name stems from the extension of thesyndrome to the heart,and lungs in addition to the already existing features.The sporadic cases are more common,although familial cases account for 2-3% of the total occurence. The aetiology is yet unclear. The search for a genetic component for the familial cases, bore no fruit.Hence our main aim was to search for a causative gene in the Iranian family exhibiting features of Goldenhar syndrome .
Methods: A linkage association study localized a region onchromosome 5 which was to found to contain the genes IRX1and IRX2. PCR analysis was carried out to amplify the coding exons, followed subsequently by Sanger sequencing.
Results: A missense mutation(C.987G>C) was detected in exon2 of the IRx-1 gene. This mutation segregated in only 3 members of the family.
Discussion: This leads us to propse that this is one of the genes that could be controlling branchial arch embryogenesis. the mutation occured near a conserved IRO-box,whose proper function has not yet been delineated.
Conclusion: This time, there is hope that further analysis,could help facilitate the prenatal testing of Goldenhar syndrome or OAVS in patients.

23. Catherine Choy

Anosmin-1 protein promotes brain tumourigenesis by mediating Integrin signalling pathways.
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Anosmin-1 protein, encoded by KAL1 gene, is an extracellular matrix (ECM) – associated protein which plays an important role in regulating the migration of olfactory and gonadotrophin-releasing hormone neurons during early brain development. There is little understanding of its role in the developed brain. Reactivation of developmental signal pathways often takes a part in tumourigenesis therefore we investigate if anosmin-1-mediated cellular mechanisms are associated with brain tumour progression. Meta-analysis and statistical gene ontogeny analysis of publicly available microarray datasets, along with expression data from a patient cohort, revealed that KAL1 mRNA is significantly up-regulated according to increasing tumour grade compared to the normal brain. This was confirmed by analysis of brain tumour tissue biopsy samples using quantitative RT-PCR. To demonstrate the tumour promoting ability of anosmin-1, cell-based functional studies were conducted using glioblastoma cell lines in vitro, showing that anosmin-1 enhances cell motility and proliferation via fibroblast growth factor 1 and urokinase plasminogen activator pathways. We also show that anosmin-1 binds to β1 integrin, a main mediator of cell adhesion and migration, and leads to increased phosphorylation of focal adhesion kinase

(FAK), protein kinase B (PKB/AKT) and extracellular regulated kinase (ERK). Knock down of KAL1 expression by shRNA significantly attenuated the cell motility and growth but increased Caspase 3/7 activity accompanied by reduction in phosphorylation of AKT. These results indicate that anosmin-1 induces integrin-mediated signal pathways to facilitate tumour cell migration and protection from apoptosis. Furthermore, increased anosmin-1 expression inhibited cell adhesion on a fibronectin matrix suggesting it can modulate the interaction of the tumour cells with the ECM. Therefore, over-expression of anosmin-1 in adult brain may contribute to the progression of malignant brain tumours through reactivation of its associated developmental pathways.

24. Ai Wern Chung

Novel atlas-based technique for longitudinal investigation of diffusion tensor tractography data: Application to healthy ageing

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Diffusion-weighted magnetic resonance imaging (MRI) measures the brownian motion of water molecules in the brain. Diffusion is predominantly governed by the underlying microstructure in the brain, i.e. white matter tracts constrict water molecules to diffuse along the fibre. Using a mathematical model known as a tensor, the direction of diffusion in space can be modelled, from which microstructure can be inferred given the orientation of the diffusion tensor (DT). By following the dominant direction of DT in subsequent imaged MRI voxels, white matter pathways can be tracked. This technique is known as tractography. Furthermore, white matter tract integrity can be investigated from calculating DT indices such as fractional anisotropy (FA, a measure of directionality in a voxel), mean diffusivity (MD, average diffusivity in a voxel) and radial and axial diffusivities (sub-divisions of MD describing average diffusion cross-sectional to, and in the direction the DT is oriented, respectively). We present a novel technique applying probabilistic diffusion tensor tractography on longitudinal data acquired over a period of two years to assess white matter structural integrity in normal, ageing subjects. Our method was able to consistently extract white matter tracts associated with working memory over time and between two ageing cohorts (middle-aged and elderly). Tract connections were found between the fronto-temporal, fronto-parietal and temporo-parietal lobes. Our study suggests a decrease in white matter structural integrity of these tracts with age could be related to the decline in working memory performance.

25. Laura Cole

Managing incontinence at home: A pilot study of preferences, effectiveness and ease of use of different types of absorbent products

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Urinary or faecal incontinence are distressing symptoms for any adult. Incontinence lowers quality of life and impacts negatively on mental health as well as creates significant practical and financial problems. Incontinence can lead to social embarrassment, restriction of leisure activity, creation of extra laundry and replacement costs for clothing and bedding, and it can be a source of conflict between individuals and their family. Absorbent products such as continence pads come in a wide range of designs and absorbencies. Studies by national users and professional groups have found wide variations in NHS advice and availability of absorbent products. Our earlier work suggests that carers and people with dementia often have preferences for types of absorbent products, that the preferences can change as levels of

abilities and mobility changes and that ineffective absorbent products increase distress and stress. There have been no studies exploring people with dementia (living at home) and their carers preferences and views of effectiveness in absorbent products. This paper reports on our EVIDEM-C study which examined acceptability, effectiveness, ease of use and issues of cost consequences of different types of absorbent products for incontinence.

26. Daniel Cooper

Identification and characterization of leptin and its receptors (Ob-R) in the skeletal muscle tissue of ten day old chick embryo

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Identification and characterization of leptin and its receptors (Ob-R) in the skeletal muscle tissue of ten day old chick embryo; Cooper, D. And Belai, A. Department of life sciences, University of Roehampton, London Leptin, a peptide hormone, associated with adipocytes, interacts with a network of leptin receptors[3]. The role of leptin is extremely important and versatile accounting for numerous biological processes such as hematopoiesis, blood pressure, fat deposition, energy expenditure, immune function, satiation, reproduction and bone mass and development[4]. Equally, skeletal muscle is known to be a metabolically active site and one of the main sites of primary lipid oxidation[2]. Skeletal muscle has long been established as one of the main targets of leptin since it accounts for a significant proportion of basal metabolic rate and thermogenesis, although the overall metabolic effects of leptin have proved to be controversial[6]. Leptin is reported to be associated with endogenous signalling factors that are responsible for controlling cellular metabolic activities, of which one of these is suggested to be mediation of energy balance and used as an indicator in energy store availability[5]. Studies conducted in which leptin was injected into the aculeate nucleus of the brain in rats have shown an increase in metabolic activities such as arterial pressure and heart rate along with sympathetic renal nerve activity[1]. This effect of leptin provides evidence that this hormone is directly involved in sympathetic outflow to tissues in the periphery thus directly influencing metabolic rate[1]. Even more importantly, leptin is suggested to have an important role as a future treatment of obesity[7]. In the present investigation, the presence or absence and pattern of distribution of leptin and its receptor (Ob-R) in the skeletal muscle of ten day old chick embryo was determined using immunohistochemical techniques. Briefly, tissue sections from the upper thigh were dissected and fixed in 4% paraformaldehyde for 2hr and washed in 30% sucrose and phosphate buffered saline (PBS). 15µm frozen sections of skeletal muscle tissue were exposed to primary antibodies specific to Ob-R, leptin as well as glial and neuronal markers PGP and GFAP. Structures specific to the immunoreactive markers were exposed to a secondary antibody conjugated to fluorophores and visualised using the fluorescence microscope. Our results identified numerous leptin receptors and its respective hormone, leptin, with a high degree of colocalization with both neuronal and glial cell markers.

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27. Rohan D'Souza

Low birth weight infants do not have capillary rarefaction at birth: Implications for early life on microcirulation

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Background: Low birth weight predicts adult essential hypertension and is linked to increased cardiovascular mortality in adult life. A reduction in capillary density (i.e. rarefaction) is a hallmark of essential hypertension, and evidence suggests that rarefaction precedes the onset of the rise in blood pressure, as it is found in normotensive individuals at high risk of developing hypertension, suggesting that rarefaction is likely to be a primary structural abnormality. We hypothesized that low birth weight infants would have significant capillary rarefaction at birth.
Methods: We studied 44 low birth weight infants born to normotensive mothers (33 were born pre-term, birth weight 1823±446 grams, and 11 were born at term, birth weight 2339±177 grams) and compared them to 71 infants born at term with normal weight (birth weight 3333±519 grams). We used orthogonal polarised spectroscopy to measure basal (i.e. functional) and maximal (i.e. structural) skin capillary densities.
Results: Low birth weight infants, whether born preterm or at term, had significantly higher functional capillary density (mean difference of 10.5 capillaries/mm2; 95%CI 6.6 to 14.4, $p<0.0001$), and higher structural capillary density (mean difference of 11.1 capillaries/mm2; 95%CI 7.6 to 14.5, $p<0.0001$) when compared to normal weight term infants.
Conclusions: Low birth weight infants born to normotensive mothers do not have capillary rarefaction at birth. These results contradict what might have been predicted from the concept of the intrauterine origins of adult disease, and suggest that microcirculatory abnormalities observed in individuals of low birth weight occur in postnatal life rather than during their intrauterine existence.

28. Colin Davidson

Can antipsychotics potentiate the effects of cocaine?

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There is a lifetime prevalence of approximately 50% of substance use disorder in schizophrenics. Further, cocaine abusing schizophrenics are at increased risk from suicide and have a greater hospitalization rate than those schizophrenics who are cocaine-free (J Nerv Ment Dis. 1993, 181:31–37). There is also evidence that the prevalence of cocaine abuse or craving is dependent upon the antipsychotic used in treatment (J Nerv Ment Dis 2005;193: 379–386). This study examines the effect of various typical and atypical antipsychotics on dopamine release and reuptake in the nucleus accumbens. We also examine combination treatment to determine whether there are any antipsychotics which potentiate the effects of cocaine. Adult male rats were killed by schedule 1. Accumbens slices (400µm) were perfused with oxygenated (95%O2/5%CO2) artificial cerebrospinal fluid at 32.5±0.5ºC. Dopamine was evoked using local electrical stimulation using bipolar tungsten electrodes and

measured using fast cyclic voltammetry at carbon fibre microelectrodes sampling at 8Hz. We used a relatively slow stimulation train (10 pulses at 10Hz, 10mA, 0.1ms pulse width) in order to allow dopamine autoreceptor activation, which normally takes about 0.5s (Synapse, 2001, 41, 301-310) thus enabling us to see greater effects with dopamine D2 antagonists. We examined the effects of metoclopramide, haloperidol, sulpiride, fluphenazine and clozapine (all 1µM) and cocaine (1 µM). In some experiments accumbens slices were treated with both an antipsychotic and cocaine for 60 min. Metoclopramide and sulpiride increased peak dopamine efflux by about 65%, while haloperidol increased peak dopamine efflux by about 50%, fluphenazine and clozapine were less effective. None of the antipsychotics had any effect on dopamine reuptake. Cocaine increased dopamine efflux by about 350% and dopamine reuptake was about 2-3 times slower. Pretreatment of accumbens slices with metoclopramide, sulpiride and haloperidol significantly increased the effects of cocaine on peak dopamine efflux while having little effect on dopamine reuptake. These data suggest that typical antipsychotics could potentiate the effects of cocaine in treated schizophrenics, however, because these drugs may also block post-synaptic dopamine receptors, further studies are needed to determine the neurochemical reasons behind cocaine abuse in schizophrenics.

29. Alison Davis

Comparison of TMEM16A and Best 3 expression and function in murine thoracic aorta.
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Introduction: Opening of Ca2+-activated Cl- channels (CACC) produces smooth muscle depolarization and increases contractility. Yet, the functional impact of these channels has always been difficult to determine because of the relatively ineffective pharmacological agents. However, since the original identification in 2008 of TMEM16A as a strong candidate for the molecular correlate of CACCs, there has been renewed interest in deciphering the functional role of these channels in a variety of cell types (see reviews Galietta, 2009; Kunzelmann et al., 2009). Moreover, an earlier CACC candidate, Bestrophin 3 (Best 3), has been shown to be important for cGMP-dependent Ca2+-activated Cl- conductance in rat mesenteric arteries (Matchkov et al., 2008). The aim of this study was to ascertain the relative abundance of Best 3 and TMEM16A in murine thoracic aorta and to determine the effectiveness of two novel TMEM16A inhibitors, tannic acid and T16inh-01 (Namkung et al., 2010 & 2011), as well as the cGMP blocker Zn2+ on □-adrenoceptor-induced contractions of mouse thoracic aorta.
Methods: Female BALB/c mice (6-8 week) were killed by overdose of pentobarbitone in accordance with Schedule 1 of the Animal (Scientific Procedures) Act 1986. Vessels were dissected and utilised in either mRNA analysis or isometric tension studies.
Results: Quantitative PCR revealed relative abundance of these genes as: Best 3 approximately equal to TMEM16A >>TMEM16B (n=3). Similar expression was observed in mouse portal vein and carotid artery. In addition, all blood vessels studied expressed a Best 3 splice variant that appeared to be specific to the vasculature (n=3). Application of ZnCl2 (100 µM) had no effect on methoxamine-induced contractions. In contrast, the T16inh-01 relaxed methoxamine-induced contractions effectively (67 ± 9% relaxation at 10 µM (n=4) although the effects were relatively slow (time to maximum relaxation = 18 ± 4 min). Tannic acid had variable effects on methoxamine contractions either producing a rapidly relaxation of ~75% within 5 mins or having no effect at all.
Conclusion: TMEM16A, but not Best 3, appears to contribute to □-adrenoceptor-induced contractions. References: Galietta. (2009) Biophys J. 16;97(12):3047-53. Kunzelmann et al.

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30. William Day

FERM domain-containing proteins in prostate cancer progression
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Prostate Cancer (PC) is one of the most frequent in men of Western countries. In later stages, PC metastasises to the bone in more than 80% of the cases. However, today's treatment and diagnosis of PC still show major limitations and the mechanism(s) underlying this predilection of PC to metastasise to bone remains undefined. The earliest difference between a confined tumour and one that gains metastatic potential is the detachment of cells from the primary tumour and the acquisition of cell motility. The establishment of metastases in secondary tissues also requires changes in cell migration and adhesion. Four-point-one, ezrin, radixin, moesin (FERM) domains are present in a variety of mammalian proteins. There are around 50 distinct FERM domains encoded by over 30 genes in the human genome, most of them with clear orthologs in other animal species, including Drosophila, C. elegans and Dyctiostelium. Based on sequence alignment between these different species, FERM domain-containing proteins (FDP) can be divided in three broad groups: talins and kindlins; ERMs, GEFs, kinases and phosphatases; myosins and Krev interaction trapped (KRIT) proteins. There is growing evidence that FDP are at the heart of a network of interacting proteins that link the actin cytoskeleton with membrane dynamics at the leading edge of migratory cells, including those involved in metastasis. As part of a screen aiming to investigate the role of FDP in prostate cancer progression we have selected 4 different genes (Talin, Kindlin, JAK (Janus Kinase) - and KRIT) to investigate their role in prostate cancer cell metastasis, using an siRNA approach in a collection of prostate cancer cell lines with different metastatic capacity. We expect that data from this screen will shed new light in the role of FDP in the interface between the plasma membrane and the underlying cytoskeleton in controlling cell migration during prostate cancer progression.

31. Ayesha De Souza

Proteomic Analyses Elucidates Protein Expression Changes in Two Canine Models of Atrial Fibrillation
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Introduction: Atrial tachycardia leads to atrial electrophysiological remodeling and increased susceptibility to atrial fibrillation (AF), highlighted by changes in ion channel function leading to action potential shortening and the promotion of atrial re-entry. Alternatively, congestive heart failure leads to atrial structural remodeling and increased susceptibility to AF, characterized by alterations in conduction properties and interstitial fibrosis. However, the underlying molecular mechanisms are poorly understood.
Methods: To identify the atrial protein changes in two canine models of AF, we applied high-throughput proteomic analysis to left atrial myocytes harvested from sham (n = 5 and 9) for atrial-tachypaced (ATP, 400 bpm x 1 wks, n = 5) and ventricular-tachypaced (VTP, 240

bpm x 2 wks, n = 8) dogs respectively. Protein extracts were subjected to 2-dimensional gel electrophoresis. Differentially expressed proteins ($p < 0.05$) were excised for identification by mass spectrometry.
Results: From our protein extracts, 124 proteins were significantly altered between sham and ATP (70 increased, 54 decreased in ATP) and 238 between sham and VTP (124 increased, 114 decreased in VTP). Among the significantly upregulated proteins were heat shock proteins (HSPs: GRP75/78 and HSP70) and structural proteins (desmin and vimentin). Metabolic enzymes predominantly changed in the VTP model (alpha/beta enolase, ATP synthase H^+ transporting mitochondrial F0, malate dehydrogenase (DH), NADH DH and ubiquinol cytochrome c reductase), although NADH DH and fatty acid binding protein were increased in ATP only. Changes in contractile proteins (myosin light chains 1/2 and troponin T) were only seen in VTP. Downregulated proteins in both models included the antioxidant, superoxide dismutase, and one of the key regulators of cardiac metabolism, pyruvate DH E1 component subunit.
Conclusions: ATP and VTP-induced atrial remodeling substantially alters the atrial proteome. Upregulated HSPs may reflect autoprotective mechanisms while downregulated antioxidants reflect oxidative stress. Perturbations to metabolic enzymes may suggest adaptations to increased metabolic requirements, with greater metabolic stress in VTP. Furthermore, structural damage along with contractile protein changes was prominent in VTP but absent in ATP. Proteomic analysis of these models of AF provides insights into molecular mechanisms underlying atrial remodeling paradigms of significant clinical relevance.

32. Sara Di Fino, Angela Gravina

Prevalence and morphological characterization of early repolarization patterns in young healthy individuals: impact of gender, ethnicity and physical activity
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Purpose: Early repolarization (ER) is a commonly observed in athletes and young healthy individuals. Recently, ER in the inferior and lateral leads has been associated with sudden cardiac arrest from idiopathic ventricular fibrillation. We studied the prevalence, distribution and morphology of ER patterns in inferior and lateral leads in young healthy individuals.
Methods: 12-leads electrocardiogram (ECG) was performed at rest in 1237 young healthy individuals (age range 13-38 years) from February to September 2011. We evaluated the impact of gender, ethnicity and physical activity on ER. Individuals were divided into physically active (exercise >2 hours/week) and sedentary. Early repolarization was defined as notched or slurred J-point elevation of at least 0.1mV from baseline, in ≥2 contiguous inferior or lateral leads; anterior ER patterns were not considered in this study. The morphology of ST-segment was classified as horizontal/descending or rapidly ascending/upsloping.
Results: The mean age of participants was 18.2 ± 4.3 years of which 979 (79%) were males, 981 (79%) were physically active and 91% were Caucasians. ER pattern was present in a total of 232 (18.7%) cases; of these 42% were in the inferior leads, 31% in lateral leads and 27% in both. Notched ER was more prevalent (64% inferior, 83% lateral, 76% infero-lateral) compared to slurred morphology, and more commonly associated with ascending/upsloping ST-segment elevation. ER was significantly more prevalent in males compared to females (20% vs 12%, p=0.003), in physically-active people compared to sedentary (20% vs 13%, p=0.0194), and in Afro-Caribbeans compared to Caucasians (48% vs 17%, p=0.0001). In addition, voltage criteria for left ventricular hypertrophy and sinus bradycardia were a common associated finding in individuals with ER pattern compared with those without (p=0.0001 and 0.002 respectively). Only 5% of individuals with ER had J-point elevation of

>0.2 mV.
Conclusion: Early repolarization is a common finding in young healthy individuals, and is more prevalent in males, physically-active individuals and those with Afro-Caribbean ethnicity. Notched ER with ascending ST-segment elevation in inferior leads was the most commonly observed orphological pattern. More research is required to understand precise long term implications of such repolarization changes in young individuals.

33. Nafi Dilaver

Anti-Mullerian Hormone (AMH) causes male sexual differentiation, but what is it doing in the ovary?
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Division of Biomedical Science, St George's, University of London
Introduction: AMH causes regression of the Müllerian ducts in the male fetus. AMH was localised to the granulosa cells (GCs) in the ovary. The highest expression of AMH is found in small antral follicles and reduces as the follicle grows. AMH has an inhibitory role in the normal ovary, inhibiting selection by reducing follicle sensitivity to FSH. Currently, AMH is used as an ovarian reserve marker and a predictor of response to induction of ovulation. AMH is highly over-produced by polycystic ovaries. All of the above suggest it is important to understand the role of AMH in the normal ovary. Hypothesis: AMH alters the regulation of a large number of genes.
Aim: To investigate the effect of AMH on gene expression in human GCs extracted from small antral follicles, taking a whole genome approach.
Methods: Ovaries were collected from women undergoing a hysterectomy with bilateral oophorectomy. Ovaries were dissected, GCs extracted and cultured for 48hrs, without or with AMH at 10ng/ml. RNA was isolated; integrity and purity were analysed followed by amplification and biotinylation. Amplified/labelled product was hybridised onto the Illumina Human HT-12 BeadChip. Microarray data was analysed and genes of interest were identified.
Results: AMH significantly up- or down-regulates 45 genes in GCs obtained from normal small antral follicles.
Conclusion: AMH significantly effects the regulation of several genes within the GCs. Phosphodiesterase-7B is significantly up-regulated, this supports the inhibitory role of AMH in follicle selection. The effects of genes up- or down-regulated in the ovary need to be determined.

34. Nafi Dilaver

Metformin inhibits human granulosa anti-Müllerian hormone production: an additional benefit in women with Polycystic Ovary Syndrome?
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Metformin is commonly used to treat women with polycystic ovary syndrome (PCOS) and in many cases this is irrespective of the presence of insulin resistance. Metformin has been shown to improve menstrual frequency, insulin sensitivity and androgen levels and we have shown direct inhibition of aromatase mRNA expression and activity. Within the ovary the 'ovarian reserve' factor anti-Müllerian hormone (AMH) is produced solely by the granulosa cells and is overproduced by these cells in the polycystic ovary (PCO). In a number of studies, metformin was also shown to reduce circulating levels of AMH. This was attributed to a new cohort of follicles growing within a normalised androgen and insulin environment. Our aim was to examine a possible further action of metformin on ovarian function by

determining whether metformin directly affects AMH expression and protein production in human granulosa cells. KGN cells (a granulosa tumour cell line), were treated with metformin (10-7M & 10-4M) alone and in the presence of insulin for 24 and 48hrs. AMH expression was determined by real time quantitative PCR (qPCR). Levels of AMH protein produced by n granulosa luteal cells treated with metformin for 48hrs were measured by ELISA (DSLabs). AMH mRNA expression after 48hrs was significantly reduced by metformin treatment alone and in the presence of insulin at both doses, (ANOVA p=0.0005). Insulin alone had no effect on AMH expression. After 48hrs 10-7M metformin reduced AMH protein by 65%. The reduction in AMH mRNA expression by metformin at both 10-7M and 10-4M was in the presence of low concentrations of insulin (0.1-1ng/ml), than with higher doses (10-100ng/ml). In summary, metformin treatment inhibited AMH mRNA expression and protein production in granulosa cells both alone and in the presence of insulin. During folliculogenesis AMH production is highest in small antral follicles and reduces as the follicle size increases. At the size at which a follicle becomes selected, AMH levels are very low or undetectable, however in women with anovulatory PCOS, the granulosa cells produce 75x more AMH which may contribute to abnormal ovarian function. Treating a subset of women with PCOS high AMH production with metformin may improve their ovulatory status.

35. John Dixon

Investigation of a continuous Iohexol infusion to measure Glomerular Filtration Rate (GFR) in healthy adult volunteers: Interim study analysis

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Aim: Validate a method of measuring Glomerular Filtration Rate (GFR) in healthy volunteers, with the eventual goal of using this method to document changes in GFR in critically ill patients with Acute Kidney Injury (AKI).

Methodology: The gold standard Iohexol clearance method was used to confirm GFR in volunteers. Following a washout period of a minimum of 4 days, subjects received the trial method, consisting of an intravenous loading dose of Iohexol over two minutes, calculated according to body mass, followed by a continuous infusion of Iohexol (Omnipaque 300) at 0.5mL/hour for 12 hours. Plasma Iohexol concentration was measured by tandem mass spectrometry at 10 time points. Iohexol concentrations were plotted on a 3-phase exponential decay graph. GFR was calculated by dividing the infusion concentration by the plateau concentration. Results were compared with 4-hour creatinine clearance (4-CrCl), and creatinine-based equations for calculating GFR. Statistical analysis was performed using the t-test.

Result: 5 healthy subjects (4 female; 4 Caucasian, 1 Afro-Caribbean; mean age 29+/-9 years) volunteered. There was no difference in mean GFR measured by the Iohexol clearance method (106.8+/-8.0 ml/min) and the Iohexol infusion method (109.8+/-11.3ml/min), P=0.64. The differences in GFR on the two occasions were similar to the intra-individual variation observed in published studies. The time to reach a plasma concentration within 10% of the plateau was 2 hours 45+/-84 minutes and within 5% was 3 hours 56+/-118minutes. GFR was overestimated by 4-CrCl (30.8+/-9.0%; P=0.01), the CKD-EPI equation (6.4+/-0.7%; P=0.08), and the Cockroft-Gault formula (17.8+/-4.3%; P=0.04). MDRD equation underestimated GFR by 9.1+/-1.0% (P=0.02).

Future work: 1) complete this study over a range of GFR in patients with Chronic Kidney Disease, 2) Our ultimate goal is to use this method in critically ill patients with AKI. The

rationale is that they will take longer to achieve a steady plasma concentration, and the final concentration achieved will be increased.
Conclusions: The continuous Iohexol infusion method of measuring GFR appears to be accurate and precise. In normal subjects, a steady plasma concentration is achieved before it is observed with creatinine changes. Creatinine-based GFR calculations are less precise.

36. James Dodd

Cognitive Function & Cerebral Functional Connectivity in COPD: A Resting State Functional Magnetic Resonance Imaging Study
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Rationale: Abnormal brain pathology is a potential systemic manifestation of COPD, with evidence of dysfunction through cognitive impairment1. Proposed factors relating to cerebral damage and cognitive dysfunction include hypoxaemia and vascular co-morbidities2. Recent work by our group using diffusion tensor imaging (DTI) suggests widespread microstructural white matter tract damage and cognitive dysfunction in non-hypoxaemic stable COPD. Resting state functional MRI (rfMRI) measures oxygen consumption of neuronal cells, and by identifying voxels that are activated in unison over time, whole-brain functional connectivity can be inferred. A direct association between functional and structural connectivity via white matter tracts in the healthy human brain has been demonstrated3. Using rfMRI we compared functional connectivity within the brains of non-hypoxaemic stable COPD versus controls.
Methods: 50 participants underwent 3T MRI (stable non-hypoxaemic COPD patients, n=25; age matched Controls, n=25). Demographic, disease severity and vascular risk measures were made in addition to full neuropsychological assessment (Table 1). During image acquisition participants were instructed to “keep your eyes open, stay awake and think of nothing in particular”. Analysis of data was done using MELODIC (FSL v4.1; www.fmrib.ox.ac.uk/fsl). Independent component analysis (ICA) was performed on all 50 subjects4 along with dual-regression analysis5. The resulting family-wise error corrected voxels were thresholded at $p < 0.01$.
Results: There was no difference in age, gender or cerebrovascular risk (FSRS) (6.1 vs 7.0 p=0.39) between COPD and controls. COPD patients had lower education attainment and performed significantly worse across all cognitive measures. The COPD patients had moderate disease, FEV1 53 % pred, SGRQ 51.6 (Table 1). RSN’s identified included the default mode network (DMN), left and right fronto-parietal (LFP and RFP), pre-frontal (PF), sensorimotor & auditory (SMA) and visual (Vis). All networks, (with the exception of visual) showed significantly reduced functional connectivity in COPD vs. controls. PF showed the greatest regional difference in functional connectivity. Figure 1. **Conclusion:** This is the first study to demonstrate that in addition to evidence of widespread white matter tract damage and global cognitive dysfunction there are also widespread differences in resting state networks and therefore functional communication between areas of the brain in stable non-hypoxaemic COPD. Table 1: Figure 1 Reference List 1. Grant, I. Arc Int Med 1982;142 2. Dodd, J ERJ;2010:35 3. Martijn P Eur Neuropsychopharmacology 2010;20:8 4. Beckmann Philos Trans R Soc London B 2005;360 5. Filippini N PNAS 2009;106:7

37. James Dodd

Cognitive Function & Cerebral White Matter Tract Microstructure in Chronic Obstructive Pulmonary Disease (COPD).

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Rationale: There is evidence to suggest that COPD leads to cognitive impairment in patients both with and without hypoxemia 1; but the pathogenesis remains poorly understood. Also relevant to potential brain pathology in COPD are common vascular comorbidities including hypertension, diabetes and older age. Diffusion tensor imaging (DTI) is a novel Magnetic resonance imaging (MRI) technique sensitive to subtle changes in white matter due to vascular damage. This is the first study to investigate white matter microstructure and tract pathology in COPD.

Methods: Participants (n=50) completed a full cognitive assessment (including executive function, working memory, episodic memory, processing speed, visuospatial ability) and 3T MRI scan. We compare 25 stable non-exacerbating COPD and 25 age-matched healthy controls. Volumes of grey matter (GMV), white matter (WMV), and white matter lesions (LV), were calculated. DTI data was analysed using tract based spatial statistics (TBSS)2.

Results: There are significant group differences between COPD patients and controls on all cognitive measures except episodic memory (executive function: $F=15.39$, $p<.001$; working memory: $F=5.94$, $p=.019$; episodic memory: $F=3.91$, $p=.054$; processing speed: $F=11.64$, $p=.001$; visuospatial ability: $F=10.10$, $p=.003$). COPD patients did not differ from healthy controls on measures of normalized GMV ($t=0.229$, $p=0.820$) or WMV ($t=-0.727$, $p=0.471$). Normalized Lesion Volume was significantly greater in patients versus controls ($t=-2.27$, $p=0.029$). DTI-TBSS revealed lower fractional anisotropy (FA) and higher mean diffusivity (MD) values throughout the brain in COPD patients versus Control subjects. Group differences in white matter integrity were observed throughout the temporal, frontal, parietal and occipital lobes and amounted to 60% of the total FA skeleton. See Figure 1.

Conclusion: This is the first paper to demonstrate that white matter integrity throughout the brain is significantly compromised in patients with COPD compared to age-matched Controls. This damage to white matter is also demonstrated by the significant group differences in white matter lesion load. No differences between patients and Controls were observed in brain volume, suggesting that group differences may be related to white matter integrity rather than atrophy. Table 1: Demographic & MRI data; Mean (SD) Reference List

1. Dodd, J. W., S. V. Getov, and P. W. Jones. 2010. Cognitive function in COPD. European Respiratory Journal 35:913-922. 2.Smith, S. M., Jenkinson, M., Johansen-Berg, H., Rueckert, D., Nichols, T. E., MacKay, C. E. et al. (2006). Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. Neuroimage, 31, 1

38. Lynne Drummond

Disordered Eating Amongst Patients with Obsessive-Compulsive Disorders and Other Anxiety Disorders

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The link between Anorexia Nervosa (AN) and Obsessive-compulsive disorder (OCD) has been recognised for over fifty years (Dubois, 1949; Palmer and Jones, 1939). This comorbidity of OCD and Eating Disorders (ED) has also been more recently reported (Fahy, Osacar and Marks, 1993; Kaye et al., 2004). Studies such as these have lead to the suggestion that both OCD and anorexia nervosa may have a common psychopathology (Hsu, Kaye and Weltzin, 1993). Although there have been many studies in eating disordered

patients reporting high levels of OCD or obsessive-compulsive personality disorder, the studies on OCD populations are more scanty. Fahy, Osacar and Marks (1993) found that, in a retrospective case note study of 105 female OCD sufferers, 11% had a history of AN. A retrospective study in South West London and St George's Mental Health NHS Trust, examining patients treated as inpatients for severe, chronic resistant OCD failed to demonstrate an unduly high prevalence of eating disorder (Raswany et al, 2006).
Method: All patients referred to the specialised Trustwide Community Treatment for OCD/BDD and other severe neurotic disorders were included in the study. Routine data were collected from all patients including measures of OCD and depressive symptomatology as well as screening for eating disorders using the SCOFF scale (Hill et al., 2009). The presence of eating disorder was suspected in those patients who had a SCOFF score >2.
Results: A total of 245 patients were included in the study and consisted of 172 patients (51% female) with OCD and 73 (58% female) with other anxiety disorders. We discovered that patients with OCD were no more likely to score positive on the SCOFF scale than those with other anxiety disorders. Women were more likely than men to score positively on the SOFF in both the OCD and the control group.
Conclusions: We demonstrated that OCD does not seem to have a higher rate of disordered eating compared with other anxious patients. We are further investigating how many patients had a true diagnosis of eating disorder using clinical interview and examination.

39. Anna El-Jouzi

Sgul's Current Research Information System (CRIS)
El-Jouzi A, Jones L
Library
The CRIS (Current Research Information System) is a web-based publications management system which provides details of our researchers' papers. This tool will be a key element in identifying suitable publications for the REF (2014). The details available in the CRIS include statistical reporting, such as bibliometrics (for example, times cited and h-index). In the future SGUL plans to integrate the CRIS with an institutional repository so that researchers will be able to deposit their papers into an open access environment via the CRIS. Other plans for the CRIS include:

- details of funding sources for research projects
- details of collaboration partners
- an institutional overview of current research to enable the creation of new collaborative groups.

40. Chris Fenner

Raised glucose concentration in endotracheal aspirates; a risk factor for early ventilator associated pneumonia in patients with traumatic brain injury
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Patients after traumatic brain injury (TBI) are particularly vulnerable to early onset ventilator acquired pneumonia (EOVAP) and recent data shows that Staphylococcus aureus (S aureus) is a very important pathogen (1). Previously we have shown in a general critical care population that patients with high concentrations of glucose in airway surface liquid (ASL) are susceptible to S aureus infection (2). Normally the concentration of glucose in ASL is <

0.5 mmol/L. Hyperglycaemia and inflammation are risk factors for increased ASL glucose concentrations (3). The aim of this study was to prospectively measure ASL glucose in ventilated critically ill patients, with and without, traumatic brain injury (TBI) and to observe the development of airway pathogens.
Methods: Patients admitted with TBI were included if they were expected to require intubation and ventilation for > 48 h and were compared with critically ill patients admitted to the general intensive care unit (GICU). Assent from relatives in accordance with the local ethics requirements was obtained. Patients were sampled daily for blood and sputum filtrate glucose concentrations, sputum microbiology and serum biochemical and haematological variables. Clinical pulmonary infection scores (CPIS) were calculated.
Results: TBI patients were younger (40 vs. 71, p=0.0018) but did not differ in severity of illness (SOFA: 7 (5-9) vs. 9 (7 – 14.5), p = 0.145). ASL glucose concentration was significantly higher in TBI patients on day 1 (2.1 (1.0-2.8) vs. 0.5 (0.3 – 1.0)) and day 2, 2.5 (1.2 – 4.1) vs. 0.3 (0.1 – 1.7). Blood glucose did not differ significantly at any time. 7 patients with TBI had S.aureus in sputum compared with no GICU patients (p=0.022). The CPIS was higher in patients with S aureus on day 2 than GICU patients (6 vs. 2.5, p=0.045).
Conclusion: Ventilated patients with TBI injury are more likely to have EOVAP caused by S.aureus than critically ill patients without TBI. It is associated with significantly increased ASL glucose concentration. Serum glucose concentrations are not increased. The exact mechanism remains to be elucidated.
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2. Philips BJ. et al (2005). Glucose in bronchial aspirates increases the risk of respiratory MRSA in intubated patients. Thorax. 60(9):761-4
3. Philips BJ et al (2003). Factors determining the appearance of glucose in upper and lower respiratory tract secretions. Intensive Care Medicine. 29(12):2204-10 This work is funded by a the BJA and RCoA.

41. Benjamin Ford

How is survival predicted by preadmission factors in PICU patients who have undergone bone marrow transplantation?
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Aims: To examine how prePICU admission characteristics predicts outcome for paediatric bone marrow transplant (BMT) patients.
Methods: Cross-reference of local PICU and BMT databases identified 54 patient episodes 2004-2009, representing 41 patients. The preadmission age, sex, underlying diagnosis, transplant type, time since transplant, and reason for admission were identified. Survivors and non-survivors were compared using Mann-Whitney and Fisher's Exact tests.
Results: Of the 41, 8 died in PICU and 12 were discharged and died later without another admission. 21 are still alive. Mortality for each episode was 20% overall. Age was similar, but there was an excess of boys in non-survivors. Survivors were admitted earlier post-transplant than non-survivors (59.7 (2.00) vs 89.1(3.33) days p=0.05). Survivors more often had autologous transplants (58% vs. 12.5% p=0.045), but those who died had more matched related or unrelated transplants. Neuroblastoma was a more common primary diagnosis in survivors (48% vs. 0% p=0.014) although haematogenous conditions were more common in non-survivors (87.5% vs. 33% p=0.014). Survivors were more often admitted with sepsis (67% vs. 25% p=0.049), but renal failure on admission predicted death (37.5% vs. 3%

p=0.019).
Conclusions: Preadmission factors have a profound effect on PICU outcome in this group. This may arise from the underlying diagnosis that required non-autologous transplantation. This alone may be responsible for our findings. They also tell us more about risks and benefits of BMT, enabling clinicians and parents to make more informed decisions and researchers to better understand how to reduce mortality in oncology children.

42. Daniel Fowler

Mycobacteria activate γδ T-cell anti-tumour responses via cytokines from type 1 myeloid dendritic cells: a mechanism of action for cancer immunotherapy
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Attenuated and heat-killed mycobacteria display demonstrable activity against cancer in the clinic; however, the induced immune response is poorly characterised and potential biomarkers of response ill-defined. We investigated whether three mycobacterial preparations currently used in the clinic (BCG and heat-killed M.vaccae and M.obuense) can stimulate anti-tumour effector responses in human γδ T-cells. γδ T-cell responses were characterised by measuring cytokine production, expression of granzyme B and cytotoxicity against tumour target cells. Results show that γδ T-cells are activated by these mycobacterial preparations, as indicated by upregulation of activation marker expression and proliferation. Activated γδ T-cells display enhanced effector responses, as shown by upregulated granzyme B expression, production of the TH1 cytokines IFN-γ and TNF-α, and enhanced degranulation in response to susceptible and zoledronic acid-treated resistant tumour cells. Moreover, γδ T-cell activation is induced by IL-12, IL-1β and TNF-α from circulating type 1 myeloid dendritic cells (DCs), but not from type 2 myeloid DCs or plasmacytoid DCs. Taken together, we show that BCG, M.vaccae and M.obuense induce γδ T-cell anti-tumour effector responses indirectly via a specific subset of circulating DCs and suggest a mechanism for the potential immunotherapeutic effects of BCG, M.vaccae and M.obuense in cancer.

43. Rupsha Fraser

Profiling cytokine and angiogenic factor production by decidual natural killer cells from first trimester pregnancies at high or low risk of pre-eclampsia.
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Decidual natural killer (dNK) cells are key players in controlling the maternal-fetal interface. dNk protein secretions will regulate placentation and uterine adaption to pregnancy, with altered secretion likely to be involved in pre-eclampsia(PE) pathogenesis. Uterine artery Doppler ultrasound was used to identify women undergoing elective termination of pregnancy, who were at most and least risk of developing PE. dNK cells were isolated and culture supernatants collected over 24h and secreted proteins characterised from both risk-groups. differences between the two groups were detected in various factors, including angiogenin, endostatin, human growth factor (HGF), placental growth factor (PlGF) and the soluble interleukin-2 receptor (sIL-2R). Analysis of secreted factors by dNK cells will be informative for understanding the role of dNK cells in normal and pathogenic pregnancies.

44. James Garnett

The mucosal glucose concentration of polarised human airway epithelial cell monolayers affects luminal Staphylococcus aureus growth.

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Glucose in the airway surface liquid (ASL) is normally maintained at a low concentration compared to glucose levels in the blood (~12.5 times lower). In healthy humans, ASL glucose concentrations rise in response to increased blood glucose (1). Patients on intensive care with elevated ASL glucose are more likely to have respiratory infection, particularly with methicillin-resistant Staphylococcus aureus (MRSA; 2). The aim of this study was to investigate the relationship between mucosal glucose concentration, glucose in the ASL and bacterial growth using an in vitro model of human airway epithelium. H441 epithelial cells were grown on transwell inserts for 10 days, at air-liquid interface, to form polarised monolayers. Cells were washed and bathed in a HCO3--buffered Krebs solution to remove antibiotics and growth supplements which could effect bacterial growth. The glucose concentration of the mucosal Krebs solution was set at 10, 20 or 40mM. For ASL glucose analysis, cells were incubated for 1 hour with the different glucose concentrations. 150µl of solution containing 0.67µg ml-1 FITC-dextran was used to wash the surface of the transwells. Glucose concentration of the washes was quantified using a Annalox glucose analyser. ASL volume was calculated by quantifying dilution of the FITC dextran using a fluorescent spectrophotometer. For co-culture studies, Staph. aureus (8325-4) were grown overnight in RPMI medium to produce a culture of ~108 colony forming units (CFU) ml-1. 50µl of 107 CFU ml-1 in glucose-free RPMI was applied to the luminal surface of H441 monolayers. The H441-Staph. aureus co-culture was incubated for 7 hours at 37°C with 5% CO2. Transepithelial electrical resistance (TEER) of the monolayers was measured using a voltohmmeter (WPI). The co-culture was then scraped from the transwells and CFU's were determined using the Miles-Misra method. Calculated ASL glucose was less than mucosal glucose concentration but increased from 0.5±0.2mM to 1.5±0.2 and 6.5±1.3mM as basolateral glucose concentration was raised from 10mM to 20mM and 40mM, respectively ($P<0.05$; n=3-5). The TEER of H441 monolayers was significantly reduced following the addition of Staph. aureus (248±44 Ω.cm2), compared to control monolayers (498±104 Ω.cm2; $P<0.05$; n=5). Altering mucosal glucose had no effect on H441 TEER. Luminal Staph. aureus growth directly correlated with mucosal glucose concentration, increasing from 8.6±8.1x107 to 1.5±0.8x108 CFU ml-1 and 3.4±1.4x108 CFU ml-1 upon elevation of mucosal glucose from 10mM to 20mM and 40mM, respectively ($P<0.05$; n=5). These results indicate that raising mucosal glucose increases glucose in ASL and that Staph. aureus utilise this glucose to promote their growth. Moreover, by reducing tight junction integrity bacteria may also increase the paracellular diffusion of glucose into the ASL. 1. Baker EH et al. (2007) J Appl Physiol 102: 1969-1975. 2. Philips BJ et al. (2005) Thorax 60: 761-764.

45. Sabiha Gati

The Prevalence and Distribution of Left Ventricular Hypertrabeculation in Highly Trained Athletes

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Aim: Left ventricular hypertrabeculation (LV HTC) is a morphological hallmark of left ventricular non-compaction (LVNC). The prevalence of LV HTC in athletes is unknown.

The aim of the study was to investigate the prevalence and significance of LV HTC in highly trained athletes.
Methods and Results: Between 2003 and 2011, 1146 athletes, aged 14-35 years, underwent 12-lead ECG and echocardiography. Echocardiograms were analysed in accordance with ASE guidelines and hypetrabeculation was defined as > 3 localised protrusions of the ventricular wall ≥3mm in thickness associated with intertrabecular recesses. Results were compared with 415 healthy controls of similar age. Athletes displayed a higher prevalence of LV HTC compared with controls (18.3% vs 9.0%; p=<0.0001). Of the athletes, 10.1% fulfilled conventional criteria for LVNC. African/Afro-Caribbean (black) athletes exhibited a higher prevalence of LV HTC compared with Caucasians (28.8% vs. 16.3%; p=0.002). Left ventricular hypertrabeculation was associated with T-wave inversion and lower indices of systolic function, however, assessment with 48 hour ECG, exercise stress test and cardiac MRI failed to identify broader features of LVNC phenotype. Follow-up during the ensuing 48.6 ± 14.6 months did not reveal adverse events.
Conclusions: The high prevalence of LV HTC in athletes, particularly amongst black athletes, suggests that the morphological anomaly represents an ethnically determined physiological epiphenomenon secondary to increased cardiac preload and afterload. Associated marked repolarisation changes and lower LV fractional shortening cannot exclude a myocardial disorder in a small minority. Prolonged longitudinal follow up in a larger cohort of athletes should identify the precise significance of LV HTC.

46. Sabiha Gati

Left Ventricular Hypertrabeculation in Afro-Caribbean individuals: An Inherited Cardiomyopathy or a Physiological Response to Increased Cardiac Preload.
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Introduction: Studies in heart failure patients of Afro-Caribbean (black) origin reveal a high prevalence (up to 30%) of myocardial trabeculations and raise the potential diagnosis of isolated left ventricular non-compaction (ILVNC). It is unclear whether the myocardial morphology is representative of ILVNC or whether it represents an ethnicity related epiphenomenon to increased cardiac preload. Pregnancy is associated with a marked increase in physiological cardiac preload. This study sought to investigate the impact of increased cardiac preload on left ventricular morphology in previously healthy black and Caucasian pregnant women.
Method: Between 2008 and 2010, 106 consecutive normotensive and previously healthy pregnant females (90% Caucasian) underwent cardiac echocardiography in the third trimester. Echocardiograms were analysed for trabeculations defined as localised protrusions of the ventricular wall ≥3mm in thickness associated with intertrabecuar recesses filled with blood from the left ventricular cavity as assessed by colour Doppler. The results were compared with 80 healthy non-pregnant females (51% Caucasian) of similar age.
Results: Pregnant black females demonstrated a higher prevalence of left ventricular hypertrabeculation (LV HTC) compared with pregnant Caucasian females (n=5; 45.5% vs n=12; 12.6%; p 0.014). Pregnant black females were 34% more likely to have LV HTC compared with pregnant Caucasian females. In contrast none of the non-pregnant females of either ethnicity exhibited any evidence of hypertrabeculaton. None of the black or Caucasian pregnant females with LV HTC showed objective features of left ventricular systolic or diastolic dysfunction. The mean EF by Simpsons method was 56.8 ± 12% v 58 ± 9.8%; p=0.792. The E/A ratio was 1.47 ± 0.33 v 1.30 ± 0.44; p: 0.218 and isovolumic relaxation

time was 81.2 ± 1.89ms v 80.6 ± 15.46ms; p: 0.6.
Conclusion: Black pregnant females exhibited a significantly higher frequency of LV HTC compared with pregnant Caucasian females with similar blood pressure in the absence objective markers of abnormal systolic or diastolic function. Based on the results of this study, it is highly likely that increased cardiac preload in heart failure is associated with an ethnically mediated myocardial response comprising of an increased number of myocardial trabeculations in black individuals and should be regarded as an epiphenomenon rather than ILVNCC outside the context of familial heart failure. The hypothesis requires prosposective longitudinal evaluation.

47. Saqib Ghani

Pre-participation cardiovascular screening in school student athletes: feasibility of large-scale school screening program in United Kingdom
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Purpose: Pre-participation cardiovascular screening (PPS) has been shown to reduce the incidence of sudden death by early identification of cardiomyopathies and heart rhythm disorders. Feasibility of large-scale PPS programs by adopting the European screening model in British schools has not yet been evaluated.
Methods: 1,475 school students aged ≥12 years underwent PPS using health questionnaire, physical examination and 12-lead ECG between February-October 2011. The ECG were analysed using the European Society of Cardiology recommendations for ECG interpretation in athletes. Persistent juvenile pattern (JP) was defined as inverted, biphasic or notched T-wave in leads V1-V3 in individuals aged ≤ 16yrs. A transthoracic echocardiogram (TTE) was performed if indicated, and referral for further investigations recommended as appropriate.
Results: Of the 1,475 participants (aged 15.8 ± 2.3yrs), 81% were males; there were 87% Caucasian, 6.5% South Asian, and 1.5% Afro-Caribbean; 73% participated in sports on average 6.9 hrs/wk. Persistent JP was observed in 83 (5.6%) school students (7.9% of cohort aged≤16yrs) with no statistically significant gender difference. After ECG and health questionnaire, TTE was performed in 117 (7.9%); 5.6% due to ECG, 2.3% based on questionnaire. The ECG changes warranting TTE included left atrial dilatation (2.5%), left/right axis deviation (1.7%), right bundle branch block (0.3%), right ventricular hypertrophy (0.3%), ventricular extra-systole (0.2%), and T-wave inversion (1.4%). ECG revealed 3 cases of Wolff-Parkinson-White and 1 case of prolonged QT interval. Transthoracic echocardiogram identified 1 student with bicuspid aortic valve, 1 with quadricuspid aortic valve, and 1 with coarctation of aorta. This reflects a diagnostic yield of 0.45%. After TTE, 79 students were cleared; false-positive rate was 5.3% (3.3% due to ECG, 2% due to questionnaire). After ECG and TTE, 38 (2.6%) students were referred for further diagnostic investigations or surveillance; to-date no significant abnormalities have been identified in this group.
Conclusion: Pre-participation cardiovascular screening in school students, when conducted in an expert setting, results in a relatively small false-positive rate. Close monitoring of persistent juvenile pattern, in isolation in asymptomatic individuals, can reduce the need for further investigations in first instance. Implementation of such school screening programs may be feasible.

48. Saqib Ghani

Prevalence and morphological characterization of early repolarization patterns in young healthy individuals: impact of gender, ethnicity and physical activity

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Purpose: Early repolarization (ER) is commonly observed in athletes and young healthy individuals. Recently, ER in the inferior and lateral leads has been associated with sudden cardiac arrest from idiopathic ventricular fibrillation. We studied the prevalence, distribution and morphology of ER patterns in inferior and lateral leads in young healthy individuals.

Methods: 12-leads electrocardiogram (ECG) was performed at rest in 1237 young healthy individuals (age range 13-38 years) between February and September 2011. We evaluated the impact of gender, ethnicity and physical activity on ER. Individuals were divided into physically-active (exercise >2 hours/week) and sedentary. Early repolarization was defined as notched or slurred J-point elevation of at least 0.1mV from baseline, in ≥2 contiguous inferior or lateral leads; anterior ER patterns were not considered in this study. The morphology of ST-segment was classified as horizontal/descending or rapidly ascending/up sloping.

Results: The mean age of participants was 18.2 ± 4.3 years, of which 979 (79%) were male, 981 (79%) were physically active and 91% were Caucasians. ER pattern was present in a total of 232 (18.7%) cases; of these 42% were in the inferior leads, 31% in lateral leads and 27% in both. Notched ER was more prevalent (64% inferior, 83% lateral, 76% infero-lateral) compared to slurred morphology, and more commonly associated with ascending/upsloping ST-segment elevation. ER was significantly more prevalent in males compared to females (20% vs 12%, p=0.003), in physically-active people compared to sedentary (20% vs. 13%, p=0.0194), and in Afro-Caribbeans compared to Caucasians (48% vs. 17%, p=0.0001). In addition, voltage criteria for left ventricular hypertrophy and sinus bradycardia were a common associated finding in individuals with ER pattern compared with those without (p=0.0001 and 0.002 respectively). Only 5% of individuals with ER had J-point elevation of >0.2mV.

Conclusion: Early repolarization is a common finding in young healthy individuals, and is more prevalent in males, physically-active individuals and those with Afro-Caribbean ethnicity. Notched ER with ascending ST-segment elevation in inferior leads was the most commonly observed morphological pattern. More research is required to understand precise long term implications of such repolarization changes in young individuals.

49. Saqib Ghani

Cardiovascular abnormalities in the potential British Olympic squad: impact of IOC recommendations for cardiovascular evaluation prior to 2012 Olympics

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Purpose: Pre-participation cardiovascular screening (PPS) has been associated with a reduction in the incidence of sudden cardiac death through identification of cardiomyopathies and heart rhythm disorders. Whereas cardiac and sporting bodies advocate PPS in athletes, there is debate over the most effective method.

Methods: We aimed to demonstrate the efficacy of PPS using 12-lead ECG in elite athletes, mostly potential participants of 2012 Olympics. Transthoracic echocardiogram (TTE) was performed in all athletes in addition to ECG. As per European Society of Cardiology (ESC)

recommendations, ECG changes were classified as training-related (Group 1) or training-unrelated (Group 2).
Results: Between 2007 and 2011, 1000 competitive athletes competing in 30 sporting disciplines (mean age 21.2 ± 5.8 years; BSA 1.86±0.23m2) underwent PPS with health questionnaire, ECG and TTE. Of these, 52% were males, and 88% were Caucasians. In 10% athletes, Group-2 ECG changes were seen in isolation or in combination. In total, 14 athletes were identified with cardiac abnormality; ECG revealed 1 athlete with Wolff-Parkinson-White syndrome, and 2 with prolonged QT-interval; TTE identified 1 athlete with dilated aortic root and aortic incompetence (returned to sport following cardiac surgery), 5 with bicuspid aortic valve, 4 with mitral valve prolapse, and 1 with pulmonary stenosis. Minor valve abnormalities included 7 mild aortic regurgitation and 1 mild-moderate mitral regurgitation. The mean LV wall thickness, LV mass and cavity dimensions were significantly greater in males compared to females (9.78mm vs. 8.52mm, p=0.0001; 230.9gm vs. 161.5gm, p=0.0001; 52.9mm vs. 48.4mm, p=0.0001 respectively). After ECG and TTE, 5.7% athletes were referred for further investigations (24-hour ECG, exercise stress test, cardiac MRI) or surveillance studies. Further evaluation did not demonstrate any significant abnormality. The false positive rate for ECG was 7.2% using ESC criteria; false negative rate for ECG was 1% and entirely due to valvular heart disease.
Conclusion: Pre-participation cardiovascular screening with ECG results in a relatively small number of athletes requiring further investigations. Echocardiography can identify structural abnormalities not detected by ECG. The prevalence of cardiac abnormalities in young athletes is low; however early identification can lead to effective treatment, and appropriate follow-up.

50. Katherine Gould

Unidentified parasites in a clinical specimen? Pathogen discovery by next generation sequencing to inform diagnosis and treatment.
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An elderly patient receiving immunosuppressive treatment was originally admitted to hospital with confusion and fevers following a period on feeling unwell. The patient was transferred to St George's with a clinical diagnosis of encephalitis. Blood cultures at the transferring hospital indicated *Listeria monocytogenes* infection. Subsequent CSF microscopy following lumbar puncture revealed numerous organisms that were morphologically suggestive of *Toxoplasma gondii* tachyzoites. The CSF was PCR positive for *L. monocytogenes* but negative for *T. gondii* and the morphologically similar protozoan *Neospora caninum*; furthermore the serology was negative for toxoplasma. The patient was treated for *L. monocytogenes* infection and presumed toxoplasma encephalitis. Repeat CSF samples demonstrated complete clearance of the as yet unidentified organism. Protozoa such as *T. gondii* and *N. caninum* are cyst forming, requiring prolonged suppressive treatment in immunocompromised patients to prevent relapse. Therefore, there was a clear clinical need to identify the parasite and confirm a positive diagnosis to inform future therapy.
In an effort to achieve parasite identification, next generation sequencing was employed in a pathogen discovery approach. This provided a comprehensive and unbiased analysis that

required no prior knowledge. DNA was purified from the first CSF sample, subjected to whole genome amplification and sequenced using the Ion Torrent Personal Genome Machine. A single sequencing run generated a total of 121 megabases of sequence, comprising of around one million DNA sequence reads of ~100bp length. Human DNA reads (~95%) were filtered from the data and discarded. The remaining reads (~50,000) were subjected to a similarity search of public databases that revealed a small proportion of these sequences had positive matches to *L. monocytogenes*. This result was consistent with previous findings from blood culture and PCR, confirming the validity of the pathogen discovery approach. However, this search did not reveal any positive matches to the sequenced *T. gondii* genome. Therefore, a large number of reads remained for which there were no matching sequences in the databases. This result may be expected if the unidentified parasite lacked any significant sequence similarity to the limited range of parasite genomes currently deposited. Ongoing directed searches continue using alternative sources of sequence data with the aim of achieving a positive identification

This case study demonstrates real-time application of next generation sequencing technology to an ongoing clinical investigation. The pathogen discovery approach provides a further tool to aid diagnosis and help inform patient care in cases where clinical questions remain following routine investigations.

51. Robert Grant

Composite performance indicators: bringing uncertainty out into the open

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Background: Despite a sceptical public and unresolved academic debate, interest in rating and ranking public service providers continues apace. In healthcare, the UK coalition government has shifted emphasis from process to outcome but familiar methodological problems remain, typified by the disparity in ratings given to Mid-Staffordshire NHS Foundation Trust by the Care Quality Commission and Dr Foster. The problems inherent in composite performance indicators have not been resolved since a report for the Royal Statistical Society warned about subjectivity and uncertainty in their composition. The extent of uncertainty needs to be openly discussed during construction; it arises from sampling error as well as the range of possible formulas to create the composite.

Methods: We illustrate graphical methods for exploring alternative formulas for a composite performance indicator and displaying the uncertainty. Anonymous data on 26 dichotomous measures of the quality of care received in 203 NHS hospitals by 10,617 people admitted following a stroke were supplied by the Royal College of Physicians of London from the national clinical audit of stroke 2008. A composite that described as much inter-hospital variance as possible was formed by a principal components analysis. This was compared with a version that adjusts for some patient covariates, and an existing composite formed by expert opinion. Simple graphs display the extent of uncertainty arising from: sampling of patients, choice of weights, order of combining patient and indicators, and covariate adjustment.

Conclusion: We contend that well-constructed and communicated composite indicators are a positive contribution in making official statistics accessible; done badly they can obscure or misrepresent the facts. These analyses show how it is possible to achieve an open representation of uncertainty and that graphs can aid discussion without requiring statistical expertise. Such presentations may help public discourse about quality of care move beyond the ubiquitous league table.

52. Andrew Gravett

The immune visibility of tumour cells can be altered upon in vitro culture with chemotherapy drugs

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In addition to the tumour cell ablating properties of chemotherapies, some have also shown remarkable capacity to modulate the immune system. Gemcitabine (GEM), is one such chemotherapy. It has been shown to affect numbers of regulatory T-cells and myeloid derived suppressor cells in human and in mouse and has shown promising synergy with dendritic cell vaccination. Initial studies from our group suggest that GEM can influence expression of cell surface molecules important for the efficient surveillance and effector function of the immune system. We have shown that GEM can increase the expression of human leukocyte antigen (HLA) class I, Leukocyte immunoglobulin-like receptor subfamily B1 and CD95 on a number of tumour cell lines from different origins in short term in vitro culture. This effect occurs on cells surviving GEM treatment. Culturing tumour cells with GEM increased beta-2-microglobulin expression but did not alter cellular HLA heavy chain concentration as assessed by Western blot. In addition to the increase of surface HLA class I and presumed subsequent quantitative increase in antigen presentation, preliminary data suggest that qualitative changes may also occur. Specifically, components of the antigen presentation machinery are altered, indicating that neo- or cryptic-epitopes may be generated and the immune response to tumour strengthened. This is yet to be investigated fully but current data may explain effects seen in previous in vivo studies and lends further credence to the idea that chemotherapy and immunotherapy should be used in combination.

53. Ruth Habibi

An Evaluation of South Thames Crossroads' Carer Peer Mentoring Service

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South Thames Crossroads' peer mentoring support service provides support for carers looking after family members suffering from a range of long-term, often aged-related conditions including dementia, stroke and terminal illnesses. Volunteer mentors give one-to-one support to carers (mentees). Qualitative methods are being used to explore mentees' and mentors' experiences of the service and their perceptions of the impact of the service. To date six volunteer mentors have taken part in a focus group and four carers have participated in semi-structured interviews. Transcripts are analysed thematically. Emerging themes identified from transcripts include: the challenges of being a carer; varying perceptions of what the role of a mentor should be; the benefits for carers including e.g. value of the service in reducing isolation and in giving carers someone outside their family to talk to. Although these are only preliminary findings, they suggest that experiences of mentoring differ between individual mentor-mentee pairs and that perceived benefits also vary. Identifying any impact of mentoring services using quantitative methods may therefore prove very difficult.

54. Hannah Harris

Predicting outcome in paediatric bone marrow transplant patients admitted to PICU

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Background and Aims: Patients surviving PICU have correctable organ dysfunction. We investigated if organ dysfunction changes after PICU admission predict outcome in paediatric bone marrow transplant (BMT) patients.
Methods: An organ dysfunction score (ODS)1 collated dysfunctional (1) and failed (2) to give an aggregate score for 5 organs from 0-10. Cross-reference of local PICU and BMT databases identified 54 patient episodes 2004-2009, representing 41 patients. ODS was calculated from notes on days 1, 3 and 5.
Results: Mean age was 7.67 (0.4) years, with 27% matched unrelated donor, 15% matched related donor, and 56% autologous transplants. Of the 54 episodes, 80% survived to PICU discharge. Mean length of stay was 4.5 (range 1-26) and 23.25 days (range 1-46) for survivors and non-survivors respectively. 46 were discharged, but 13 readmitted. Eventual death occurred in 20, with 8 deaths on PICU, 12 after discharge. For the last episode the mean admission ODS was 3.9 and 5.0 in survivors and non-survivors respectively (p=0.02). The pattern of change in ODS over the first days was examined. All (N=14) improving at D3 survived, as did 7/8 who had not worsened by D3. Of those worse at D3 and D5, 8/14 and 5/10 still survived PICU. Of all organ failures, renal was most predictive of death.
Conclusions: This BMT PICU admission series shows that rapid improvement in organ dysfunction predicts survival. However those who deteriorate over the first 3 or 5 days are still as likely to survive as not. 1 Wilkinson CCM, 14(4):271-274

55. Shahnaz Hassan

Effect of the anti-epileptic drug retigabine, on anoxia-evoked dopamine efflux in the caudate nucleus

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Retigabine (ezogabine, D-23129, Trobalt, Potiga) is an anticonvulsant, developed by Valeant Pharmaceuticals and GlaxoSmithKline, as a treatment for partial epilepsies. It was approved by the EMA and FDA this year. Retigabine activates potassium channels, specifically Kv7 (KCNQ). In preclinical tests Retigabine suppresses seizures induced by electroshock and chemicals. Here we tested Retigabine in an in vitro model of ischaemia, where anoxic depolarization evokes massive amounts of dopamine (DA) efflux. We hypothesized that a drug which dampens down nerve activity might delay the onset of anoxic depolarization and consequently may be of use in stroke. Male Wistars (9 weeks), were killed by cervical dislocation and coronal striatal slices (400µm) were cut. Following equilibration (21ºC), slices were transferred to the slice chamber and perfused continuously with aCSF (33ºC; 100ml/h). Following 35min equilibration, the perfusion medium was switched to either a DMSO control or Retigabine (1µM) then at 45min the solution was switched to ischaemic aCSF, containing either 0 or 1µM retigabine. DA levels in the dorsolateral caudate were measured by fast cyclic voltammetry at carbon fibre microelectrodes. Voltammetric scans (-1.0 to +1.4V vs. Ag/AgCl, 480V/s) were performed at a frequency of 1 Hz. An increase in the current signal at +600mV, together with a corresponding reduction peak at -200mV, were characteristic of DA detection in the caudate. Perfusion with ischaemic aCSF typically evoked a large increase in DA from the slice and four aspects of the DA release were measured: (1) time to onset of DA release from the initiation of ischaemia (T-on); (2) time taken to reach maximum DA release after the onset of release (T-peak); (3) maximum extracellular DA concentration (peak-DA); and (4) mean rate of DA release ($\delta DA/\delta T$). We found Retigabine to increase T-on i.e. the slice appeared tolerant to the effects of oxygen and glucose deprivation. T-peak increased while peak DA and $\delta DA/\delta t$ both decreased. These changes can all be considered neuroprotective because large extra-vesicular concentrations of

dopamine are neurotoxic. These data suggest that retigabine could be useful as a prophylactic pharmacotherapy in stroke

56. Lynsey Hawker

Evolving mobility at St George's Library - An adaptive strategy to supporting user demand for health information on the move.

Lynsey Hawker, Verity Allison
Library, Information Services, St George's University of London

Our poster presents current projects being undertaken by the library to explore, develop and support emerging mobile technologies in the field of healthcare. The rise in mobile websites, apps and indeed in devices that support them opens up a number of opportunities and challenges for accessing information and research. As Librarians we need to find ways of promoting and supporting our users in using these new tools to further enable research but ensure, at the same time that these new and evolving ways of accessing information continue to allow researchers access to high quality literature.

57. Vanessa Ho

Actions Of Calcium-sensing Receptor Ligands In Mesenteric, Femoral and Pulmonary Arteries From The Rat

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The cell surface, extracellular calcium-sensing receptor (CaR), which is activated by Ca2+ ions, has been reported in the endothelium, smooth muscle and perivascular sensory nerves, and thus could contribute to vascular control. Relaxation elicited by increasing [Ca2+]o (from 1 to 5mM) is thought to be mediated by CaR in perivascular sensory nerves expressing Transient Receptor Potential Vanilloid type1 receptors (TRPV1). Our recent study (Thakore & Ho, 2011) confirmed that mesenteric relaxation to Ca2+ is sensitive to the negative allosteric modulator of CaR, calhex231 and functional desensitization of sensory nerves by capsaicin (a potent TRPV1 agonist). However, we also reported that Ca2+ and positive allosteric modulators of CaR (calcimimetics) could differ in their mechanisms of action, including the involvement of CaR (Thakore & Ho, 2011). Here, we further investigated the relaxant effects of [Ca2+]o and the two calcimimetics, calindol and cinacalcet in different vascular regions. Small mesenteric, intrapulmonary and femoral arteries from male Wistar rats (250-350g) were mounted in a wire-myograph for tension recording. In mesenteric arteries bathed in physiological solution containing 1mM Ca2+, CaCl2 (1.5-5mM) induced relaxation that was inhibited by 3µM calhex231, confirming our previous data. Ruthenium red (10µM), which is a non-selective TRPV blocker, also reduced the maximal relaxation to Ca2+. By comparison, smaller Ca2+-induced relaxations and a smaller calhex231-sensitive component were seen in femoral arteries. Interestingly, femoral relaxation to Ca2+ was attenuated by ruthenium red but potentiated by capsaicin. On the other hand, in intrapulmonary artery, increasing [Ca2+]o predominantly resulted in small contraction, which was enhanced by calhex231, perhaps due to an underlying, CaR-mediated relaxation. However, capsaicin treatment had no effect. The relaxant effects of Ca2+ were mirrored by those of calcimimetics in the different vascular regions; with rank order of potency and efficacy, mesenteric>femoral>>pulmonary artery. In fact, in intrapulmonary artery, calindol caused contraction, followed by relaxation at higher contractions. To conclude, the vascular action of Ca2+ and calcimimetics differ greatly depending on the vascular regions. Our data also question the role of CaR and capsaicin-sensitive nerves in Ca2+-induced relaxation in

regions beyond the mesenteric circulation. Thakore P & Ho WS (2011). Br J Pharmacol 162: 749

58. Vanessa Ho

Age-related Changes In The Vascular Action Of The Endocannabinoid, 2-AG
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The endocannabinoid, 2-arachidonoylglycerol (2-AG) is known to modulate vascular tone and blood pressure. However, there are inconsistent and conflicting data regarding its action as a vasodilator. A large variation in its potency and efficacy has been reported. This might, at least partly, be explained by the susceptibility of 2-AG to be hydrolysed into arachidonic acid in the vascular wall (Ho & Randall, 2007). In small mesenteric arteries, we found that inhibitors of monoglycerol lipases and cyclooxygenase potentiate vasorelaxation to 2-AG, indicating that local catabolism inactivates 2-AG (Ho & Randall, 2007). Here, we hypothesize that another contributing factor is the age of the laboratory animals used. We therefore examined the vasorelaxant effects of 2-AG in isolated small mesenteric artery obtained from younger (12-16 weeks) and older (25-28 weeks) Wistar rats (all male). In younger rats, relaxation to 2 AG was attenuated by endothelium removal. 2-AG responses were much smaller and independent of the endothelium in older rats. Interestingly, in older rats, the presence of the cyclooxygenase inhibitor, indomethacin partially restored the 2-AG relaxation. Inhibition of monoglycerol lipases with methyl arachidonoyl flurophosphonate (MAFP) caused a slightly greater potentiation. The resultant responses were similar to those obtained in the presence of both indomethacin and MAFP, suggesting that the lipases and cycloogenases share a common pathway. In endothelium-denuded vessels from older rats, the combined treatment of MAFP and indomethacin similarly potentiated 2-AG relaxation. In fact, these potentiated responses to 2-AG (by MAFP and indomethacin) were more potent than the control relaxation obtained in younger rats, especially in the absence of the endothelium. On the other hand, in older rats, relaxation to noladin ether, the metabolically stable analogue of 2 AG, was not affected by MAFP and indomethacin. To conclude, we propose that the vasorelaxant property of 2-AG is greatly reduced by aging, probably due to enhanced catabolism in the vascular smooth muscle. 2-AG is rapidly hydrolysed by MAFP-sensitive lipases to arachidonic acid, which is in turn metabolised by cyclooxygenase. This probably results in vasocontractile prostanoids, which are known to be prevalent in aging arteries. Ho WS & Randall MD (2007). Br J Pharmacol 150: 662

59. Louise Hogan

Characterisation of bovine leukocyte Ig-like receptors
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Leukocyte Immunoglobulin-like receptors (LILR), are innate immune receptors involved in regulating both innate and adaptive immune functions. LILR show more interspecies conservation than the closely related Killer Ig-like receptors, and homologues have been identified in rodents, primates, seals and chickens. The murine equivalents, paired Ig-like receptors (PIR), contain two additional immunoglobulin domains, but show strong sequence

and functional similarities to human LILR. The bovine genome was recently sequenced, with preliminary annotations indicating that LILR were present in this species. We therefore sought to identify and characterize novel LILR within the Bos taurus genome, compare these phylogenetically with LILR from other species and determine whether they were expressed in vivo. Twenty six potential bovine LILR were initially identified using BLAST and BLAT software. Phylogenetic analysis using MEGA5 software, indicated that 16 of these represent novel bovine LILR. Protein structures defined using protein BLAST predict that the bovine LILR family comprises seven putative inhibitory, four activating and five soluble receptors. Preliminary expression analysis was performed by mapping the predicted sequences with raw data from total transcript sequence generated using Genome Analyzer IIx (Illumina) to provide evidence that all 16 of these receptors are expressed in vivo. The bovine receptor family appears to contain receptors which resemble the six domain rodent PIR as well as the four domain LILR found in other species indicating co-evolution of both receptor types within the same species.

60. Mehnaz Hossain

Markers of telomeric crisis and immortalization in melanoma progression.

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Background and objectives: Abnormal mitoses are characteristic of cancer cells. A common cause of these appears to be telomeric crisis. Crisis occurs in cells that have bypassed cellular senescence (irreversible cell cycle arrest after extended division, mediated by p16 and p53 pathways), and proliferated further. This leads to very short, dysfunctional telomeres, which can be ligated giving dicentric chromosomes. Consequences include large-scale chromosomal rearrangements, anaphase bridges, tripolar and other abnormal mitoses, and apoptosis. Rare cells may overcome crisis and become immortal by re-expressing TERT, a subunit of telomerase, required to maintain telomeres. Primary melanomas appear to have evaded senescence but possibly not crisis, as they often fail to yield immortal cells when explanted. Here we aimed to test whether and when crisis occurs in melanoma progression, and whether immortalization is associated with metastasis.

Methods: Routine paraffin sections of archival lesions from St. George's Healthcare Trust were used, including approximately 20 each of radial growth phase (RGP), vertical growth phase (VGP), and metastatic melanomas (from lymph nodes and skin). Haematoxylin and eosin-stained sections were viewed for scoring of abnormal mitoses, with emphasis on anaphase bridges (relatively specific for crisis). Also noted were giant and multinucleate cells, and later nuclear blebs and chromatin bridges between nuclei. Currently, unstained sections are being immunostained for TERT, and for additional crisis markers.

Results: RGP melanomas showed very few mitoses, with no anaphase bridges, but some multinucleate cells and chromatin bridges. One abnormal mitosis only was observed in one RGP melanoma. In VGP melanomas, multinucleate cells, tripolar mitoses, nuclear blebs and chromatin bridges all appeared common, and anaphase bridges were seen in up to 40% of anaphases. Surprisingly, similar features were seen in most metastatic lesions, with similar percentages of anaphase bridges.

Conclusions: Some RGP melanomas may be entering crisis. VGP melanomas have extensive features of crisis, consistent with the rarity of immortality in culture. Even some metastases show features of crisis, suggesting that melanoma cells may metastasize before full immortalization. Marker expression data should clarify these findings.

61. Amanda Host

Placental expression and secretion of cytokines and angiogenic factors in first trimester pregnancies at increased risk of pre-eclampsia

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Objectives: This study aimed to profile cytokines and angiogenic factors produced by placental villous tissue from first trimester pregnancies characterised by their risk of developing pre-eclampsia (PE) if the pregnancy had progressed.

Methods: Uterine artery Doppler ultrasound scans were carried out on women attending clinic for elective surgical termination of pregnancy. Pregnancies were defined as those at most risk of PE (>25%) or at least risk of PE (<1%) based on the mean uterine artery resistance index and the presence or absence of bilateral notching. Placental tissue was either immediately snap-frozen and homogenised or cultured for 24 hours after which the medium was replaced and cultured for a further 48 hours and collected. Levels of angiopoietin-1 (Ang-1), angiopoietin-2 (Ang-2), activin A, vascular endothelial growth factor (VEGF), interleukin-8 (IL-8) and interleukin-6 (IL-6) were quantified by ELISA or by multi-analyte profiling (Luminex).

Results: Activin A, Ang-1, IL-8 and IL-6 secretion did not differ between placental villous culture supernatants from low risk versus high risk placentae. Secretion of Ang-2 and VEGF from high risk placentae was significantly greater than low risk placentae; however, no difference in Ang-2 or VEGF expression was observed in tissue lysates. When samples were analysed by gestational age, there was a significant decrease in Activin A and Ang-2 secretion from low risk but not high risk placentae over time. In contrast, there was a significant increase in IL-8 secretion from high risk but not low risk placentae.

Conclusion: This study demonstrated differences in the secretion of angiogenic factors between tissue obtained from first trimester pregnancies classified as at least or at most risk of developing PE. Differences in placental secretory characteristics between the two groups may contribute to the imbalance of angiogenic factors found in maternal serum of pre-eclamptic pregnancies.

62. Patrick Houston

Design and validation of novel mini-microarray for large scale Staphylococcus aureus population analysis.

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Design and validation of novel mini-microarray for large scale Staphylococcus aureus population analysis. The ability of Staphylococcus aureus to transfer virulence and resistance determinants via horizontal transfer of mobile genetic elements (MGE) is an important factor in the evolution of successful lineages. Studying the genetic barriers which control how strains are becoming increasingly virulent and resistant to antibiotics is essential to understanding how S. aureus populations are evolving. Comparative genomics using complex whole genome microarrays have previously been used to investigate the distribution of MGEs and candidate genes controlling their transfer within S. aureus populations. Due to the relative high cost of this method it is unsuitable for large scale population studies. Therefore, in the present study we describe the design, optimisation and validation of a high-throughput, low cost, precipitate reaction based (biotin) mini-microarray system (Alere, Germany). The custom array design comprises 204 probes (~30 mer) specifically designed to investigate the distribution of MGEs such as bacteriophage, pathogenicity islands, plasmids, transposons and staphylococcal cassette chromosomes. Moreover, probes targeting genes

which likely block or permit MGE transfer, hsdS variants for lineage determination and species-specific controls were included. Validation of custom primer-probe combinations using eight sequenced S. aureus strains gave clear positive signals for corresponding target sequences. This method was subsequently used to conduct large scale population analysis on methicillin-resistant Staphylococcal aureus (MRSA) isolates collected in St. George's Hospital, London between 1999 and 2009. The distribution of MGEs, factors which may block or facilitate horizontal transfer, and their influence on evolution of successful or dominant lineages over time will be discussed.

63. Christopher Howe

Energy expenditure estimation during walking in overweight and obese adults from the ActiTrainer accelerometer and heart rate

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Background: Gold standard methods of measuring energy expenditure (EE) are impractical and expensive (Brouchard et al., 1983). Conversely, several studies have shown that accelerometers can provide good estimates of EE in healthy (Fudge et al., 2007) and overweight individuals (Jacobi et al., 2006). The accuracy of EE prediction can be enhanced using a combination of heart rate (HR) and accelerometer counts (AC) (Fudge et al., 2007).
Purpose: To determine the accuracy of the ActiTrainer accelerometer (alone and in combination with HR) to predict EE in overweight and obese adults during walking exercise.
Methods: Ten overweight and obese adults (mean ± sd body mass index 30.0 ±4.5 kg/m2, age 38 ±16 years, n = 7 males and 3 females) completed a continuous incremental walk on a treadmill starting at 4 km•h-1 increasing by 0.5 km•h-1 every 5 min for a total of 30 min. Oxygen uptake and carbon dioxide production were measured throughout indirect calorimetry and actual EE during the final minute of each stage was calculated using the Weir equation (Weir. 1949). AC and HR were continuously measured using an ActiTrainer accelerometer. EE was predicted from AC using the Freedson equation (Freedson et al., 1998) and the values compared to the output from the indirect calorimeter. Novel prediction equations were generated by simple and multiple linear regression using AC and a combination of AC and HR.
Results: Estimated EE from AC using the Freedson equation was higher than EE measured by approximately 3Kcal•min-1 for all walking speeds ($P < 0.01$). There was no difference between EE estimated using the prediction equation derived from AC in the current study and EE measured using indirect calorimetry ($P = 0.67$). However, there was a tendency for combined AC and HR data to over predict ($P = 0.06$) EE compared to the indirect calorimetry method.
Conclusion: Data from the current study suggests that AC from the ActiTrainer accelerometer can be used to predict mean group EE during walking exercise in overweight and obese adults. Further research is required to investigate the reliability of these equations to predict EE during walking exercise in the field.

64. Franklyn Howe

Assessment of brain tissue involvement in Systemic Lupus Erythematosus from correlative analysis of ^{1}H Magnetic Resonance Spectroscopy and Diffusion Tensor Imaging

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Systemic Lupus Erythematosus (SLE) is an autoimmune disease that frequently produces neuropsychiatric symptoms; however the clinical symptoms are diverse, and it is hard to quantify cerebral involvement to monitor disease progression and treatment response. The aim of this study was to investigate the metabolic and structural changes in the brain of a cohort of SLE patients using ^{1}H Magnetic Resonance Spectroscopy (MRS) and Diffusion Tensor Imaging (DTI). Metabolite levels were quantified by ^{1}H MRS in parietal and frontal brain regions of lupus patients and correlated with fractional anisotropy (FA) and median diffusivity determined by Diffusion Tensor Imaging in the same voxels. Partial linear regression controlling for the grey-white matter fraction in each voxel to correlate metabolites with diffusion parameters was performed with SPSS. The frontal voxel showed the expected positive correlation of FA with NAA ($r = 0.359$, $p = 0.017$, one-tailed) and most likely an irreversible neurodegenerative process. In addition we observed a correlation between ESR and mI in the parietal voxel ($r = 0.523$, $p = 0.019$, one-tailed) and here were highly significant correlations between frontal and parietal tCho ($r = 0.677$, $p < 0.001$) and mI ($r = 0.51$, $p = 0.0010$) but not for NAA and tCr. mI is consider to have a role in osmoregulation and so may directly represent cerebral inflammation associated with increased ESR. Hence the striking correlation between tCho and mI in frontal and parietal regions may be derived from variability in sub-clinical disease-flares across all patients. Longitudinal studies combining multi-voxel MRS with tract-based analysis of DTI may help characterize the heterogeneity of the cerebral disease process and sequence of events that lead to irreversible neuronal damage and so allow improved clinical management.

65. Yanmin Hu

Antibiotic discovery-New method which focuses on dormant bacteria

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New classes of antibiotics are needed. Between 1940 and 1962 more than 20 new classes of antibiotics were marketed. Since then, only two have reached the market. All current classes have been discovered by the Fleming method which targets log phase bacteria. These compounds are powerful antibacterial agents against multiplying bacteria, but are relatively weak against non-multiplying or dormant bacteria. Both multiplying and non-multiplying bacteria, and indeed spores in some species of bacteria are found side by side in human infections. The presence of non-multiplying bacteria and spores increases the period of chemotherapy required for a cure, and this increases the emergence of resistance. Unfortunately, when the proportion of resistant strains reaches an unacceptably high level, the antibiotic becomes redundant. Some infections contain particularly troublesome non-multiplying bacteria. The classical example is tuberculosis which requires 6 months of chemotherapy to attain cure. Other infections such as Staphylocuccus aureus and E. coli also have remarkably antibiotic tolerant slowly or non-multiplying subpopulations of bacteria and are associated with enhanced mortality.

Methods: By targeting non-multiplying bacteria throughout the discovery process, A small chemical compound was identified with potent activity against non-multiplying Staphylococcus aureus and E. coli.
Results: Numerous hits were obtained, of which one, called HT61, is quinolone-derived with a molecular mass of about 400 Daltons. It is active against non-multiplying bacteria, including methicillin sensitive and resistant as well as Panton-Valentine leukocidin-carrying Staphylococcus aureus. It also kills mupirocin resistant MRSA. In addition, it is active against Bacillus cereus spores. The mechanism of action of the drug is depolarisation of the

cell membrane and destruction of the cell wall. The speed of kill is within two hours. In comparison to conventional antibiotics, HT61 kills non-multiplying cells more effectively, 6 logs versus less than one log for major marketed antibiotics. HT61 kills methicillin sensitive and resistant S. aureus in the murine skin bacterial colonization and infection models. No resistant phenotype was produced during 50 serial cultures over a one year period. The antibiotic caused no adverse affects after application to the skin of minipigs. It is now in clinical trials.
Conclusion This is a new concept for antibiotic discovery. By targeting non-multiplying bacteria throughout the discovery process, many new classes of antibiotics have been identified, of which one has been developed through preclinical and is now in clinical trials. This new method has the potential to generate many new classes of antibiotic which are active against non-multiplying bacteria and spores. They may be able to reduce the rate of emergence of resistance, shorten the duration of therapy, and reduce relapse rates.

66. Jorg Huber

Does 'morningness' correlate with breakfast eating frequency in a national UK sample?
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Introduction: 'Morningness' has been found to be related to mental and physical health, to career success and breakfast behaviour. As part of an earlier experimental study we observed a moderate correlation between morningness and breakfast eating frequency, indicating that those who are morning-active are more likely to eat breakfast. However to date there have been no studies that have investigated breakfast consumption in relation to morningness, as a measure of circadian rhythm in a large, representative sample.
Objectives: To test the hypothesis that morningness statistically predicts breakfasting frequency and to explore the role of mental health, personality characteristics and 'breakfast beliefs' as possible correlates of breakfasting in a large UK sample.
Method/Design: A UK representative, web-based survey of 1,068 adults was conducted combining standardised scales and self-designed questionnaire statements. The Composite Morningness Questionnaire was used to assess personal preferences for morningness and eveningness respectively. The measure is used as an indicator of individual circadian rhythms. Wellbeing was assessed using the WHO-5 Wellbeing scale. Non-parametric statistical correlations and logistic regressions were run.
Results: Morningness correlates with breakfasting frequency ($r = 0.24$). Morningness also correlates with conscientiousness ($r = 0.21$), wellbeing ($r = 0.32$; all p-values <0.0005) and BMI ($r = 0.15$; $p < .005$). Logistic regression analysis compared those who never breakfast with those who eat breakfast every day. Gender and socio-economic status did not reach significance, but morningness, time spent watching TV and age were identified as significant predictors of breakfast frequency ($p < 0.05$).
Conclusions: Individuals who are active in the morning are more likely to eat breakfast. Older people are more likely to eat breakfast. Hours watching TV as an indirect measure of physical activity levels has a negative effect on breakfast frequency: those watching more TV are less likely to eat breakfast.

67. Jorg Huber

Factor structure, reliability and validity of the 10-item Connor Davidson Resilience Scale (CD-RISC) in an English Type 2 diabetes sample

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Resilience is defined as a characteristic of an individual which helps this person to adjust and cope successfully in the face of adversity. The concept is attracting increasing interest in relation to successful adaptation to chronic conditions, reducing the risk of co-morbid mental health problems. In order to study the usefulness of a resilience measure in a group of patients with a serious chronic condition, we evaluated the psychometric properties of the short 10 item Connor-Davidson Resilience scale, which contains elements of hardiness, self-efficaciousness and optimism amongst others, in a sample of English Type 2 diabetes patients. We recruited a sample of 173 patients (86 males; mean age 59 ± 14.5 years) with disease duration of 6.7 years ± 6.9. Exploratory principal components analysis resulted in one factor explaining 61% of variance. Confirmatory factor analysis (AMOS 19.0) supported the one factor solution proposed in the literature. Chi-square for assessing model fit was highly significant (Chi-square = 83.1; p <.0005). However, the comparative fit index CFI of .956 and the root mean square error of approximation RMSEA of .089 suggest good and acceptable fit respectively. Cronbach's alpha reliability is excellent (α = .93). Females, younger and less educated patients and those with a history of mental health problems are less resilient. Resilience also correlates with depression and anxiety, and diabetes specific distress in the expected directions (all p-values < .05). Therefore, the scale is very suitable for English Type 2 diabetes patients, with excellent reliability and good validity, and its brevity being an additional advantage.

68. Chris Huggins

Characterisation of transcriptional and post transcriptional properties of vascular interstitial and cultured smooth muscle cells
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Phenotypically modulated vascular smooth muscle cells (VSMCs) are considered a key component of the remodelling of blood vessels during injury or disease such as atherosclerosis or restenosis after stent implantation. Various in vitro models are used for studying mechanisms of phenotypic modulation of vascular smooth muscle cells and the established culture of VSMCs (cVSMCs) is often used for this purpose. Vascular interstitial cell (VICs), recently found in wall of various blood vessels, including veins and arteries, likely represent resident phenotypically modulated VSMCs under normal physiological conditions. In this study we compared the expression of proteins and corresponding genes for number of VSMCs-specific markers in freshly dispersed contractile VSMCs, VICs and cVSMCs from rat aorta using immunocytochemistry and comparative real-time RT-PCR from separately collected cells. ANOVA test for three variables was used for statistical analysis. We observed that VICs, similarly to cVSMCs, display the presence of sparse αSM-actin-enriched stress fibres, while in VSMCs this protein is more tightly packed with higher density in superficial region of the cell. We also found that compared to contractile VSMCs both VICs and cVSMCs show a decrease in gene and protein expression for smoothelin, myosin light chain kinase (MLCK) and SM22α. The expression of αSM-actin and smooth muscle myosin heavy chain (SM-MHC) was significantly decreased in cVSMCs compared to contractile VSMCs, however, compared to VSMCs it was not decreased in VICs. Our study demonstrates that both VICs and cVSMCs display the features of the putative phenotypically modulated VSMCs such as the presence of actin-enriched stress fibers and decrease in

expression of contractile VSMCs markers such as smoothelin, MLCK and SM22α compared to contractile VSMCs. However, it was observed that the expression of two major contractile proteins αSM-actin and SM-MHC (the only specific marker of smooth muscle cell type), was decreased only in cVSMCs while it was not decreased in VICs compared to contractile VSMCs. The results obtained in this study suggest that mechanism of governing the phenotypic modulation could be different or altered in the cultured VSMCs comparing to those in native phenotypically modulated VSMCs.

69. Nikolaos Ioannou

Responses of human pancreatic cancer cells to treatment with Insulin-like growth factor receptor (IGF-IR) tyrosine kinase inhibitor NVP-AEW541 alone and in combination with anti-EGFR mAb ICR62 or cytotoxic drugs.

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Aberrant expression and activation of growth factor receptor signalling pathways including epidermal growth factor receptor (EGFR) and the insulin-like growth factor receptor (IGF-IR) have been reported in a wide range of epithelial cancers and have been associated with increased cell growth, migration and invasion, angiogenesis and cell survival. Of the growth factor receptor inhibitors, the EGFR inhibitor erlotinib has been approved for the treatment of pancreatic cancer, but its overall therapeutic efficacy is of short duration. In some studies, signalling with the IGF-IR has been associated with resistance to therapy with the EGFR inhibitors. In this study, using the Sulforhodamine B colorimetric assay, we investigated the sensitivity of a panel of human pancreatic cancer cell lines (PT-45, AsPC1, PANC1, MiaPaca2, BxPC3, Capan1 and FA6) to treatment with IGF-IR tyrosine kinase inhibitor NVP-AEW541 alone or in combination with anti-EGFR monoclonal antibody ICR62 and cytotoxic agents (i.e. 5-FU, doxycycline and gemcitabine). We also investigated the association between the expression levels of IGF-IR and EGFR in these tumour cells, determined by flow cytometry, and their responses to treatment with NVP-AEW541 and/or ICR62. At concentrations above 5μM, NVP-AEW541 inhibited completely the growth of most of the pancreatic cancer cell lines and with IC50 values ranging from 342nM (FA6) to 2.73μM (PT45). At maximum concentration of 200 nM used in this study, ICR62 did not have any effect on growth of the human pancreatic tumour cell lines. In addition, treatment with a combination of ICR62 and NVP-AEW541 did not enhance the inhibitory effect of the single agent in pancreatic cancer cells. Interestingly, treatment with a combination of NVP-AEW541 and gemitabine was found to be synergistic for ASPC1 and PANC1 cells, additive for BXPC3, Miapaca2 and PT-45 cells, but antagonistic for FA6 and CAPAN1 cells. We also examined the cell cycle distribution of BXPC3 cells following treatment with NVP-AEW-541 and found an increase in the population of cells in sub-G and G0/G1 phases. Interestingly, gemcitabine treatment of BXPC3 cells was accompanied by an increase in the populations of cells in both the sub-G1 and S phases of the cell cycle suggesting different modes of action. We conclude that dual targeting of EGFR and IGF-IR in pancreatic cancer cells by a combination of ICR62 and NVP-AEW541 is not superior to treatment with a single agent. Further studies on the identification of predictive markers for response to treatment with the IGF-IR inhibitor and cytotoxic drugs together with their therapeutic potential when used in combination are warranted.

70. Ricky James

Estimating prevalence of using doping and herbal supplementation with hormonal boosting effects among UK club level athletes

Ricky Jamesa, Tamás Nepuszb, Declan P Naughtona, Andrea Petroczia
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Estimating prevalence of using doping and herbal supplementation with hormonal boosting effects among UK club level athletes Ricky Jamesa, Tamás Nepuszb, Declan P Naughtona, Andrea Petroczia a School of Life Sciences, Faculty of Science, Engineering and Computing, Kingston University b Department of Biological Physics, Eötvös Loránd University, Hungary Background Performance enhancing drugs (doping) are well documented for their positive and negative effects on the body and assumed to be widely used among athletes at all levels, particularly in sub-elites [1]. Whilst it is important that relevant governing bodies gain insight into the prevalence of doping use in athletic and fitness populations, owing to the clandestine nature of such behaviour, it is difficult to obtain reliable and valid information on prevalence [2]. Therefore the aim of this pilot project was to test, compare and contrast a recently developed non-random model, Simple Sample Count (SSC) [3], for obtaining estimates for the use of doping and herbal supplements with hormonal boosting effects. **Methods:** Following ethical approval, 513 participants (58.7% male) from various sports clubs across the UK were asked to complete an anonymous survey containing SSC, social projection and simple network scale up on the use of doping and hormonal boosters, along with basic demographics. Athletes in the sample represented a variety of sports but mainly consisted of football, rugby, boxing and track and field with the highest educational level being undergraduate and A levels or equivalents.
Results: Alarmingly high prevalence rates were present for both substance categories. Doping prevalence was estimated for 19.8 ± 9.3%. Hormonal boosters were used by 54.0 ± 9.7%. SSC prevalence estimate for doping was in keeping with those obtained via social projection (13.8% in own sport and 26.1% in all sports) and network scale up (1.6% for known and an additional 15.7% for suspected doping).
Conclusion: The SSC showed good consistency with social projection and network scale up results. Such approach may be successfully employed in estimating prevalence for transgressive and/or socially sensitive behaviour in sport and beyond. The estimated prevalence for both doping and supplements in this population underscores the need for prevention.

71. Carl Jenkinson

Dietary substances inhibit the key steroidogenic testosterone glucuronidating enzyme UDP-glucurononsyltransferase (UGT)2B17.

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The anabolic steroid testosterone is commonly abused by athletes owing to its effects on muscle growth and performance. UDP-glucurononsyltransferase (UGT)2B17 is the key enzyme involved in the glucuronidation of testosterone for elimination by excretion in urine (Jakobson et al. 2008). Excreted testosterone glucuronide serves a marker for the testosterone/epitestosterone (T/E) ratio used in sport to detect testosterone abuse by athletes (Sottas et al. 2010). Alterations in the glucuronidation of testosterone, through inhibition in the UGT2B17 enzyme, could change the T/E ratio and mask testosterone abuse. Previous reports reveal that two non steroidal anti-inflammatory drugs inhibit with the two key glucuronidating enzymes UGT2B15 and UGT2B17 (Sten et al. 2009). However there is no

current knowledge of the interaction of dietary samples on the inhibition of testosterone glucuronidation. The aim of this study was to analyse a number of dietary substances for inhibition of the key steroidogenic enzyme UGT2B17. Analysis of testosterone glucuronidation was performed using hepatic (UGT)2B17 supersomes with candidate dietary inhibitors by analysis of residual testosterone using high performance liquid chromatography. The results from these studies reveal that components of commonly consumed dietary substances green and white tea samples and red wine inhibited the UGT2B17 enzyme, therefore inhibiting testosterone glucuronidation. The level of testosterone glucuronidation reduced to 70-85% of the control value with the addition of the tea samples. With the addition of red wine the level of glucuronidation reduced to 60% activity of the uninhibited control over 90 minutes. In addition, several catechin compounds commonly found in teas and phenolic compounds commonly found in red wine where found to inhibit UGT2B17 testosterone. The degree of inhibition was more pronounced at lower levels of testosterone. These results highlight the interactions of a number of dietary compounds and substances on testosterone glucuronidation and the competitive inhibition being expressed by some of the compounds. In summary, these results also show that these compounds could have an impact on the validity of the current method in detecting testosterone abuse in sport. Key words – Testosterone, UGT2B17, Glucuronidation, Red wine, Tea, HPLC. Jakobson Schulze J, Lundmark J, Garle M, Skilving I, Ekström L, Rane A. Doping Test Results Dependent on Genotype of Uridine Diphospho-Glucuronosyl Transferase 2B17, the Major Enzyme for Testosterone Glucuronidation. The Journal of Clinical Endocrinology & Metabolism, 2008, 93(7): 2500-2506. Sottas PE, Robinson N, Saugy M. The athlete's biological passport and indirect markers of blood doping. Handbook of Experimental Pharmacology. 2010, 195, 305-326. Sten T, Finel M, Ask B, Rane A, Ekström L. Non-steroidal anti-inflammatory drugs interact with testosterone glucuronidation. Steroids 2009, 74(12): 971-977.

72. Thomas Jepps

Impaired vascular Kv7 function in animal models of hypertension.
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Background: Voltage-gated potassium (K+) channels encoded by KCNQ genes (Kv7 channels) are present functionally in vascular and non-vascular smooth muscle cells (Yeung et al., 2007; Greenwood and Ohya, 2009). However, nothing is known about the functional impact of these channels in vascular disease. The aim of the present study was to compare the effect of structurally different activators of Kv7.2-7.5 channels: S-1, retigabine and BMS-204352, on blood vessels from normotensive and hypertensive animals.
Methods and Results: Isometric tension recordings were performed on segments of mesenteric artery and thoracic aorta and the coronary blood flow was studied using the Langendorff heart preparation, in which rats were anaesthetised with i.p. injection of 50 mg/kg sodium pentobarbitone according to the Danish guidelines for animal experiments. Blood vessel segments from normotensive rats were relaxed by all three Kv7 activators with potencies of BMS-204352= S-1 >retigabine. In the Landendorff isolated-heart BMS-204352 and S-1 dose-dependently increased coronary perfusion at concentrations between 0.1-10 µmol L-1 whereas retigabine was effective at 1-10 µmol L-1. The ability of these agents to relax precontracted vessels and increase coronary flow was considerably impaired in tissues isolated from spontaneously hypertensive rats (SHRs). For example, segments of mesenteric artery from normotensive rats were relaxed by S-1 with an EC50 of 2.5 µmol L-1 but in segments from SHRs S-1 had an EC50 of 47.2 µmol L-1. Of the 5 KCNQ gene isoforms the expression of KCNQ4 was reduced (~3.7 fold) in the SHR aorta. Kv7.4 protein levels were

~50 % lower in aortae and mesenteric arteries from SHRs compared to the normotensive vessels. A similar attenuated response to S-1 and decreased Kv7.4 abundance was observed in mesenteric arteries from mice made hypertensive by angiotensin II infusion compared to normotensive controls.
Conclusions: In two different models of hypertension the functional impact of Kv7 channels is dramatically down-regulated. References: Yeung et al. (2007) Br. J. Pharmacol., 151:758-770. Greenwood and Ohya (2009) Br. J. Pharmacol., 156:1196-203.

73. Trupti Jivram

Generation 4 (G4) - Implementing an Online Interactive Curriculum using Virtual Patients
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Aim: Problem-based learning is well established in medical education. Students work through paper patient cases and explore possible investigations, diagnoses and treatments, generating learning objectives, in groups of eight with a facilitating tutor. The 'Generation 4' project explored the extent to which virtual patients (VPs) could be used to transform the existing PBL curriculum and mimic the role of the practitioner, allowing 'safe practice' in real life to avoid fatal mistakes. Techniques used Original paper cases were rewritten to fit the VP model and the online interactive VPs replaced existing paper-based cases, allowing students optional routes through a case, making clinical decisions and exploring the outcomes of those decisions. Students played the cases by projecting them on an interactive whiteboard which allowed a wide range of e-tools to be integrated into the VPs to enrich the learning opportunities including wikis, videos, and web-traces. Formative assessments VPs were designed around the topic of the week, providing students with additional opportunities to widen their understanding of the subject and assess their knowledge.
Results and conclusion: Students strongly supported the new developments; Tutors believed these resources improved the student experience and increased discussion. A controlled trial demonstrated an significant increase in student exam performance, which will be presented in the poster. G4 has led to a more adaptive, personalised, competency-based style of learning which more closely matches the role of the practitioner. The PBL experience has been transformed by a range of interactive technologies built around a core of virtual patients which extends the learning opportunities available within the PBL tutorial.

74. Jiwa NS[1], Bridges LR[2], Esiri MM[3], Hainsworth AH[1]

VEGF receptor in vascular smooth muscle myocytes of small penetrating arteries in aged human brain.
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Background: VEGF receptor 2 (VEGFR2) is a major mediator of vascular re-modelling, and is at the centre of several vascular pathologies, including atherosclerosis, and blood brain barrier dysfunction. VEGFR2 function is well known in endothelial cells and also in neurons. We recently observed VEGFR2 in vascular smooth muscle cells (VSMC) of small arteries in human brain. Here we aim to test the hypotheses that VEGFR2 in VSMC of small penetrating arteries is associated with: i) age, ii) vessel sclerosis.
Methods: Paraffin sections of human caudate/putamen from aged cases with neuropathologically-diagnosed small vessel disease (n=15, mean (SD) age: 80 (11) y), aged

control cases with minimal brain pathology (n=11, age: 83 (7) y), and young controls (n=5, age range 11-40 y), were labelled immune-histochemically for VEGFR2. Donated brain tissue was primarily from the OPTIMA cohort. In small arterial vessels (10-200 micron outer diameter, data from 281 vessels) VEGFR2 abundance in the VSMC layer was graded by three independent, blinded observers using a 4 point scale, and sclerotic index (S.I.) was estimated.
Results: In aged cases, VEGFR2 was routinely observed in VSMC. Abundance of VEGFR2 was greater in aged cases than in young controls ($p<0.05$) but did not differ significantly between SVD cases and aged controls. Median S.I. for SVD brains was 0.46 (IQR: 035-0.60), significantly greater than that of aged controls (0.39, IQR 0.30-0.48) and young controls (0.39, IQR 0.30-0.50). VEGFR2 abundance was not correlated with S.I. or with in-life systolic blood pressure ($p>0.05$).
Conclusion: VEGFR2 expression in VSMC of penetrating arteries was positively associated with age. This suggests a possible role for VEGFR2 in vascular smooth muscle cells of aged brain.
NSJ is a recipient of the Jane White prize. Supported by Alzheimer's Research UK, St George's Hospital Charity and the Neuroscience Research Foundation.

75. Elwira Kaminska

Identification of putative biomarkers in cancer therapy
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Clinical Sciences, St. George's, University of London Medical Biomics Centre, St. George's, University of London Health and Medical Sciences, University of Surrey
A recent clinical trial of dendritic cell (DC) vaccination in late stage melanoma has shown elevated markers of inflammation in the serum of patients not responding to therapy. Additional biomarkers have been identified using in silico methods, which are linked to inflammation and have immuno-modulatory abilities. Briefly, pre-vaccination sera were analysed for a large range of inflammatory molecules using cytometric bead array technology. Increased levels of apoC3, TNF α, SCF, MiP1 α and IL-12p40 were seen in patients not responding to the therapy. An in silico platform was then employed to identify additional novel biomarkers. Using Ariadne Pathway Studio software we identified a number of potential markers with a high level of connectivity between identified inflammatory markers and their regulators. One of these putative markers, Apolipoprotein E (ApoE) was then assayed in the patient serum using ELISA and shown to be significantly increased in the non-responding patients compared to the responders. ApoE has been show to modulate immune cell function and to regulate tumour progression. ApoE mediates antigen presentation by DC to NKT cells and has been shown to protect again LPS mediated sepsis. ApoE self-derived peptides can induce monocytes differentiation into DCs and enhance T cell response. This project has used an in silico methodology to predict potential biomarkers of DC vaccination responsiveness in tumour bearing patients. One new predicted potential molecule has been shown to be increased by conventional immunological methods validating this approach to biomarker discovery.

76. Said Khelwatty

Co-expression and predictive value of CD44, CD133 and p-glycoprotein for response to treatment with anti-EGFR mAb ICR62 and irinotecan in human colorectal tumour cells
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The presence of a small sub-population of cancer stem cells (CSCs) has been suggested to be responsible for not only the initiation and progression of tumour cells, but also for resistance to treatment with conventional therapies. While CD133 and CD44 have been suggested as stem cell markers in various normal and cancer tissues, their roles in the biology of colorectal cancer and their importance as predictive markers for response to therapy with EGFR inhibitors and cytotoxic drugs remain unclear. We investigated the expression level and predictive value of CD133, CD44 and p-glycoprotein (p-Gp), a drug-resistance antigen, in response to treatment with anti-EGFR monoclonal antibody (mAb) ICR62 and cytotoxic drugs. A panel of eleven human colorectal tumour cell lines and a wide range of techniques (e.g. FACS analysis, SRB growth inhibition assay) were employed in this study. Of the cell lines examined, HCT116, HT29, CCL-228 and DiFi cells were highly CD44 positive (i.e.> 95% of tumour cell populations) while CCL-225 and Colo-2 were CD44 negative. In contrast, the percentage of CD133 positive cell populations was much lower in the majority of colorectal tumour cells examined with only Caco-2 cells expressing CD133 marker in more than 95% of the tumour cell population. The expression of P-Gp was found to be the highest in Colo-2 with mean fluorescence intensity (MFI) of 53, followed by CCL-225 (MFI=49) and Caco-2 (MFI=12). We found no significant association between the expression of CD44, CD133 and p-Gp and response to treatment with anti-EGFR mAb ICR62. Interestingly, Caco-2 cells which contained high levels of all the three markers were least sensitive to treatment with irinotecan (IC50 = 26μM). Our results suggest that the expression of CD44, CD133, and p-glycoprotein in colorectal tumour cells is not associated with the response to treatment with anti-EGFR mAb ICR62.

77. Gwenan Knight

What makes a successful hospital MRSA clone?

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CoMPLEX, UCL

The small number of clones responsible for the vast majority of MRSA infections differ country by country, for reasons that are as yet unknown. Across the UK, and at our hospital, St George's Healthcare NHS trust, a change in dominant MRSA clone from CC30-SCCmecII to CC22-SCCmecIV was seen in the early 2000s. To understand why, we investigated MRSA incidence, clonal type, antibiograms, infection control policies, clonal population modelling and fitness. This revealed three important factors governing the success of an MRSA clone. Firstly, a successful clone maintains a range of antibiograms, exchanging resistances between isolates rather than accumulating them. Selection is thus for the clonal population and not a single highly resistant isolate, suggesting that S. aureus has a novel evolutionary dynamic. Secondly, if several MRSA clones are able to hold and shuffle resistances, the fittest will dominate. Surprisingly, a difference in fitness between clones was uncovered as measured by independent growth in high nutrient broth, competition and desiccation survival assays. This was incorporated in a simple deterministic model, which allowed us to show that once resistance is gained by the fittest clone it will dominate. This was reflected in our MRSA populations within the newly dominating CC22 clone. Thirdly, a near universal level of ciprofloxacin resistance appears to be crucial in allowing MRSA clones to succeed. This represents an Achilles heel for MRSA populations as shown by a significant correlation between a drop in hospital-wide ciprofloxacin prescribing and MRSA infection incidence at our hospital in 2007.

78. Katie Lane

Measurement of Hepatic Drug Metabolism in Critically Ill Adults

Katie Lane(1), John Dixon(1), Denise McKeown(2), David Holt(2),Iain MacPhee(1), Barbara Philips(1)

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Aim: CYP2D6 is the Cytochrome P450 enzyme subtype responsible for the metabolism of 25% xenobiotics. We aim to develop a method of accurately assessing hepatic drug metabolism by CYP2D6 in critically ill adults using tramadol as a 'probe drug'.

Hypothesis: We suggest that a single measurement, 4 h after intravenous administration of tramadol is a reliable indicator of integral plasma midazolam exposure or area under the curve (AUC) in critically ill patients.

Methodology: A prospective study of 10 critically ill adults was conducted at St George's. Patients taking medications potently inhibiting or inducing CYP2D6 were excluded. An intravenous bolus of tramadol was administered and samples collected at multiple time points for 8 hours. Correlation between 4 h tramadol concentration and AUC was determined. Serum tramadol concentration was determined by high performance liquid chromatography with tandem mass spectrometry.

Results: Four hour tramadol concentration correlated with AUC of a graph of concentration vs time, $r = 0.982$, $p < 0.0001$. This was the strongest correlation with AUC of the time points tested. Conclusions: A single time-point determination of tramadol concentration 4 hours post-intravenous bolus appears to be a reliable marker of integral tramadol exposure and hence CYP2D6 function. This method will be used in further studies to determine the effect of AKI on hepatic drug metabolism in the critically ill.

79. Lamis Latif

Induction and differentiation of neuronal cells from postnatal neural crest-like stem cells

Latif L

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Neural crest stem cells (NCSCs) have attracted global interest due to their potential to form a number of important cell types in the body such as neurons, melanocytes and smooth muscle cells. Acquiring primary NCSCs from embryonic mice has proved to be a meticulous process, which is why we focused on the use of other readily available cell lines, the murine NC-m6 cells. These are thought to possess similar characteristics to NCSCs, and are currently the models being used, in association with research of NCSCs. Previous research has suggested that the NC-m6 cell line may have been able to differentiate into pre-Schwann cells. To further these results, this investigation set out with the following objectives: (a) to investigate whether or not the addition of TGFβ1 and NRG1 caused NC-m6 cells to produce neuronal cells, (b) to establish the individual effect each growth factor had in neuronal cell production (c) to establish whether long term growth in medium containing the aforementioned factors caused further differentiation of the NC-m6 cell line. Cell proliferation, morphology, and presence of neuronal cells using an antibody marker specific for neurons, which has never been used before in the NC-m6 cell type were all used to examine this. In regards to the generation of neuronal cells it was established that NC-m6 cells grown in medium containing NRG1, TGFβ1, and FCS, led to the formation of cells that stained positive, and showed the presence of filaments using the neurofilament neuronal marker antibody (NNM). It was also discovered that growth in media containing TGFβ1 alone caused cells to differentiate into neuronal cells, as opposed to NRG1, which greatly

increased cell proliferation, but did not produce as many neuronal cells. Finally, long term growth in media containing both growth factors caused marked differentiation of NC-m6 cells into neuronal cells, as morphologically the cells had become bipolar, and clear filaments could be seen after immunostaining. The exact subtypes have yet to be determined. These results draw attention to the differentiative potential of NC-m6 cells into neuronal cells, which is a property akin to NCSCs, and although further research is necessary to determine the exact subtypes, it is evident that the NC-m6 cell line serves an excellent model for the study of NCSCs.

80. Karin Leslie

Reduced Levels of HIF 1α and Antioxidant Enzyme Activity in Pregnancies at Higher Risk of Developing Preeclampsia.

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Background: The first trimester placenta develops in a low oxygen environment. Until 10-12 weeks the maternal spiral arteries are plugged by trophoblast and there is little blood flow into the intervillous space. Premature onset of maternal blood flow and defective placentation have been associated with early pregnancy loss, preeclampsia and IUGR. Uterine artery Doppler provides a non invasive proxy measure of successful placentation. High resistance flow can identify those pregnancies at highest risk of developing preeclampsia. This study aimed to investigate whether first trimester pregnancies characterised as being at highest risk of developing preeclampsia showed evidence of alterations in the oxygen environment and oxidative stress.

Methods: First trimester placental tissue was obtained with informed consent and ethical approval from women undergoing termination of pregnancy. Doppler ultrasound was performed prior to surgery: high resistance was defined as mean RI≥0.85 (95th centile). Protein expression was quantified by western blotting and antioxidant enzyme activity assayed.

Results: In pregnancies with high resistance indices compared with normal resistance; 1)Levels of placental HIF1α protein were significantly lower. ($p=0.022$, $n=35$) 2) Placental glutathione peroxidase activity was significantly lower. ($p=0.007$, $n=20$) 3) There was no evidence of any alteration in placental tissue markers of oxidative stress (nitrotyrosine residues, 4- hydroxy nonenal , malondialdehyde, heat shock protein 70) ($n=34$).

Conclusions: The reduced level of HIF-1α may indicate that pregnancies at increased risk of preeclampsia are exposed to higher first trimester oxygen levels than normal, which could have consequences for angiogenesis and placental development. Although high resistance pregnancies had lower levels of antioxidant enzyme activity this did not result in higher levels of markers of oxidative stress. Lower antioxidant defences may contribute to the development of oxidative stress in the second and third trimester or influence the ability of the placenta to react to changes in oxygen tension.

81. Wai Liu

Supernatants from lymphocytes stimulated with Bacillus Calmette-Guerin can modify the antigenicity of tumours and stimulate allogeneic T-cell responses.

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Background: Reduced expression of class 1 human leucocyte antigens (HLA1) is often a mechanism by which tumours evade surveillance by the host immune system. This is often

associated with an immune function that is unable to mount appropriate responses against disease, which can result in a state that favours carcinogenesis.
Methods: In the current study, we have explored the effects of Bacillus Calmette-Guerin (BCG) on the cytokine output of leucocytes, which is a key determinant in generating antitumour action, and have also assessed the effect of these cytokine cocktails on HLA1 expression in solid tumour cell lines.
Results: BCG potently activated a broad range of leucocytes, and also enhanced the production of cytokines that were Th(1)-predominant. Supernatants from BCG-treated leucocytes significantly increased the expression of HLA1 on the surface of cancer cell lines, which correlated with increased cytolytic T-cell activity. We also showed that the increased HLA1 expression was associated with activation of intracellular signalling pathways, which was triggered by the increases in the Th(1)-cytokines interferon-γ and tumour necrosis factor-α, as counteracting their effects negated the enhancement.
Conclusion: These studies reaffirm the role of BCG as a putative immunotherapy through their cytokine-modifying effects on leucocytes and their capacity to enhance tumour visibility.

82. Angela Loyse

Comparison of early fungicidal activity (EFA) of high dose fluconazole, voriconazole, and flucytosine, as second drugs given in combination with amphotericin B, for the treatment of HIV-associated cryptococcal meningitis
Loyse A, Wilson D, Meintjes G, Jarvis J, Bicanic T, Bishop L, Rebe K, Bekker L-G, Wood R, Harrison T
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Background: HIV-associated cryptococcal meningitis is associated with an estimated 600,000 deaths worldwide per year. Current standard initial therapy consists of amphotericin B (AmB) plus flucytosine (5-FC), but 5-FC remains largely unavailable in Asia and Africa. Alternative, more widely available, and/or more effective antifungal combination treatment regimens are urgently needed. **Methods**: 80 HIV-seropositive, antiretroviral naïve patients presenting with cryptococcal meningitis were randomised to 4 treatment arms of two weeks duration: Group 1) AmB (0.7-1mg/kg) + 5-FC (25mg/kg four times daily); Group 2) AmB (0.7-1mg/kg) + Fluconazole 800mg daily ; Group 3) AmB (0.7-1mg/kg) + Fluconazole 600mg twice daily; Group 4) AmB (0.7-1mg/kg) + Voriconazole 300mg twice daily. The primary end point was the rate of clearance of infection from the cerebrospinal fluid (CSF), or early fungicidal activity (EFA), as determined by results of serial, quantitative CSF cryptococcal cultures.
Results: There were no significant differences in the rate of clearance of cryptococcal CFU from the CSF between the four treatment groups: the mean (SD) EFA (log CFU/mL CSF/day) for treatment groups 1, 2, 3 and 4 were -0.41 (0.22), -0.38 (0.18), -0.41 (0.35), and -0.44 (0.20), respectively. Overall mortality was 12% (9/78) at 2 weeks and 29% (22/75) at 10 weeks with no significant difference between groups. There were few laboratory abnormalities related to the second agents given; in particular there were no significant (≥ grade 3) rises in alanine transaminase titre or falls in neutrophil count.
Conclusion: There was no significant difference in EFA between AmB in combination with fluconazole and AmB plus 5-FC for the treatment of HIV-associated cryptococcal meningitis. AmB plus fluconazole 800-1200 mg/day represents an immediately implementable alternative to AmB plus 5-C. AmB plus voriconazole is an effective alternative combination in patients not receiving interacting medications.

83. Luisa Madeira

Hydroponic Plant Culture for the Production of a Monoclonal Antibody raised against MUC-1

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Plants offer an inexpensive alternative to traditional systems for the production of recombinant monoclonal antibodies. However, cultivation of transgenic plants in the field raises regulatory concerns regarding product quality and uniformity. Contained hydroponic cultivation of plants is a highly controlled production platform in which recombinant pharmaceuticals can be produced not only in the vegetative tissues of the plant, but also by the process of rhizosecretion. Rhizosecretion is potentially an appealing process as recombinant proteins can be harvested over the entire lifetime of the plant, and, most importantly, downstream processing is simplified as extraction is from simple hydroponic medium rather than complex plant tissues. Although advances have been made in the use of rhizosecretion for expression of recombinant pharmaceuticals, the principal limitation of the system has been the relatively low yields that can be obtained. Here, we present the development of a hydroponic system for tobacco plants, for the production of M12, a monoclonal antibody raised against the epithelial tumour marker MUC-1. Optimization of rhizosecretion levels for M12 was obtained by manipulation of medium compounds and mechanical stimulation of roots. Initial yields were enhanced 60-fold, reaching on average 30 µg/ml/week, although a yield of 100 µg/ml has been observed, giving rise to optimism that there is further room for improvement. Antibody purification by Protein A was achieved with 73% recovery. The glycosylation profile of the purified antibody was assessed by MALDI MS/MS and 3 glycoforms were detected, all of the complex type. The production system developed is simple, inexpensive and potentially rapid to scale up, with the development of an automated fluid handling system. This low-tech approach addresses many regulatory concerns and would be readily transferable to low-income regions.

84. Maria Manoussaka

Occult replication of a conditionally-live attenuated SIV profoundly upregulates T effector memory cell frequency

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Background: The most potent protection against infection with virulent SIV, including protection against mucosal challenge, is conferred by "vaccination" with live attenuated virus. Although this approach is precluded for HIV because of safety concerns, understanding the mechanisms of superinfection resistance may inform rational vaccine design.
Methods: In order to uncouple antigen exposure from active viral replication we compared peripheral and intestinal T cell phenotype and SIV peptide-specific responses following infection of macaques with wild type SIVmac239, attenuated SIVmac239Δnef or with a doxycycline (dox)-conditional replication variant of SIVmac239 Δnef designated SIVrtTA. Global (antigen-non-specific) T cell phenotype was assessed for central memory (Tcm) (CD28+CD95+) and effector memory (Tem) (CD28-CD95+) and SIV-specific T cell responses were measured by detection of TNF-α, IFN-γ, IL-2 and IL-17.
Results: In animals in which on-going virus replication was permitted (SIVrtTA + dox &

SIVmac239Δnef), the proportion of CD4 and CD8 Tcm were reduced in the majority of animals while Tem proportions increased. In animals infected with SIVrtTA in which dox had been withdrawn for 8 weeks prior to analysis, these changes were not seen. Moreover animals infected with wild type virus had elevated CD4 and CD8 Tcm. Analysis of gut mucosal homing (α4+β7+ and β7+) T cells showed similar polarised changes.
Conclusion: Overall, we found that active replication of SIVrtTA and SIVmac239Δnef had a profound impact on global T cell phenotype and antigen-specific polyfunctionality in the periphery and the gut. The use of these SIV mutants will contribute to the understanding of the mechanisms of superinfection resistance.

85. Saraid McIlvride

Impact of prenatal androgens on glucocorticoid metabolism in a sheep model of PCOS
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Polycystic ovary syndrome is one of the most common endocrine disorders affecting women of reproductive age. The major symptoms are hyperandrogenism and polycystic ovaries. However, PCOS is also associated with the metabolic syndrome. The aetiology of PCOS is still unknown. One theory is that the hyperandrogenism is caused by disruption of cortisol feedback to the HPA-axis causing a consequent increase in production of adrenal androgens. Cortisol metabolism is mediated by the 11βHSD isoenzymes. There is some evidence to suggest that these enzymes are dysregulated in women with PCOS. The aim of this project was to test the hypothesis that cortisol metabolism would be disrupted in a sheep model of PCOS. The primary objective of this study was to measure the activity of the 11βHSD isoenzymes in adipose tissue of prenatally androgenised lambs. Scottish Greyface lambs were exposed to prenatal androgens, either by direct fetal injection or via maternal injection during gestation, in order to induce a PCOS-like phenotype. Radiometric conversion assay was utilised to measure the activity of the 11βHSD isoenzymes in tissues of 10-12 week old prenatally androgenised lambs. Prenatal androgenisation of lambs directly in utero caused an increase in NAD+-dependent cortisol metabolism in adipose tissue of male lambs. There was no effect in female lambs, or in lambs exposed to androgens via maternal route of injection. In conclusion, prenatal androgens caused an increase in NAD+-dependent oxidation of cortisol by the 11βHSD2 isoenzyme in male lambs.

86. Denise McKeown

Raltegravir in Preterm Neonates: Preloading Prior to Birth and Delayed Clearance Following Transplacental Transfer from HIV-1 Positive Women.
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Background: Raltegravir (RAL), has been successfully administered to achieve a rapid reduction in maternal viral load (mVL), preventing MTCT of HIV in pregnant women who present late to clinic, or have drug resistant HIV-1.1,2 McKeown et al1 reported the transplacental transfer of RAL and the need for further investigation into the potential for preloading prior to delivery and on neonatal drug clearance.
Methods: Blood samples were collected from mother and neonate close to delivery and post-delivery from the neonate. Roche Taqman 2.0 assay was utilised to monitor the mVL until delivery. RAL concentrations were quantified using LC-MS/MS.
Results: Case 1: spontaneous vaginal delivery at 29 weeks gestation; undetectable mVL at

delivery. RAL regimen initiated 22.5h prior to delivery. The maternal and neonatal RAL concentrations were 300ng/mL (post-maternal dose (PMD): 10.5h; post-delivery (PD): 0h) and 602ng/mL (PMD: 11h; PD: 0.5h), respectively. Case 2: emergency CS at 33 weeks + 2 days gestation; undetectable mVL at delivery. RAL regimen initiated at 22 weeks gestation. The maternal and neonatal RAL concentrations were 2318ng/mL (PMD: 6h; PD: 0h) and 3781ng/mL (PMD: 7h; PD: 1h), respectively. Neonatal RAL concentration of 312ng/mL was detected 92h PD. Case 3: emergency CS at 30 weeks + 3 days gestation; mVL of 55copies/mL at delivery. RAL regimen initiated 14h prior to delivery. The maternal and neonatal RAL concentrations were 64ng/mL (PMD: 3h; PD: 1h) and 120ng/mL (PMD: 4h; PD: 2h), respectively. Neonatal RAL concentration of 67ng/mL was detected 63h PD. To date, all infants remain HIV-1 negative by DNA PCR, with no reported adverse events.
Conclusions: Effective transplacental transfer of RAL, with higher concentrations detected in neonates, was observed for all cases. Cases 1 and 3 detectable RAL concentrations were achieved in both mother and neonate, despite therapy being initiated 22.5h and 14h prior to delivery, respectively. This shows the potential for RAL to preload the fetus. McKeown et al1 reported a sub-therapeutic neonatal RAL concentration 72h PD (40weeks + 1day). Higher neonatal RAL concentrations were observed for cases 2 and 3, 92h and 63h PD, respectively, which may reflect immature UGT1A1 mediated excretion by glucuronidation in preterm neonates. References: 1. McKeown D.A. et al. AIDS 2010;24(15):2416-8. 2. Pinnetti C. et al. J Antimicrob Chemother 2010;65(9):2050-2.

87. Evangelia Mitsopoulou

Medical curriculum enrichment through repurposing and sharing multi-type content across European academic institutions

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Aim: The mEducator Best Practice Network (BPN), a European Union co-funded project under the eContentplus programme, aims to explore existing standards for describing medical education content to enable it to be discovered, retrieved, shared and reused across European academic institutions. By the end of the project, the provided state-of the art medical educational resources will be described by a proposed metadata standard while two alternative solutions for educational discovery and retrieval on the web will be compared and evaluated as part of its best practise nature. Techniques used The project methodology consisted of four stages: Content Preparation All content partners provided examples of medical educational resources of different types. St George’s contributed a number of interactive clinical scenarios. Content Standardisation All provided content was described using a customised metadata schema, which builds upon and extends existing standards for describing medical education resources. Content Discovery and Retrieval Two alternative solutions, based on Web 2.0 "mash-up" technologies and semantic web services, were developed. Evaluation An evaluation strategy for the metadata schema and the two solutions was agreed, including methods such as functional evaluation grids, end-user evaluation with controller scenarios, expert review scenarios, templates and exemplary cases, questionnaires for usability and metadata accuracy and heuristic/expert evaluation and review of the search/discovery functionalities.
Results: The expected results of the project are a standardised metadata schema, recommendations on how to apply e-learning standards and best practices for sharing medical education resources, and a simple IPR (Intellectual Property Rights) scheme for educational content provided and repurposed in academic networks.

Conclusions: Medical and healthcare education can be modernised/improved by means of new technologies. Given the increasing importance of sharing resources, the use of web technology can facilitate the efficient interchange of medical educational resources across different academic networks. This exchange can lead in turn to an enriched curriculum. The critical evaluation of semantic web architecture and a web 2.0 framework provides evidence that the technological nature of a sharing mechanism impacts its effectiveness and the results it retrieves.

88. Francisco Molina-Holgado

The endocannabinoid system through a neuroimmune network regulates neurogenesis: The involvement of HIF-1alpha and IL-1beta signalling pathways

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Cross-talk between inflammatory mediators and neural stem cells (NSC) might have important consequences for neural development and brain repair. Although signalling through the endocannabinoid (eCB) system has been implicated in many aspects of neural development, its role in NSC remains elusive. Recent findings from our laboratory have demonstrated that the eCB system cross talks, in a bi-directional manner, with signalling from a neuroimmune network in controlling the self-renewal and differentiation of NSC. Both eCB system and neuroimmune network signals were sufficient and independent of the other to inhibit proliferation or differentiation of NSC Specifically, eCBs promoted the generation or migration of NSC through the cannabinoid CB1 or CB2 receptors activation in collaboration with hypoxia inducible factor 1 alpha (HIF-1alpha and interleukin 1 (IL-1) signalling pathways. HIF-1alpha and IL-1beta are important inflammatory mediators that are involved in neurodegeneration. However they are also involved in brain repair and recovery. Emerging evidence indicates that HIF-1alpha is a potent signal that induces NSC proliferation. In contrast, IL-1□□modulates lineage specification of NSC. Here we examine how the eCB system is capable of cross-talk with signalling from HIF-1alpha and IL-1 system in controlling the self-renewal and differentiation of NSC respectively. These findings raise the possibility that the eCB system could have potential as a novel strategy for promoting brain repair. Supported by University of Roehampton (UK), Instituto de Salud Carlos III, grant number 08/1999 (Spain), Fundacion Mutua-Madrileña (Spain) and MRC (UK)

89. Francisco Molina-Holgado

Role of interleukin-1 receptor antagonist (IL-1ra) in neurogeneis: A new potential target for brain repair

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Recent reports suggest that there is a synergy between the immune system and neural stem cells (NSC) to promote functional recovery since immune cells help to maintain neurogenesis in germinal centres of the adult central nervous system (CNS) even under non-pathological conditions. Evidence is emerging that the interleukin-1 receptor antagonist (IL-1ra), an endogenous antagonist for the actions of IL-1 in the brain, is a potent signal that induces

neural stem cell proliferation and migration. NSC cultures cells were prepared from the cortex of day 16 (E16) C57BL6 mice (wild type,WT) or IL-1 β knock-out (IL-1β -/-) mice. NSC were exposed to recombinant murine (rm) IL-1ra (20, 40, or 60ng/ml, t= 7days), the specific CB1 antagonist AM251 (1 □M, t=7days), or to the CB2 antagonist AM630 (1 μM, t= 7 days) for cell proliferation (serial dilution) assays. In addition we study the effects of IL-1ra (60ng/ml, t=24h) on BrdU incorporation. Experiments were performed using a pulse of BrdU (10 μM, t-=6h) after 24 of culture passage. NSC were exposed for 24 hours to IL-1ra alone or in combination with the inhibitor of diacylglicerol lipase (DAGL) activity RHC-80267 (5μM). We show that NSC self-renewal is controlled by bi-directional cross-talk between the endocannabinoid system and the IL-1ra signalling pathway. IL-1ra increases By blocking endogenous IL-1 ra activity, we demonstrate that the IL-1 system is critical for the proliferation of NSC. Moreover, this IL-1ra proliferative effect on NSC is mediated by the endocannabinoid system as demonstrated with the pharmacological blockade of CB1/CB2 cannabinoid receptors using specific antagonist (AM250 or AM630) or the DAG lipase inhibitor RHC80267. Overall these data suggest a novel mode of action for the endocannabinoid system in NSC proliferation that is coupled to IL-1ra signalling and that may be of therapeutic interest in the emerging field of brain repair. EM-H and FM-H are funded by Gobierno de Castilla-La Mancha (Fundación para Investigación Sanitaria en Castilla-La Mancha; FISCAM, G-2009-C/06).FMH is supported by University of Roehampton.

90. Francisco Molina-Holgado

Cannabinoid receptors direct neurogenesis through neuroimmune networks: Role of interleukin-1 receptor antagonist (IL-1ra) and interleukin-6 (IL-6)

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Neuroimmune networks and the brain endocannabinoid system (eCB) contribute to the maintenance of neurogenesis. Activation of cannabinoid receptors suppress chronic inflammatory responses through the attenuation of pro-inflammatory mediators. Moreover, the eCB directs cell fate specification of neural stem cells (NSC) in the central nervous sytem (CNS). The aim of this study is to understand better the relationship between the eCB, the immune system and NSC biology, in order to develop therapeutical strategies on CNS diseases that may facilitate brain repair. NSC derived from the stem cell line COR-1 express functional CB1 and CB2 cannabinoid receptors and the neural stem cell marker SOX-2. First, we detected the presence of functional CB1 and CB2 cannabinoid receptors and the SOX-2 marker by immunoblotting in cell homogenates obtained from adherent NSC cultures. Secondly, we have investigated the role of CB1 and CB2 cannabinoid receptors in the control of NSC proliferation and in the release of immunomodulators that lead the NSC fate. Specifically, in the neural stem cell line COR-1, CB1 and CB2 cannabinoid receptors modulate FGF-2 (20ng/ml, t=24h) induced stem cell proliferation by the release of interleukin-1 receptor antagonist (IL-1ra) and interleukin-6 (IL-6). The specific antagonists for CB1 (SR-141716A, 1microM, t=24h) or CB2 (SR-144528, 1microM, t=24h) cannabinoid receptors, abolish or decrease (by 50% approximately) respectively, NSC proliferation, indicating a critical role for both CB1 and CB2 receptors in the proliferation of COR-1 stem cells. Next, we study if endogenous or exogenous cannabinoid agonists interact with the CB1 and CB2 cannabinoid receptors modulating neural stem cell self-renewal or NSC fate specification through diverse downstream neurogenic pathways and immune system

modulators. Both cannabinoid agonists ACEA (0.5 microM, t=24h) or JWH-056 (0.5 microM, t=24h) induced a significant increase of IL-1ra in COR-1 cells (by 50% approximately). This effect was abolished when the COR-1 stem cells were pre-treated with the above specific antagonist for CB1 or CB2 receptors. As opposed to the above cytokine, only CB2 receptors modulate the release of IL-6, a pleiotropic cytokine, which display a degree of functional redundancy due to the presence of a glycoprotein 130 (gp130), in their receptors. Endocannabinoids appear as new participants connecting the immune system and neurogenesis. Thus, the endocannabinoid system, which has neuroprotective and immunomodulatory actions mediated by different signaling cascades in the brain, could assist the process of proliferation and differentiation of embryonic or adult neural stem cells, and this may be of therapeutic interest in the emerging field of brain repair. EM-H and FM-H are funded by Gobierno de Castilla-La Mancha (Fundación para Investigación Sanitaria en Castilla-La Mancha; FISCAM, G-2009-_C/06). MQU-Le is supported by Instituto de Salud Carlos III (Ministerio de Ciencia e Innovación of Spain, Exp. Nº CA09/00609).FMH is supported by University of Roehampton.

91. Martina Muggenthaler

Propionic acidaemia in the old order amish presenting as isolated dilated cardiomyopathy

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Propionic acidaemia (PA) is one of the most frequent organic acidaemias worldwide and caused by deficiency of the mitochondrial enzyme propionyl CoA carboxylase (PCC). The disease is inherited in an autosomal recessive mode and results from mutations in either the PCCA or PCCB genes, encoding both subunits of the enzyme (α and β).The phenotype varies from a severe neonatal form with episodes of ketoacidosis, developmental delay and neurological symptoms which often ends in coma and death, to a milder, chronic form with less severe developmental retardation. Cardiac manifestations, namely dilated and hypertrophic cardiomyopathy, are less common. We describe an Old Order Amish family with three siblings presenting with isolated severe dilated cardiomyopathy (DCM) in early adulthood. Prior to presentation with DCM the only significant symptom in all three was vomiting on exercise. The first sibling was diagnosed with DCM six months after giving birth to her fourth child at a routine check-up. Her brother was found to have DCM whilst in hospital with pneumonia at the age of 24. He subsequently had an internal defibrillator fitted and has recently undergone cardiac transplantation. Another brother died suddenly at the age of 23 whilst singing in church. His Echocardiogram had shown a mildly dilated left ventricle six months prior to his death. Whole exome sequence analysis of the first affected sibling revealed the non-synonymous variant c.1606 A>G (Asn536Asp) in the PCCB gene on chromosome 3, an area which had been highlighted as the probable disease locus with homozygosity mapping. This variant has previously been reported in association with mild forms of propionic acidaemia and was shown to segregate with the disease phenotype of DCM in this family.One asymptomatic sibling aged 15 was also found to be homozygous and is currently undergoing cardiological assessment. To our knowledge this is the first report of three siblings with propionic acidaemia presenting with isolated DCM in adulthood. We speculate that there might be additional genetic variations in this family leading to mitochondrial dysfunction and disruption of the respiratory chain in homozygous carriers of the PCCB mutation resulting in the long-term development of cardiomyopathy.

92. Reena Murgai

Investigating the Expression and Function of Scar Proteins in Post-Ovulatory Wound Repair
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Introduction: Metaplastic transformation of Ovarian Surface Epithelial (OSE) cells is suggested to give rise to Epithelial Ovarian Cancer. However, the underlying mechanism which results in tumourigenesis is unclear. It is proposed that at the ovarian surface, the ruptured follicle results in tissue injury requiring repair which may disrupt the normal ovarian matrix and contribute to cancer formation. There is an increased risk of tumour formation at other anatomical sites with chronic wounding. An understanding of post ovulatory wound repair and its contribution to cancer may be gained by applying current knowledge of wound repair in other anatomical sites to the ovaries.
Objectives: The first aim was to characterize scar forming proteins (Collagen types I, III and IV and α-Smooth Muscle Actin (α-SMA) in post-ovulatory wound repair in the mouse using immunohistochemistry. The second part of the project aimed to analyse the effects of matrix proteins on mouse OSE cell proliferation.
Results: Collagen I and III were most abundant encircling the pre-ovulatory follicles. Collagen III had increased expression in the corpus luteum suggesting up regulation following ovulation. α-SMA fully encompassed the pre-ovulatory follicles but the ovarian surface was not stained positive when at the surface of the ovary. Culturing mouse OSE cells with matrix proteins did not affect cell proliferation. However, the cells underwent epithelial-mesenchymal transition.

93. Vivek Nama

Structural capillary ratefaction precedes the onset of preeclamsia
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Background: Microvascular rarefaction, defined as reduced vascular density, is a consistent finding in essential hypertension. Functional and structural capillary rarefaction occurs in individuals with sustained and borderline essential hypertension, and in their normotensive offspring. Women who develop preeclampsia are at increased risk of hypertension in later life. We hypothesised that capillary rarefaction precedes the onset of preeclampsia and could play a role in its pathogenesis.
Methods: In this longitudinal cohort study we recruited 322 consecutive Caucasian women, of which 305 subjects completed the study. We used intravital video-microscopy to measure basal (i.e. functional) and maximal (i.e. structural) skin capillary densities according to a well-validated protocol and measured plasma angiogenic and anti-angiogenic factors. Subjects were studied at 5 consecutive predetermined visits.
Results: Preeclampsia occurred in 16 women (mean onset at 35.6±4.8 weeks) and 272 women had normal pregnancy. In women with normal pregnancy significant reduction in maximal capillary density occurred at 27-32 weeks but had resolved by the puerperium (mean change-4.4 capillaries/field, 95%CI –8.3 to –0.5). In contrast, in women who later developed preeclampsia, structural rarefaction was greater and occurred earlier at 20-24 weeks (mean change: -7.0 capillaries/field, 95%CI –12.6 to –1.4), and persisted into the puerperium. We also found that the change in soluble Endoglin from 11-16 weeks to 27-32 weeks was significantly correlated with the change in structural capillary density.
Conclusion: This is the first study to show that significant structural capillary rarefaction

precedes the onset of preeclampsia. Capillary rarefaction could play a role in the pathogenesis of this disease.

94. Vanda Nemes

Recording and analysing speech perseveration in Alzheimer's Disease
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Division of Clinical Sciences, St George's University of London Signal Processing and Control Group, ISVR, University of Southampton
Frequent repetition of language events (perseveration) is a common presenting feature of Alzheimer's disease (AD) and becomes more prominent as the condition progresses. Recording the occurrence and severity of perseverative speech could serve as a marker of real-world cognitive performance, contributing to early diagnosis and monitoring of disease-modifying treatments. Continuous audio speech recording in a real world setting would, however, violate privacy and would be difficult to interpret without manually segmenting the various sources of recorded language (i.e. patient and interlocutors). If, however, speech were captured by recording energy fluctuations from the vocal apparatus (bone conducted speech) using an accelerometer, the data would derive exclusively from the patient, would be devoid of linguistic meaning, and could be automatically analyzed using a signal processing approach. In this study we aimed to develop a methodology to record and interpret bone-conducted speech in a real world environment, allowing possible repetitive speech patterns to be detected and quantified. In the first stage of the study, we carried out proof of concept experimental testing on healthy subjects. An accelerometer was affixed to the skin above the Temporo-Mandibular Joint and used to record speech-related vibrations while the subjects read aloud a script with embedded repeated short questions and statements. Audio recording via a conventional microphone served as a reference to validate the accelerometer data. Recorded signals were initially pre-processed to reduce background noise and remove silence periods. A variety of signal features were then extracted from each recorded signal and combined using Principal Component Analysis to obtain the one-dimensional representation of the feature vector. Finally, frequently occurring patterns in this condensed feature vector were detected using a motif-discovery algorithm. In the next stage of the study we will record bone conducted speech in patients with possible or probable AD to establish the feasibility and tolerability of the proposed methodology in a natural environment. Future work will include larger subject populations, with the aims of: correlating rates of perseveration with stage and progression in dementia; calibrating treatment effects from established interventions; and defining distinct patterns of perseverative speech. If successful, the technique could form the basis of a device for monitoring performance in, for instance, trials of disease modifying drugs for AD.

95. Claire Nightingale

Risk markers for coronary heart disease and type 2 diabetes in childhood: comparison of Indian children living in India and the UK
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Introduction: UK Indian adults have higher risks of coronary heart disease (CHD) and type 2 diabetes (T2D) than Indian and UK European adults. With growing evidence that CHD and T2D risks begin before adulthood, we compared risk factor patterns in Indian children living

in India and the UK. Methods: We compared markers of adiposity and cardiometabolic risk in 9-10 year-old Indian children in the Mysore Parthenon birth cohort study, India (n = 538) and in the cross-sectional Child Heart Health Study, England (n = 483), which used comparable survey methods in 2007-8 and 2004-7 respectively. Small mean age and gender differences between studies were adjusted for in analyses.
Results: UK Indian children were taller and had markedly higher levels of BMI (mean difference 3.2 kg/m2, % difference 22%, 95%CI 20 to 25%) combined skinfold thickness (% difference 36%, 95%CI 29 to 44%), LDL-cholesterol (mean difference 0.4, 95%CI 0.3 to 0.5 mmol/L), systolic BP (mean difference 11.3, 95% CI 9.9 to 12.8 mmHg) and fasting insulin (% difference 141%, 95%CI 121 to 163%). These differences were similar in boys and girls; differences in LDL-cholesterol, blood pressure and insulin remained marked after adjustment for adiposity markers and pubertal status. **Conclusions:** Substantial differences in cardiometabolic risk between UK Indian and Indian children are apparent before puberty. They do not depend on differences in adiposity and are likely to have an environmental basis. Strategies for chronic disease prevention need to include measures to combat the emergence of chronic disease risks in childhood or earlier.

96. Ifeanyichukwu Okike

Bacterial meningitis in babies 0-90days of age: A UK and Republic of Ireland prospective study

I.O. Okike[1], K. Henderson[2], R. Blackburn[2], B.Muller-Peabody[2], A. Johnson[2], P.T. Heath[1].
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Background and aims: Meningitis in the first 3 months of life is associated with significant mortality and morbidity. Previous UK studies were conducted in the 1980s and 1990s. It is important to define the current burden of disease and how they are being managed and identify any opportunities for improvements in diagnosis and management in order to prioritise treatment and prevention strategies and establish an evidence based management guideline.
Methods: Cases are identified prospectively by active surveillance through the British Paediatric Surveillance Unit (BPSU), routine microbiological surveillance through the Health Protection Agency, and via parents of cases through meningitis and Group B streptococcus (GBS) support charities. The surveillance period is July 2010 – July 2011 (burden of disease study) and September 2011- March 2012 (healthcare delivery study-HCD). For the HCD study, a pack containing an information sheet, consent form and parental questionnaire is sent to a named local Paediatrician for onward forwarding to the parents of babies identified through the above sources. This study is based on a detailed medical notes review and parent – completed questionnaire. With parental consent, recruited babies will also be contacted at two years of age for a neurodevelopmental assessment. **Results:** We received 484 reports through the BPSU orange card system and present results on the first 200 cases meeting the case definition, England 173(86.5%), Wales 10 (5%). 115 (57%) were male and the median age of disease was 14 days (range 0-88). 154 isolates were obtained from the cerebrospinal fluid and blood; Group B Streptococcus (GBS) 77/154(50%), Streptococcus pneumonia 16/154 (10%) and Gram negatives other than E coli 16/154 (10%). Case fatality was 15/200(7.5%). Research and Development approval has been granted in 37 NHS Trusts and ten babies have been recruited into the on going HCD study and the target is 100.
Conclusions: There remains an unchanged significant burden of meningitis in the first 3 months of life. New strategies for prevention are required. The HCD study will provide data

on the timing of events (signs and symptoms) leading up to the diagnosis. Both studies will contribute to new guidelines on the management of neonatal meningitis.

97. Ifeanyichukwu Okike

Bacterial meningitis in babies 0-90 days of age: a UK and Republic of Ireland prospective study

I.O. Okike[1], K. Henderson[2], R. Blackburn[2], B.Muller-Peabody[2], A. Johnson[2], P.T. Heath[1].
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Conclusion: There remains a significant burden of meningitis in the first 3 months of life. GBS is the most common causative pathogen. New strategies for prevention are required.

This study is funded by the Meningitis Research Foundation and was presented in the 2011 WSPID Congress in Melbourne Australia

98. Ifeanyichukwu Okike

The immunogenicity of a novel A (H1N1) vaccine in HIV-infected children

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Background: In October 2009, the United Kingdom Department of Health recommended vaccination of high-risk groups, including children with HIV, with a novel, oil-in-water AS03B adjuvanted Influenza A (H1N1) vaccine (PandemrixTM). There were no published data available regarding the immunogenicity of this vaccine in such children.

Objectives: This study evaluated the immunogenicity of the adjuvanted Influenza A (H1N1) vaccine in HIV-infected children immunised according to national recommendations and assessed the impact of vaccination on individual CD4 counts and HIV viral loads.

Methods: HIV-infected children attending outpatient appointments between 01 November and 31 December 2009 were offered two doses of H1N1 vaccine three weeks apart and a blood test before and 3 weeks after the second dose of vaccine. Serum antibody responses were determined by a haemagglutination inhibition (HAI) assay using standard methods.

Results: Of the 39 children recruited for vaccination, 31 (median age 11.2, range 3.0–17.9

years) received both doses of vaccine and provided pre- and post-vaccination blood samples. Eight children (26%) had baseline HAI titres ≥1:32. After vaccination, 29 children (94%, 95% CI, 78.6–99.2%) had HAI titres ≥1:32 (seroprotection), of whom 27 (87.1%, 95% CI, 70.1–96.4%) had also had a four-fold rise in titres (serocon-version). In the univariate analysis, post-vaccination geometric mean titres (GMTs) were higher among the 21 children receiving highly active anti-retroviral therapy compared with the 10 treatment-naïve children (GMT 406 [95% CI 218–757] vs. 128 [49–336]; P = 0.035), but this was no longer statistically significant when adjusted for prevaccine GMTs. There was no significant impact of vaccination on CD4+ T cell count or HIV viral load.
Conclusion: The AS03B-adjuvanted pandemic Influenza A (H1N1) vaccine is highly immunogenic and appears to be safe in HIV-infected children.

99. Pia Ostergaard

Use of exome sequencing and linkage data to identify GATA2, the gene responsible for Emberger syndrome
Ostergaard P, Simpson M, Connell F, Brice G, Mansour S, Mortimer P, Trembath R & Jeffery S.
Medical Genetics, Division of Biomedical Sciences (PO, SJ), South West Thames Regional Genetics Unit (GB, SM), and Cardiac and Vascular Sciences (PM), St. George's University of London. Genetics and Molecular Medicine, King's College London (MS, RT). Department of Clinical Genetics, Guy's Hospital (FC).
This is an example of how Exome sequencing data (using the Agilent human All Exon Capture Assay and sequenced on the Illumina GAIIx platform) combined with linkage analysis performed on data from a genotyping array (Illumina, Human-Linkage12 panel with 6,090 markers) led to the identification of the causative gene, GATA2, for Emberger syndrome. Subsequent analysis of other Emberger patients identified a further seven mutations in GATA2. Emberger syndrome is an autosomal dominant primary lymphoedema associated with a predisposition to acute myeloid leukemia (AML). GATA2 is a transcription factor which plays an essential role in gene regulation during vascular development and hemapoietic differentiation. Our findings indicate that haploinsufficiency of GATA2 underlies primary lymphoedema and predisposes to AML in this syndrome.

100. Ege Ozkan

The Genetic Studies of Inherited Eye Diseases A Report from GeneSEARCH
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Genetic factors contribute to different types of inherited eye diseases which could cause blindness at different stages of life. More than 60% of the diagnosed cases with blindness in the newborns are affected by inherited eye diseases, for instance congenital cataract, glaucoma, retinal dystrophy, optic atrophy or eye malformations. Consanguinity increases the risk of inheriting these conditions as an autosomal recessive trait. Conducting genetic research in eye disorders enhances the understanding of the eye on molecular pathology level in consanguineous families. GeneSEARCH programme is established in 2008 based at SGUL which is an international collaboration between Iranian universities and SGUL. This is focused on Iranian families with high percentage of consanguineous marriages. We established a cohort with 50 Iranian consanguineous families with at least 4 - 5 affected cases who have inherited eye diseases. We mapped 6 novel loci for autosomal recessive inherited eye diseases including retinal degeneration, optic atrophy and congenital cataract. Also,

currently we have been able to identify a novel phenotype for Bardet-Biedl Syndrome and a form of retinis pigmentosa. This is a valuable cohort for novel eye diseases gene identification as well as beneficial for patients and their family's carrier and genetic testing.

101. Tom Pinnell

The effect of some cathinones, including mephedrone and bupropion, on dopamine release and reuptake in the rat accumbens brain slice.

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Division of Biomedical Science, St George's University of London, *TICTAC

During the last year, a class of drugs called the cathinones, has made headlines due to their abuse liability and potential involvement in drug-related deaths in the UK. Many of these chemicals, which were freely available via the internet, have now been made controlled substances. A recent report highlighted our lack of research into these drugs (AMCD, 2010) and the present study attempted to shed light on the mechanisms of action of some of these chemicals. Adult male rats were killed by schedule 1 in accordance with The Animals (Scientific Procedures) Act (1986). Accumbens slices (400µm) were perfused with oxygenated (95%O2/5%CO2) artificial cerebrospinal fluid at 32.5±0.5ºC. Dopamine was evoked using local electrical stimulation using bipolar tungsten electrodes and measured using fast cyclic voltammetry at carbon fibre microelectrodes sampling at 8Hz to ensure we accurately measured both dopamine release and reuptake events. We examined the effects of mephedrone (1, 10 & 30 µM) diethylpropion (1, 3, 10, 30 & 100 µM), bupropion (0.1, 1 & 10 µM) and ethcathinone (0.1, 1 & 10 µM) on both basal and electrically evoked dopamine, and also dopamine reuptake. In some experiments we examined cocaine (0.1, 1 & 10 µM) and amphetamine (3, 10 & 30 µM) by way of comparison. Mephedrone had a similar effect to amphetamine in that it caused dopamine efflux in the absence of electrical stimulation, presumably via reverse transport. Diethylpropion, bupropion and ethcathinone caused concentration-dependent increases in electrically evoked dopamine (max ~225% of baseline) and concentration-dependent slowing of dopamine reuptake (max ~250% of baseline). These effects on electrically evoked dopamine efflux are larger than expected for drugs which had been described as amphetamine-like (Santamaría & Arias, 2010). Indeed, the effects of diethylpropion, ethcathinone and burpopion on electrically evoked dopamine are more similar to those of cocaine than amphetamine. References. ACMD report (2010) Consideration of the cathinones. Available at: http://www.homeoffice.gov.uk/publications/drugs/acmd1/acmd-cathinodes-report-2010
Santamaría, A. and Arias H. (2010) 'Neurochemical and behavioral effects elicited by bupropion and diethylpropion in rats', Behavioural Brain Research, 211 (1), pp. 132–139.

102. Grisha Pirianov

Rosiglitazone negatively regulates c-Jun N-terminal kinase and toll-like receptor 4 proinflammatory signalling during initiation of experimental aortic aneurysms

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Division of Clinical Sciences, St George's, University of London

Objective: Development and rupture of aortic aneurysms (AA) is a complex process involving inflammation, cell death, tissue and matrix remodeling. The thiazolidinediones (TZDs) including Rosiglitazone (RGZ) are a family of drugs which act as agonists of the nuclear peroxisome proliferator-activated receptors and have a broad spectrum of effects on a number of biological processes in the cardiovascular system. In our previous study we have

demonstrated that RGZ has a marked effect on both aneurysm rupture and development, however, the precise mechanism of this is unknown.
Methods and Results: In the present study, we examined possible targets of RGZ action in the early stages of Angiotensin II-induced AA in apolipoprotein E-deficient mice. For this purpose we employed immunoblotting, ELISA and antibody array approaches. We found that RGZ significantly inhibited c-Jun N-terminal kinase (JNK) phosphorylation and down-regulated toll-like receptor 4 (TLR4) expression at the site of lesion formation in response to Angiotensin II infusion in the initiation stage (6-72h) of experimental AA development. Importantly, this effect was also associated with a dramatic reduction in production of TLR4/JNK-dependent proinflammatory chemokines MCP-1 and MIP-1□.
Conclusion: These data suggest that RGZ can modulate inflammatory processes by blocking TLR4/JNK signalling in initiation stages of AA development.

103. Elena Polycarpou

Resveratrol metabolites inhibit colon cancer cell growth

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Introduction: Resveratrol (3, 5, 4' trihydroxy trans stilbene) is a polyphenol found in grapes, berries and other plants with chemopreventive and anti-inflammatory actions in rodent models of colitis and colon cancer. The limited bioavailability of resveratrol after ingestion suggests that its metabolites may be active. Here we test the effects of the major metabolites resveratrol 3-O-D glucuronide (RV3G) and resveratrol 4'-O-D glucuronide (RV4G) on the growth of HCT116, CCL228 and Caco-2 colon cancer cell lines. Changes in p53 expression in HCT116 cells (the other cell lines have mutated p53) were also investigated.
Methods and Materials: Cells were grown in 96 well plates (or as polarised monolayers in permeable Snapwells for 21 days) and growth assessed by a neutral red uptake assay. Amounts of p53 tumour suppressor protein in HCT116 cells after treatment were detected by Western blot and quantified by densitometry. Resveratrol and its metabolites were from Bertin Pharma (France). Results and conclusions The growth of unpolarised Caco-2, HCT116 and CCL 228 cells was inhibited by resveratrol, RV3G and RV4G after 48 hours (IC50 9-23 □M for all three compounds, n=3). In polarised cells, resveratrol or RV3G also inhibited cell growth when added from the apical, but not basolateral side. p53 expression was measured in HCT-116 cells and was increased by resveratrol but not by its metabolites. We suggest that resveratrol metabolites are effective at inhibiting cancer cell growth at concentrations measured in the plasma after ingestion of resveratrol, by a mechanism independent of increased p53 expression.

104. Marcus Pond

A pan-pathogen microarray for detection of microbiological associations with symptomatic urethritis in males

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In addition to its known microbiological aetiology, urethritis in men may be linked with other genital tract organisms, as yet unidentified in its pathogenesis. We used a microarray, with capacity to detect 22 genital tract organisms, in order to determine the association of symptomatic urethritis with infection or carriage of these organisms. 129 patients were asked to provide an extra first void urine specimen or give permission for their residual urine specimen submitted for Chlamydia trachomatis NAAT testing to be utilised. Patients were categorised into three self-reported symptom groups: definite symptoms of urethritis (discharge and/or dysuria), category 1(C1) n=80; non-specific symptoms of urethritis (e.g. minimal urethral discomfort), category 2 (C2) n=26; and asymptomatic category 3 (C3) n=23. Total urine nucleic acid was extracted and subsequently used for PCR coupled microarray analysis. Organisms were defined as present or absent using an online data analysis method. In a pre-planned analysis, the following categories were compared for prevalence of organisms: C1 versus C2 and C3 combined; C1 and C2 combined versus C3 using Fisher's exact test. One or more organisms were detected in 74% (n=95) of patients and two or more organisms in 33% (n=42). The prevalence of organisms known to cause urethritis in this largely symptomatic cohort was: 16% (n=21), 9% (n=12) and 5% (n=6) for C. trachomatis, Mycoplasma genitalium and for Neisseria gonorrhoeae respectively. Escherichia coli was the most prevalent organism detected with a prevalence of 18% (n=23). The presence of M. genitalium was statistically associated with C1 and C1 and C2 combined (p=0.03 and 0.01 respectively). In symptomatic patients, C. trachomatis, Ureaplasma urealyticum, and Gardnerella vaginalis appeared to be more prevalent than in asymptomatics although not statistically significantly. Lactobacilli where detected in 1.3% and 4% of patients with C1 and C2 symptoms respectively, compared with 17% of asymptomatic patients. The absence of lactobacilli was associated with urethritis symptoms, either C1 alone or C1 and C2 combined (p= 0.03 and p= 0.01) respectively. Using a polymicrobial microarray approach we have demonstrated that symptomatic urethritis is associated with depletion of lactobacilli. This confirms early work using urethral swabs. The temporal nature of Lactobacilli depletion in relation to the onset of symptomatic urethritis needs to be investigated further.

105. Marcus Pond

Investigating a clinical case of XDR-TB: comprehensive drug resistance and isolate profiling by whole genome sequencing.

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Drug-resistant tuberculosis (TB) remains a major challenge to global health and in turn to healthcare in the UK. In 2009 there were 8,286 notified tuberculosis cases in the UK, of which 8% of isolates were resistant to one first line drug and 1.2% of isolates were multi-drug resistant (MDR). Detection of drug resistance at initial diagnosis would allow timely treatment with effective antibiotics to improve patient outcomes, reduce onwards transmission, avoid further resistance developing and minimise associated healthcare costs. However, there is currently a lag between initial diagnosis and confirmation of drug resistance, partly due to slow growth of the organism for phenotypic testing but also due to limitations of existing diagnostic tools.

In this case study, whole genome sequencing was used to examine an extensively drug resistant (XDR) TB isolate, in which resistance had developed to both first-line and second-line drugs, thus seriously limiting treatment options. A previous study had shown that

identifying mutations in key genes associated with resistance may also be able indicate successful modifications to the treatment regimen (1). The clinical history of the XDR-TB patient included numerous presentations with active disease, prior successful treatment with different drug combinations plus interim episodes of non-compliance. The primary aim of the genomic analysis was to address the urgent clinical need to comprehensively determine the genetic basis of resistance so that previously reported phenotype-genotype associations may guide rational modifications to the limited treatment options available.
Genome sequencing of the isolate using an Ion Torrent Personal Genome Machine confirmed mutations in genes associated with reported phenotypic drug resistance; current knowledge representing ~900 SNPs in >45 genes collated in the TBDReaM database (2). Furthermore, as whole genome sequencing is not limited to analysing pre-defined genes or mutations, additional genomic analysis permits detailed typing and characterisation of the isolate. Such extended analysis confirmed this isolate was of the Beijing type, a lineage associated with higher virulence, transmission and drug resistance in outbreaks worldwide. Future analysis of additional isolates may help dissect transmission chains, aid contact tracing and investigate reactivation/ re-infection events.
Ongoing work aims to shorten the diagnostic window further by genome analysis directly from sputum samples or following only a short period of culture. Reducing the time to administration of appropriate treatment has benefits for both the patient and the healthcare provider. Whilst initially thought of as a high-tech, expensive solution to the problem, the clinical application of next generation sequencing for MDR-TB is cost-effective when considered in context of the total care package required for treatment of these patients.

106. Oleksandr Povstyan

Reduced P2X receptor - mediated responses in renal vascular myocytes of spontaneously hypertensive rats.

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The aim of this work was to compare responses induced by stimulation of P2X receptors (P2XRs) with 10 μM of *αβ*-meATP in renal vascular smooth muscle cells (RVSMCs) freshly isolated from spontaneously hypertensive rats (SHR) and their normotensive control, the Wistar Kyoto (WKY) rats. RVSMCs were isolated from arcuate and interlobular arteries of rat kidney. P2XR-mediated cationic current (I_{P2X}) was recorded using amphotericin B perforated patch-clamp method. Changes of $[Ca^{2+}]_i$ in fluo-3 loaded RVSMCs were visualised using fast (33-40 Hz) *x-y* confocal imaging. Data are presented as mean ± S.E.M. The data groups were compared using unpaired Student's *t*-test. We found significant ($p<0.01$) reduction of I_{P2X} in RVSMCs from SHR: the peak current density was on average 57±7 pA/pF (n=18) in RVSMCs from SHR and 101±12 pA/pF (n=25) in RVSMCs from WKY. The peak amplitude of the *αβ*-meATP -induced $[Ca^{2+}]_i$ transients was also significantly ($p<0.001$) reduced in SHR RVSMCs: mean $\Delta F/F_0$ was 1.6±0.1 (n=89) in RVSMCs from SHR and 3.9±0.2 (n=95) in RVSMCs from WKY. Relative contribution of Ca^{2+} entry through P2XRs to the *αβ*-meATP -induced elevation of $[Ca^{2+}]_i$ was decreased in RVSMCs from SHR, while contribution of Ca^{2+} entry via voltage-gated Ca^{2+} channels was increased. In spite of decreased sarcoplasmic reticulum (SR) Ca^{2+} load (tested with 10 mM caffeine) in RVSMCs of SHR, relative contribution of the SR Ca^{2+} release to the *αβ*-meATP -induced $[Ca^{2+}]_i$ mobilisation was similar in both groups. Nevertheless, 100 μM ryanodine reduced the *αβ*-meATP -induced $[Ca^{2+}]_i$ transients significantly stronger in SHR RVSMCs, while the effect of 30 μM 2-APB was similar in both groups. Our results suggest that in SHR: (1) both Ca^{2+} entry and Ca^{2+} release following P2X receptor activation in RVSMCs were

significantly reduced and (2) the main cause of the Ca^{2+} release reduction was decrease of the SR Ca^{2+} load resulting from an enhanced ryanodine receptor - mediated Ca^{2+} leak. Reduction of P2XR-mediated signals may underlie impairment of sympathetically driven and autoregulatory responses in renal vasculature leading to glomerular damage in hypertension.
Supported by WT (042293, 074724) and & BHF (PG/08/062/25382 and FS/06/077).

107. Harry Pritchard

Ageing Circulating Endothelial Progenitor Cells in Abdominal Aortic Aneurysm
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Background: Abdominal Aortic Aneurysm (AAA) is defined as a 50% dilation of the aortic diameter. AAA rupture leads to mortality and it has been shown that rupture is preceded by a burst of medial neovascularisation. Endothelial Progenitor Cells (EPC) is a population of circulating mononuclear cells within the peripheral blood, that originate within the bone marrow, with the potential to intergrade into the endothelium in order to repair the vessel or aid in angiogenesis. Telomeres are nucleic acid domains found at the end of chromosomes, consisting of the repeating hexamer DNA sequence TTAGGG. Telomere lengths decrease at each at each cellular division and the cells become senescent once the telomere length becomes too short.
Objectives: To quantify circulating EPCs, and EPC-stimulating serum cytokine concentration, within patients with AAA and control patients. To also assess the relative telomere length within AAA and control patients. Method – Peripheral blood mononuclear cells (PBMC) were left to adhere to fibronectin and EPCs were characterised by uptake of Dil-ac-LDL and binding of FITC-Lectin. ELISA quantified cytokines and relative telomere length was established by qPCR.
Results: There was a significant increase in circulating EPC within AAA patients compared to controls. There was a trend to higher levels of VEGF and G-CSF within AAA patients. Furthermore there was a decrease in T/S ratio within AAA patients.
Conclusion: An increase in circulating EPCs within AAA patients, with a trend of an increase in VEGF and G-CSF. AAA patients also have shorter EPC telomere length, therefore closer to becoming senescent.

108. Rajendra Prasad Raghuraman

Foetal origin of cardivascular diseases: do twin infants with low birth weight have capillary rarefaction?
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Objectives: Low birth weight (LBW) has been strongly associated with an unfavourable constellation of hypertension, coronary artery disease, obesity, and insulin resistance in adulthood. The foetal origin of cardiovascular diseases (CVD) is a widely accepted concept and capillary rarefaction is one of the proposed mechanisms in LBW. Capillary rarefaction is a consistent finding in adult hypertension. We hypothesized that adverse in utero conditions and in particular placental ischaemia might impair microcirculatory growth and cause capillary rarefaction in the newborn twin babies.
Methods: We studied 13 pairs of twin infants (8 preterm, 5 at term) born to normotensive mothers (mean age: 7.17±7 days, mean birth weight; 2133±539 grams) and 38 term normal birth weight singletons born to normotensive mothers (mean age: 1.55±2.5 days, mean birth

weight: 3346±531 grams). We used orthogonal polarized spectroscopy to measure basal (ie, functional) and maximal (ie, structural) skin capillary density at the plantar surface of the infant toe. RESULTS: Twin infants had significantly higher functional capillary density (37±3.5 vs 28±6.0, mean difference of 8.9 capillaries per mm2, 95% CI; p<0.0001) and higher structural capillary density (41±3.5 vs 33±4.5, mean difference of 8.3 capillaries per mm2, 95%CI; p<0.0001) when compared to the normal birth weight term singletons. The significance in the capillary density remained the same even after adjustment for age and skin temperature. The difference in structural capillary density was much higher in preterm twins (22%) than the term twins (16%) when compared to the NBW singletons. **Conclusion:** Newly born twin infants with LBW do not have capillary rarefaction at birth. Further longitudinal studies are imperative to explore the timing of capillary rarefaction in LBW infants that occurs remote from the birth so that an early medical intervention may prevent the occurrence of CVD in LBW individuals.

109. Rajendra Prasad Raghuraman

Capillary remodelling in infants borm to hypertensive pregnancy: novel concepts of early life microcirulation

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Objective: Capillary rarefaction is pathognomonic of essential hypertension. We have previously shown that much of the capillary rarefaction in essential hypertension is due to the structural absence of capillaries. We have also shown significant capillary rarefaction in normotensive adult offspring of hypertensive parents, suggesting a familial predisposition in which capillary rarefaction represents a primary vascular abnormality that antedates the onset of sustained elevation of blood pressure. We hypothesized that infants born to mothers with hypertensive disorders of pregnancy would have significant capillary rarefaction at birth.
Methods: We studied 22 such infants (mean gestational age 37.1±3.3 weeks, mean birth weight 2585±916 grams) and compared them to 40 normal birth weight infants born at term to normotensive mothers (mean gestational age 39.7±1.1 weeks, mean birth weight 3337±525 grams). We used orthogonal polarised spectroscopy to measure basal (i.e. functional) and maximal (i.e. structural) skin capillary densities according to a well-validated protocol.
Results: We found that term infants born to hypertensive mothers had significantly lower maximal capillary density (mean difference of –5.0 capillaries/mm2; 95%CI –9.4 to –0.6, p=0.025). Conversely, preterm infants with low birth weight (LBW) born to hypertensive mothers had significantly higher basal and maximal skin capillary densities compared to term infants.
Conclusion: While these results add further support to our belief that capillary rarefaction in essential hypertension is likely to be a primary vascular abnormality; they also add novel insights suggesting that the intrauterine environment exerts powerful influences on the remodelling of the microcirculation in LBW preterm infants of hypertensive mothers.

110. Hariharan Raju

Diagnostic role of exercise tolerance testing in familial premature sudden cardiac death

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Background: Investigation of blood relatives for evidence of an inherited cardiac condition is advocated following an unexplained sudden cardiac death (SCD). Aim: We determined the

diagnostic yield of exercise tolerance testing (ETT) in investigation of inherited cardiac conditions following familial premature SCD.

Methods: Between 2006 and 2010, we evaluated 308 blood relatives of 148 SCD victims, who completed at least 3 minutes of the Bruce protocol. ETTs were analysed for: QT prolongation; Brugada type 1 pattern; ST depression: blood pressure (BP) response;multiple ventricular ectopics or arrhythmia. Individual pathological phenotypes were determined by a combination of 12-lead ECG, echocardiogram, 24-hour holter monitor, with additional MRI, CT coronary angiography and genetic mutation analysis, as appropriate.

Results: Thirty (9.8%) patients had an abnormality during ETT, details of which are summarised in Figure 1. All ETTs with abnormal QT prolongation and dynamic Brugada pattern were associated with diagnoses of long QT syndrome and Brugada syndrome respectively. An example of dynamic Brugada phenotype is given in Figure 2. Ventricular ectopy was seen in 15 patients, of whom 5 demonstrated phenotypic cardiomyopathy or channelopathy on further investigations. No patients with significant ST depression had evidence of coronary abnormalities on imaging. No hypotensive BP response was seen, but exertional hypertension was associated with systemic hypertension.

Figure 1

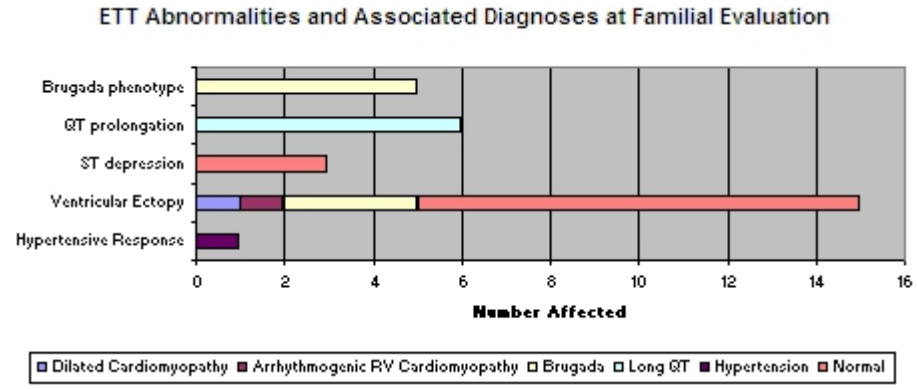
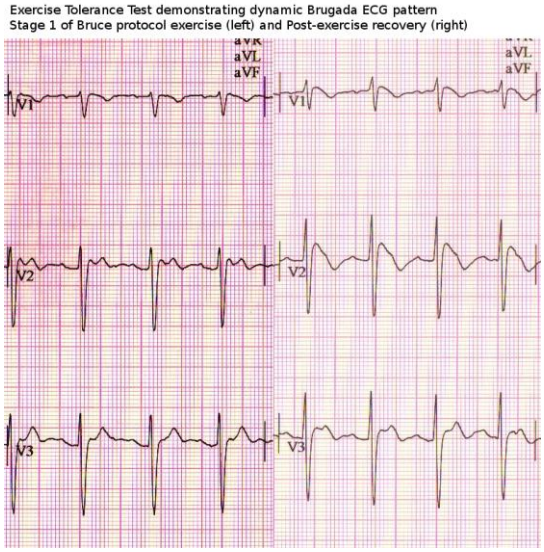


Figure 2



Conclusion: The ETT is a useful diagnostic adjunct when evaluating relatives of victims of premature SCD. Reliable diagnostic indicators include inappropriate QT prolongation and dynamic Brugada pattern. Ventricular ectopy is non-specific, but is associated with both cardiomyopathic and channelopathic processes in a significant minority. ST segment

depression, however, is unhelpful and should be viewed in the context of the patient's cardiovascular risk profile.

111. Hariharan Raju

Ethnic variation in QT interval amongst highly trained athletes

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Background: Studies in Caucasian (white) athletes indicate that a significant proportion exhibit an isolated prolonged corrected QT interval (QTc), raising concerns for potentially false diagnoses and disqualification from competitive sport. The prevalence of prolonged QTc interval in athletes of African/Afro-Caribbean (black) descent is unknown. However, this ethnic group generally exhibits a high proportion of ECG repolarization changes and increased left ventricular wall thickness, that may impact on QTc.
Aim: We aimed to assess the impact of ethnicity on QTc in young elite athletes.
Methods: We assessed 3035 elite athletes, aged 14-35 years, who were participating at national and international level in a variety of sporting disciplines. Athletes were evaluated with ECG and 2D echocardiography. Athletes diagnosed with structural heart disease or hypertension were excluded from analysis.
Results: Demographic and cardiological results are summarized in Table 1. Black male athletes exhibited shorter QTc than white male athletes, but QTc was similar amongst black and white female athletes. Bivariate analysis revealed that none of T wave inversions, ST segment elevation, or left ventricular wall thickness were associated with QTc. No ethnic difference was observed in prevalence of QT prolongation, as defined by ESC Sports Consensus criteria (male >440ms; female >460ms).

Table 1 **Characteristics of Athletes Evaluated**

	Black Male (n=901)	White Male (n=1652)	Black Female (n=122)	White Female (n=360)
Mean Age, years	22 ± 5	17 ± 4	21 ± 5	18 ± 4
Mean Heart Rate, bpm	61 ± 12	56 ± 10	63 ± 10	59 ± 9
Mean QRS duration, ms	88 ± 14	96 ± 10	84 ± 10	88 ± 9
Mean LV wall thickness, mm	10.6 ± 1.6	9.4* ± 1.2	9.2 ± 1.2	7.9* ± 2.9
ST segment elevation, n (%)	570 (63.3%)	406 (24.6%)	20 (16.3%)	64 (17.8%)
T wave inversions, n (%)	204 (22.6%)	66* (4.0%)	18 (14.6%)	15* (4.2%)
Mean QTc (Bazett's), ms	393 ± 26	404* ± 20	407 ± 25	412 ± 27
QTc >440ms, n (%)	20 (2.2%)	49 (3.0%)	13 (10.6%)	39 (10.9%)
QTc >460ms, n (%)	4 (0.4%)	7 (0.4%)	1 (0.8%)	5 (1.4%)

Means presented as mean ± standard deviation
* = $p<0.001$ white vs black athletes
Conclusion: Despite demonstrating a higher prevalence of repolarization changes and morphological left ventricular hypertrophy, black athletes do not exhibit a longer QTc than white counterparts. Based on ESC Sports Consensus criteria, prevalence of a long QTc in black and white athletes is similar, obviating the need for ethnicity specific criteria for defining a long QTc.

112. Felix Raschke

Analysis of 1H MRSI data of brain tumours using LCModel and whole tissue representations
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Abstract: Proton magnetic resonance spectroscopic imaging (1H MRSI) can determine a brain tumour's spatial varying metabolic profile, which has the potential to aid diagnosis and monitor the effects of treatment. There is still a need for an automated and easy to use analysis tool for 1H MRSI data that could return estimated tissue proportions for each voxel to assess glial brain tumour grade, heterogeneity and infiltration. In this study we apply a recently published novel methodology for the classification of single voxel 1H MR brain tumour spectra [1] to 2D short echo MRSI data of 29 brain tumour patients with eleven WHO grade II (G2), five III (G3) and thirteen IV gliomas (G4). The widespread analysis tool LCModel [2] is used to fit mean spectra (M) of different tumour grades and normal white matter (NWM) instead of individual metabolite spectra. To account for tumour heterogeneity within each tumour grade and white matter variation, a variability term (V) was calculated for each tissue type and added into the analysis. LCModel then gives an estimate of proportions of the tissue spectra for each MRSI voxel. Previous studies have shown that G3 gliomas share spectral characteristics with both G2 and G4 [3]. Therefore, for simplicity of the proof-of-principle method we only include G2 and G4 representations in the LCModel analysis calculated with principal component analysis from single voxel data used in ref. [1]. The results are displayed in RGB colormaps and histograms showing the fitted tissue proportions for each MRSI dataset with red representing G4, green G2 and blue NWM. Colormaps and tissue histograms show clear differences between G2 and G4 gliomas and abnormal voxels reaching within normal appearing brain in all cases. G3 gliomas are not clearly distinguished but show G4 characteristics. An additional G3 basis set might improve the differentiation. Further work is needed to further understand the variability within different tumour and tissue types and including additional components in the basis set and correlation of the results to other MR modalities such as diffusion tensor imaging and diffusion weighted imaging and patient outcome.
References [1] Raschke F et al. NMR Biomed in print DOI:10.1002/nbm.1753 [2] Provencher SW Magn Reson Med 30:672-679; 1993. [3] Opstad KS et al. NMR Biomed 20:763-770; 2007. Acknowledgements This work is funded by CRUK & ESPRC Cancer Imaging Programme at the Children Cancer and Leukaemia Group (CCLG), in association with the MRC and Dept of Health (England), Grant C7809/A10342.

113. Zahra Riaz

Elucidating the role of a novel gene from the 22q11 region implicated in the pathogenesis of schizophrenia
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Chromosome 22q11.2 deletion syndrome (22q11DS) is associated with a 10-fold increase in susceptibility to schizophrenia. Schizophrenia is a chronic, psychiatric disorder characterised by a variety of symptoms, which may include hallucinations, paranoia, delusions, and cognitive defects. Schizophrenia affects 1% of the world's population and imposes significant medical, social and financial burdens on individuals and society. Genetic and environmental risk factors contribute to the manifestation of schizophrenia. However, the genetic causes of schizophrenia are poorly understood and much current debate revolves around potential defects in neurodevelopment. Analysis of individual genes from the 22q11.2 region will improve our understanding of their contribution to schizophrenia susceptibility, both in 22q11DS and in the general population. The novel gene, GNB1L, lies within the 22q11.2

region and therefore one copy is deleted in almost all 22q11DS patients. In addition, some patients with schizophrenia, but without 22q11DS, have altered levels of GNB1L within the brain. Gnb1l is expressed in the developing and adult mouse brain and hemizygous deletion of Gnb1l in transgenic mice leads to behavioural changes that are also seen in patients with schizophrenia. Taken together, these data suggest a role for GNB1L in the pathogenesis of schizophrenia. The cellular function of GNB1L is unknown, but bioinformatic analysis suggests that GNB1L mediates protein-protein interactions. We have therefore used a yeast-2-hybrid assay to identify potential interacting partners of GNB1L. We have discovered that GNB1L interacts with HIPK1, a protein crucially involved in neurodevelopment and recently linked with the developmentally important wnt-signalling pathway. We are now further exploring GNB1L interaction with HIPK1, and its potential role within the wnt-signaling pathway.

114. Suman Rice

Metformin inhibits follicle-stimulating hormone action in human granulosa cells: relevance to polycystic ovary syndrome.

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Results of several studies have demonstrated that metformin can induce regular menstrual cycles and increase ovulation in women with polycystic ovary syndrome(PCOS). We have previously shown that part of the efficacy of metformin's actions is due to a direct action on ovarian steroidogenesis, by inhibition of granulosa cell expression and activity of aromatase. Metformin inhibited insulin-stimulated aromatase via activation of ERK-1,2 signalling pathway which negatively regulates CYP19 expression. However, the interaction of metformin with other pathways that are involved in aromatase production remain to be elucidated. Chief amongst these is the FSH-stimulated cAMP/PKA pathway. In order to investigate this KGN cells were cultured with 10-7M metformin (16ng/ml), FSH at 5 & 10ng/ml metformin, forskolin (FSK) at 10 & 25 M metformin for 48hrs. Reverse transcribed mRNA was quantified for aromatase and FSHR expression and normalized to L19. To investigate aromatase promoter II (PII) activity, cells were transfected with a PII-specific reporter construct as well as 5ng/ l of Renilla expression vector as a transfection control. After serum-starvation, cells were treated as described above except the FSH doses were extended to 1, 2.5 and 20ng/ml. Luciferase reporter assays were carried out using the Dual-Glo Luciferase Assay System. FSH increased aromatase mRNA expression in a dose-dependent manner which was attenuated by the presence of metformin. FSH, especially at 1ng/ml, was able to markedly enhance PII-driven aromatase expression, which was significantly reduced by metformin. However metformin did not decrease FSK-stimulated aromatase levels, indicating that the inhibitory effect of metformin on FSH action maybe upstream of cAMP signalling i.e on the FSH receptor (FSHR). Surprisingly, metformin alone was able to markedly reduce basal FSHR levels. A high FSK dose(25 M)also suppressed FSHR mRNA and interestingly the addition of metformin reduced FSHR mRNA levels even further. In the liver, metformin has been shown to act at a cellular level to phosphorylate CBP (CREB-binding protein) and bring about the disassembly of the CREB transcriptional complex, and it remains to be determined if a similar mechanism exists in granulosa cells. To conclude, metformin reduced FSH-stimulated aromatase expression and FSHR levels. This is of concern given its widespread use in anovulatory PCOS.

115. Tiziana Rossetti

The role of the SVZ in ischemic conditions

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The occlusion of a cerebral artery often results in neural deficit and/or patient death. A partial recovery frequently follows non-fatal stroke and it is possible that this may involve the activation of subventricular zone (SVZ) progenitors. To clarify the role of SVZ neurogenesis in stroke recovery, lentiviral vectors (LVs) were used to label and modulate neurogenesis in the mouse transient Middle Cerebral Artery occlusion model (tMCAo; ref. 1). We produced four different LVs, each of them carrying two expression cassettes: one encoding eGFP, and another encoding either precursor glial cell-derived neurotrophic factor (GDNF), the non-toxic C-terminal tetanus toxin fragment C (TTC; ref. 2), an shRNA targeting Cyclin D1 or an empty shRNA expression cassette (control). Vector effectiveness was previously demonstrated in vitro (GDNF, TTC) or assessed on NIH3T3 cells by western blot and growth kinetics (shRNA against Cyclin D1). 8 to 10-week-old male mice were used for in vivo work; animals were injected in the SVZ with one of the various LVs and two weeks later some of the animals underwent tMCAo. The optimal duration of tMCAo was assessed as 1 hour in preliminary experiments. LVs labelled the SVZ and progenitor migration effectively by eGFP expression, in physiological as well as pathological conditions. Sensory-motor recovery was monitored for 30 days post-tMCAo surgery, and afterwards brain histology was performed. Survival and neurological assessment of tMCAo animals injected with LVs, showed some positive trends in treatments expected to induce SVZ expansion. In addition, sensory-motor recovery was significantly decreased for the shRNA_CyclinD1 group (* $p<0.05$). Immunofluorescence analysis demonstrated a predominance of Iba1 and DCX expressing cells produced by the SVZ after tMCAo.

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116. Jonathan Round

Can chest radiographs predict outcome in oncology patients admitted to paediatric intensive care?

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Studies have shown that children with malignancies admitted to paediatric intensive care (PICU) have higher mortality and morbidity. A retrospective case control study was used to identify differences in chest radiograph (CXR) appearance at admission to PICU of oncology patients who died compared to survivors. Unplanned oncology admissions who died on PICU between 2004 to 2009 (11 patients) were compared with unplanned oncology admissions who survived their PICU episode and were matched for age and sex over the same time period (20 patients). Admission CXRs were retrieved from PACs database, anonymised, and reported by a consultant paediatric intensive care specialist. Comparison of CXRs showed that consolidation or opacity in ≥ 3 zones was significantly higher in the non- survivor patients compared to survivors (63.6% vs 15% ($p = 0.013$) and 90.9% vs 45% ($p = 0.02$) respectively. Admission CXRs may be useful as a tool to predict mortality, guide treatment and assisting research.

117. Claire Ryall

Investigation into the role of secreted protein, acidic and rich in cysteine (SPARC) in beta cell growth and signalling

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Introduction: Pancreatic islet transplantation is an experimental treatment for patients with diabetes. Expansion of primary islet beta cells in vitro for potential transplant use is currently limited with present protocols. Identification of novel regulators of beta cell growth would improve this approach. SPARC (Secreted Protein Acidic and Rich in Cysteine), an extracellular protein, modulates cell-matrix and cell-cell interactions. SPARC can regulate cell growth and motility, but a role for SPARC in the regulation of pancreatic beta cell growth has not yet been examined.
Methods: The sulphorhodamine B (SRB) assay measuring total protein content was used to determine the effect of SPARC treatment on insulin-like growth factor (IGF)-1- and hepatocyte growth factor (HGF)-induced INS-1 cell growth. Western blot analysis was used to determine the effect of SPARC on IGF-1- and HGF-induced Akt and extracellular signal-regulated kinase (ERK) activation in INS-1 cells.
Results: In INS-1 cells, SPARC treatment (20μg/ml) inhibited IGF-1- and HGF- induced cell growth by approximately 20 per cent ($P < 0.01$).Western blot analysis revealed that SPARC (2.5μg/ml) reduced IGF-1 stimulated Akt phosphorylation to basal levels. In contrast, SPARC did not affect HGF-stimulated ERK phosphorylation in INS-1 cells at the concentrations used.
Conclusions/Summary: Our results show that SPARC inhibits IGF-1- and HGF- induced proliferation of INS-1 beta cells. We have further shown that SPARC inhibits IGF-1-induced Akt phosphorylation in INS-1 cells. SPARC is therefore a novel regulator of beta cell growth.

118. Tariq Sadiq

Can point-of-care antimicrobial susceptibility testing help contain the spread of resistance in Neisseria gonorrhoeae?
Howell-Jones R, Lowndes CM, Ison C, Pond M, Dave J, Butcher PD, Sadiq ST on behalf of eSTI2 consortium.
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Antimicrobial resistance is a major problem in the treatment and prevention of Neisseria gonorrhoeae. Increasing levels of resistance drive empirical treatment towards more expensive and broad spectrum antimicrobials and will likely lead to combination and more prolonged therapies in the near future. Advances in rapid molecular diagnostic technologies and improved understanding of the genetic determinants of resistance are now making genetic based antimicrobial susceptibility tests feasible. The majority of gonococcal infections in England and Wales remain susceptible to penicillin (79%) and ciprofloxacin (65%) (data from 2009). Whilst demographic and behavioural variables (e.g. gender, sexuality, sexual contact abroad) may be of value in immediately identifying subgroups with sufficiently high susceptibility to enable safe empirical use of these antibiotics, if available, point-of-care susceptibility tests might be a more accurate, acceptable and economically effective approach. Through improved clinical management and by complementing the use of new treatment regimens, susceptibility tests could potentially impact on antimicrobial

resistance selection pressures. The eSTI2 consortium is building translational capacity, in collaboration with industry, to develop point-of-care tests for both STI diagnosis and antimicrobial susceptibility for N gonorrhoeae. There will be many challenges in the development pathway for these tests including technological innovations, determining optimum use of markers and how best to integrate tests into clinical care. Phenotypic susceptibility testing of cases will remain important as the molecular basis of resistance to many antimicrobials is not fully understood and new resistance mechanisms continuously evolve. This poster has been previously presented at the Health Protection Conference 2011.

119. Cynthia Sam

Chamber specific expression of sarcoplasmic reticulum Ca2+-ATPase (SERCA2a) in healthy and diseased rat hearts

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Background and Aim: Depolarisation of cardiac cells causes calcium (Ca2+) release from an intracellular store known as the sarcoplasmic reticulum (SR). The transient elevation in intracellular Ca2+ concentration that results, causes cardiac contraction. SERCA2a allows cardiac relaxation to occur when it pumps Ca2+ back into the SR thus refilling the Ca2+ store for the next contraction. The abundances of SERCA2a differ in atria and ventricles of various species since relaxation times are shorter in atria (Freestone et al, 1999). We show that SERCA2a expression in healthy and diseased rat hearts is differently distributed in specific heart chambers.

Methodology: Left and right chambers of atria and ventricles from rats induced to manifest heart failure (shunt) and sham operated Wistar rats, as well as spontaneously hypertensive rats (SHR) and their normotensive controls (WKY) were snap frozen and homogenised. The aorto-caval shunt was used to induce heart failure via volume overload (Scheuermann-Freestone et al, 2001). Western blotting was carried out and the protein abundance was quantitatively determined.

Results: Sham operated animals have a higher abundance of SERCA2a than shunt operated animals although this did not reach statistical significance in atrial tissue (P=0.07 in atria; P=0.03 in ventricles). The left atria of sham operated animals showed the highest abundance of SERCA2a (29% of total SERCA2a). WKY left ventricular heart tissues showed a slightly raised abundance of SERCA2a (1.26 ± 0.13 densitometer units) compared to SHR (1.01 ± 0.13 densitometer units). Ventricular tissue possesses less SERCA2a than atria in both normal and pathological animal populations.

Conclusion: SERCA2a is expressed in a chamber specific manner in the cardiac tissue samples of both healthy and diseased heart. The lower abundance of SERCA2a in heart failure tissues is due to the alterations in Ca2+ handling proteins that results in degradation of the cardiac pump function (Spann et al, 1967). SERCA2a is also expressed more in WKY compared to SHR for all four heart chambers which relates to less Ca2+ release in the SHR animals and consequently less forceful contractions of the hypertrophied heart (Li et al, 2005). We would like to thank the BPSRG Kingston University and acknowledge Dr Roland Vetter and Dr Andrew Snabaitis

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mouse due to chronic volume overload. Eur. J. Heart Fail. 3, 535-543. J.F. Spann Jr, R.A. Buccino, E.H. Sonnenblick, E. Braunwald. (1967) Contractile state of cardiac muscle obtained from cats with experimentally produced ventricular hypertrophy and heart failure. Circ. Res. 21 341-354. S-Y Li, K.L Golden, Y. Jiang, G-J Wang, J.R Privratsky et al. (2005) Inhibition of sarco(endo)plasmic reticulum Ca2+-ATPase differentially regulates contractile function in cardiac myocytes from normotensive and spontaneously hypertensive rats. Cell Biochem and Biophys. 42, 1-12.

120. Sebastian Sethe

Research and the Law
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Legal, ethical and commercial considerations shape research trajectories in significant ways. This poster gives an overview over the main legal, regulatory, ethical and strategic issues that researchers are advised to consider, highlights some common issues, and identifies some recent legal and regulatory developments in research governance.

121. Iltaf Shah

A novel LC-MS/MS assay to account for epimers, isobars of vitamin D and its application to Alzheimer's disease.
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Background: Insufficient vitamin D levels have been linked to a wide range of diseases including several major neurological conditions, including the putative roles and levels of vitamin D in dementia. Owing to inter laboratory, inter method variations, overlapping peaks and identical masses of epimers and isobars, considerable discrepancies in results are frequent. The primary aim of this study was to accurately identify and quantitate vitamin D metabolites through chromatographic separation from its epimers and isobars and to investigate the relationships or differences between vitamin D forms and cognitive state (MMSE scores), along with calcium levels.
Methods: Blood samples were collected from 5 healthy male Caucasian volunteers and vitamin D metabolites along with co-eluting epimers and analogues were quantified. AD subjects were grouped as A: untreated (n= 26) and B: treated with acetylcholinesterase inhibitors (AChEI) (n= 44) and C: control (n= 35), based on the cognitive function and treatment. Levels of vitamin D metabolites along with epimers and isobars were measured using liquid chromatography-mass spectrometry (LC-MS) and calcium measurements were conducted using inductively coupled plasma-mass spectrometry (ICP-MS).
Results: The new method allowed chromatographic separation and quantification of vitamin D metabolites, along with their epimer 3-epi-25OHD3 and isobars 1-∝-hydroxyvitamin-D3 (1∝OHD3), and 7∝hydroxy-4-cholesten-3-one (7∝C4).
In AD subjects, no relationship was observed between MMSE score, calcium and any form of vitamin D. There was no significant difference in calcium levels among the three groups. For Group A, the levels of both total 25-hydroxtvitamin-D (25OHD) (14.79 +/- 13.31 nmol/mL,)

and 25-hydroxyvitamin-D2 (25OHD2) (undetected) were lower compared to the control (40.50 +/- 23.46 and 9.58 +/- 6.23 nmol/mL) and treated groups (32.89 +/- 28.58 and 6.84 +/- 7.86 nmol/mL) p=0.004 and p<0.001, respectively. No significant differences were found in the levels of the primary forms vitamin D2 and vitamin D3.
Conclusions: This specific, reliable, reproducible and robust method provided valuable insight into the vitamin D status of AD sufferers with 25OHD being significantly lower in patients suffering from AD arising from extremely low levels of 25OHD2 along with low levels of 25OHD3**.** Treatment with AChEI appears to reverse this deficit.

122. Syeda Amena Shah

Quantitative analysis of mephedrone and its metabolites using liquid chromatography tandem mass spectroscopy: application to human hair
Syeda A.B. Shah, Nawed I.K. Deshmukh, James Barker, Andrea Petroczi, Paul Cross, Roland Archer, Declan P. Naughton
School of Pharmacy and Chemistry, Kingston University, London, UK School of Life Sciences, Kingston University, London, UK School of the Environment, Natural Resources and Geography, Bangor University, United Kingdom
Recent abuse of designer drugs such as mephedrone has presented a requirement for the introduction of sensitive, reliable and reproducible methods for the detection of these controlled drugs in different matrices. Hair analysis can provide information on the prior use of drugs. This study focuses on the development of a validated method for the quantitative analysis of mephedrone and its two metabolites; 4-methylephedrine and 4-methylnorephedrine in human hair. The calibration curve was found to be linear in the range 5 pg/mg to 100 pg/mg for mephedrone and 10 pg/mg to 150 pg/mg for 4-methylephedrine and 4-methylnorephedrine. Mephedrone had an R2 value of 0.999 with 0.990 for its two metabolites. The method was successfully validated for the intraday precision, interday precision, limit of detection (LOD), accuracy and extraction recovery. The LOD and lower limit of quantification (LLOQ) for mephedrone were 2.5 pg/mg and 5 pg/mg respectively. For both 4-methylephedrine and 4-methylnorephedrine, the LLOD was 5 pg/mg and the LLOQ was 10 pg/mg. Five out of 154 hair samples were confirmed to be positive for mephedrone. Out of those five samples, mephedrone could be successfully quantified in only one sample, but detected in the other four samples. The proposed metabolism for mephedrone was derived by following a similar pathway that has been used previously for methcathinones and other methamphetamines. The outlined method can be valuable for future long term detection of mephedrone and its two metabolites in hair.

123. Tanya Shaw

MALDI mass spectrometry imaging reveals a significantly altered proteome during wound repair and scar formation
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Scar formation is the inevitable and currently untreatable consequence of tissue damage, and misregulation can lead to the development of pathological scarring as in hypertrophic and keloid scars. In developed countries alone, it is estimated that around 100 million people each year will be left with a scar following surgery; moreover, there are thought to be 11 million people with keloid scars. Despite the high incidence and debilitating nature of both normal and keloid scars, we still lack effective treatments. The lack of understanding about exactly which molecules are contributing to, or are responsible for, scar formation is impeding

progress in developing targeted therapeutics for their treatment. This project used MALDI Mass Spectrometry Imaging to identify a signature of proteins expressed during scar formation, and we have the ultimate goal of using this approach to identify novel molecules implicated in skin fibrosis. Scars resulting from excisional skin wounds made to the dorsal skin of adult male mice were collected at 7 or 14 days post wounding, freshly frozen, cryosectioned, coated with an energy absorbing matrix optimised for the detection of proteins in the 2-20kDa range, and irradiated with a nitrogen laser beam at 80 micron intervals across the tissue sections. The resulting mass spectra revealed a significantly and reproducibly altered proteomic signature in scarred versus normal dermis. Specifically, proteins having mass-to-charge values (m/z; approximately equivalent to mass in Daltons) of ~2797, ~4743, ~6284 and ~6657 were expressed at relatively higher intensities in scar vs normal tissue on Day 7, and the up-regulation of 3 of these 4 peaks had resolved by Day 14. Peaks at m/z ~4970 and ~6284 showed a significant up-regulation in scar tissue on Day 14 post wounding. On-going work is focused on: 1) identification of the protein(s) represented by the differentially-expressed peaks; 2) extrapolation and confirmation of our findings in human scar tissue; and 3) comparison of the "scar signature" observed in skin to other fibrotic tissues. The results of this work are hoped ultimately to be of prognostic, diagnostic, and/or therapeutic value.

124. Nabeel Sheikh

Do we need ethnicity-specific guidelines for pre-participation screening of athletes?
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Aims: Physical activity is associated with ECG phenotypes that overlap with those observed in conditions predisposing to sudden cardiac death. In 2005 European guidelines were produced to help differentiate ECG changes reflecting physiological adaptation to exercise from those that should prompt further investigations. These were updated in 2010 resulting in improved specificity in predominantly Caucasian cohorts (white athletes; WA). We sought to examine the performance of the 2010 guidelines in athletes of African/Afro-Caribbean origin (black athletes; BA).
Techniques: Electrocardiograms of 923 male BA were evaluated to determine the proportion of individuals requiring further investigations based on 2005, compared to 2010 guidelines. The same evaluation was performed for a cohort of 1711 male WA and 209 patients with hypertrophic cardiomyopathy (HCM). "Refined Criteria" were also evaluated, consisting of an upper limit of 470 msec for QTc and removing the following as abnormalities: 1. isolated voltage criteria for left atrial enlargement (LAE); 2. Isolated voltage criteria for right ventricular hypertrophy (RVH); 3. T-wave inversions (TWI) in V1/2 in WA and V1-V4 in BA.
Results: Using 2005 guidelines, 549 BA (59.5%) had a positive ECG requiring further investigation compared to 846 WA (49.4%). In comparison, 398 BA (43.1%) had a positive ECG using 2010 guidelines versus 216 WA (12.6%). All HCM patients met the criteria for a positive ECG, regardless of the guidelines used. Using "Refined Criteria", the number of BA with a positive ECG fell to 161 (17.4%) and WA to 93 (5.4%). Five patients with HCM had ECG LAE (2.4%); all were symptomatic apart from 1 (0.5%). Five patients with HCM ECG LAE in combination with LVH but no other abnormalities on their ECG; all were symptomatic apart from 1 (0.5%).
Conclusions: Updated guidelines significantly reduce the number of positive ECG results in

WA, but less so in BA, emphasizing the need for ethnicity specific criteria to be developed. Refining criteria based on exercise-related physiological changes results in further reduction in positive ECGs. Findings in patients with HCM suggest that isolated ECG LAE, or in combination with ECG LVH alone, may be regarded as physiological rather than pathological in asymptomatic athletes.

125. Nabeel Sheikh

Ethnic Differences in the Phenotypic Expression of Hypertrophic Cardiomyopathy
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Purpose: Hypertrophic Cardiomyopathy (HCM) is a heterogeneous condition with variable phenotypic expression. Current studies are based on predominantly Caucasian cohorts (white patients; WP), and the phenotypic manifestations of HCM in individuals of African/Afro-Caribbean origin (black patients; BP) are not fully realized. Data in athletes and hypertensive patients indicate that black ethnicity is associated with a greater prevalence of repolarisation abnormalities on the ECG as well as a greater magnitude of left ventricular hypertrophy (LVH), highlighting the importance of defining the HCM phenotype in this ethnic group.
Methods: Between 2001 and 2010, 155 consecutive patients with HCM (52 BP, 103 WP) were assessed in 3 specialist cardiomyopathy clinics. All underwent comprehensive evaluation including 12 lead ECG and echocardiography.
Results: Black patients exhibited significantly different echocardiographic patterns of LVH, with more concentric (44.2% vs. 30.1%) and apical (28.8% vs. 11.7%) hypertrophy compared to WP, who exhibited more asymmetric septal hypertrophy (57.3% vs. 25.0%) (p=0.004). Black patients exhibited a similar magnitude of LVH compared to WP (maximum left ventricular wall thickness 17.3±4.9 mm vs. 18.8±4.1 mm, p=0.069). Relating to ECG repolarisation abnormalities, BP exhibited significantly more T wave inversions in the lateral leads (76.9% vs. 60.2%, p=0.038) and deep (≥ 0.2 mV) T wave inversions (69.2% vs. 51.5%, p=0.035). Black patients also displayed more ST segment depression (50.0% vs. 35.0%, p=0.071), although this was not statistically significant. In contrast, WP had significantly more pathological Q waves (23.3% vs. 9.6%, p=0.039).
Conclusions: Ethnicity appears to exert a significant effect on the ECG and echocardiographic patterns in patients with HCM. A significant proportion of black patients exhibit concentric LVH, highlighting the diagnostic challenges in distinguishing HCM from hypertensive heart disease and physiological adaptation to exercise in black individuals. The greater prevalence of deep T wave inversions and T wave inversions in the lateral leads underscores the importance of further evaluation of black individuals with such ECG repolarisation abnormalities.

126. Nabeel Sheikh

Ethnic differences in repolarisation patterns and left ventricular remodelling in highly trained male adolescent (14-18 years) athletes
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Purpose: Studies in adult, black athletes (BA) demonstrate a high prevalence of ECG repolarisation changes and echocardiographic left ventricular hypertrophy (LVH) that may overlap with hypertrophic cardiomyopathy (HCM). The prevalence of ECG repolarisation

changes and echocardiographic LVH in adolescent BA, the group most vulnerable to exercise-related sudden death from HCM, is unknown.
Methods: This study evaluated 219 male adolescent BA (14-18 years, inclusive) with 12-lead ECG and 2-D echocardiography. Results were compared with 1440 male adolescent WA. Athletes with T wave inversions and morphological LVH were invited for further investigations.
Results: ST segment elevation was more frequent in BA (63.5% vs. 14.9%, $p<0.001$), while ST segment depression was exceedingly rare in both groups. T wave inversions (21.5% vs. 2.9%, $p<0.001$) and deep T wave inversions (11% vs. 0.3%, $p<0.001$) were commoner in BA. Black athletes demonstrated greater left ventricular wall thickness (10.4±1.6 vs. 9.4±1.2 mm, $p<0.001$) compared to WA. Twenty-three (10.5%) BA exhibited a left ventricular wall thickness >12 mm versus only 6 (0.4%) WA ($p<0.001$). None of the athletes exhibited the broader phenotype of HCM on further investigation. In multivariable analysis black ethnicity was the strongest independent predictor for the presence of T wave inversions (OR 3.56, 95% CI 1.56-8.13, $p=0.003$) and LVH (OR 3.17, 95% CI 1.77-5.71, $p<0.001$).
Conclusions: As with adult athletes, T wave inversions and LVH were more prevalent in adolescent BA compared to WA. These findings have important implications in the pre-participation screening era since extrapolation of ECG and echocardiographic criteria, solely derived from Caucasian cohorts, would result in 25.6% of BA requiring further investigations for cardiac pathology.

127. Vishvesh Shende

Astroglial plasticity is implicated in hippocampal remodelling in adult rats exposed prenatally to dexamethasone

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Prenatal dexamethasone treatment is associated with neurodevelopmental abnormalites and increased risk of neuropsychiatric disorders in adulthood (Yossuck et al., 2006). We investigated the long-term effects of prenatal dexamethasone administration on hippocampal remodelling in 3 months old male Sprague-Dawley rats whose mothers were treated with dexamethasone (E16-E19, 0.5µg/ml in drinking water) (McArthur et al., 2007). Dexamethasone-treated (DEX) and control (drinking water without dexamethasone) groups (each n=6) were compared. All animal procedures were carried out in accordance with the United Kingdom Animals (Scientific Procedures) Act of 1986. Total brain volume was measured by means of peripheral quantitative computed tomography (Stratec Medizintechnik, Germany). Hippocampal volume was estimated by means of Cavalieri principle (Stereoinvestigator, MicroBrightField Inc) and expressed as % of total brain volume. The optical fractionator method was used to estimate cell numbers in the hippocampal CA1, CA2, CA3 and dentate gyrus (DG). GFAP-immunoreactive astroglial cell morphology was investigated. Primary processes were traced using Neurolucida and their length and numbers were estimated and further analysed by Neurolucida Explorer MicroBrightField Inc). The efficiency and reproducibility of sampling and volume estimation were checked by Gundersen coefficient of error and coefficient of variation. Total brain volume was not affected by the treatment. The relative volume of the dorsal hippocampus showed a moderate, by 8%, but significant reduction in DEX vs control ($p<0.005$). Dexamethasone had no effect on the cell density in the CA1, CA2, CA3 and DG. Morphological analysis indicated that numbers of astroglial primary processes were not affected in any of the hippocampal subregions analysed but significant reductions in the total primary process length were observed in CA1 by 32% ($p<0.005$), CA3 by 50% ($p<0.001$) and

DG by 25% ($p<0.01$). Mean primary process length values were also significantly decreased in CA1 by 25% ($p<0.005$), CA3 by 45% ($p<0.001$) and DG by 25% ($p<0.005$). No significant astroglial morphological changes were found in CA2. We conclude that the observed reduction in dorsal hippocampal volume and morphological impoverishment of astroglia, consistent with their atrophy, reflect long-term changes in astroglial plasticity in response to prenatal dexamethasone exposure.

128. Zhanzhong Shi

Erythropoietin-induced activation of Rho/ROCK/MLC2 in a model of tumourigenesis
Shi ZZ, Yuen HF, Percy MJ, Dunlop EA, El-Tanani MK, Lappin TRJ
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Erythropoietin (Epo) is the major regulator of erythropoiesis and its cognate receptor (EpoR) has been shown to be functionally active in non-erythroid tissues, including tumours. Results of recent meta-analyses have raised concerns about the potential adverse effects of erythropoiesis stimulating agents (ESAs) for the treatment of anaemia in cancer patients. We have modified a benign non-invasive rat mammary cell line, Rama37, which expresses low levels of endogenous EpoR, to constitutively express high levels of human EpoR (Rama37-28). In these cells pharmacological levels of Epo (10 U/ml) activated three major signalling pathways (Jak2/STAT5, PI3K/Akt and Ras/ERK) associated with cell survival and proliferation. Increased cell invasion, migration, adhesion and colony formation were found in the Rama37-28 cells compared to Rama37 parental controls. To determine if Epo directly influences cell invasion via Akt we investigated Rho GTPase activity, which plays a fundamental role in regulating cell morphology, adhesion and motility. Myosin light chain 2 (MLC2) is phosphorylated by ROCK, a Rho kinase, and can be used as a marker for cell invasion. Epo was found to increase phosphorylation of MLC2 in Rama37-28 cells but not in Rama37 parental cells. It is known that aberrant expression of ROCK is related to tumour metastasis and poor clinical outcome, and it is plausible that exogenous ESAs may enhance tumour metastases through this pathway.

129. Zhanzhong Shi

Silencing of ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1) in acute leukaemia cell line
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Ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1) is an essential member of the UCH family which plays important roles in cell proliferation, cell cycle control, apoptosis and cell signalling, etc. Previous studies in our group using suppression subtractive hybridization had found that UCH-L1 was undetectable in acute myeloid leukaemia cell line UT-7 cells while it was over-expressed in non-small cell lung carcinoma cell line H838 cells, and over-expression of UCH-L1 in H838 cells was related to increased tumour metastasis and anti-apoptotic properties. But the mechanisms underlying the silencing of UCH-L1 in UT7 cells has not been elucidated. Studies from other groups have shown that UCH-L1 gene expression was silenced by DNA hypermethylation in tumours such as colorectal carcinoma and

multiple myeloma cells. In the current study, UT-7 cells were treated with 5'-azacytidine (5'-Aza) for up to 4 days. The alteration of expression of UCH-L1 at mRNA level was detected by real-time quantitative reverse-transcription polymerase chain reaction (Q-PCR). The effect of 5'-Aza on UT-7 cell survival was evaluated. It was shown that the expression of UCH-L1 in UT-7 cells treatment with 5'-Aza (5 μM) for 4 days increased by 2.59 × 105 fold compared to that of 0 hour control as determined by Q-PCR (Ct values 39.55 ± 0.79 vs 20.6 ± 2.36) ($p = 0.0099$), but similar to the positive control, H838 cells (Ct values 20.6 ± 2.36 vs 19.71 ± 0.15). More apoptotic cells were detected in UT-7 cells treated with 5'-Aza (5 μM) for 6 days compared to the time match control as determined by flow cytometric analysis (42.3% vs 9.8%). In conclusion, DNA hypermethylation leads to UCH-L1 silencing in UT-7 cells and might provide further molecular basis of demethylation therapy for acute myeloid leukaemia.

130. Vikram Sinai Talaulikar

A novel hysteroscopic technique for the accurate biopsy of decidua parietalis and basalis: Implications for progress in research on the early events of human placentation.
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Background: A better understanding of the biology of human implantation and early placentation is not only desirable from a purely scientific point of view, but it could shed light on a number of disorders of pregnancy that include miscarriage, intrauterine growth restriction and preeclampsia. Discrepancies in the results from studies of early events in human trophoblast invasion of decidua have been acknowledged and research into such early pregnancy events is hampered by ethical constraints, poor access and the accuracy of biopsy material. We describe a novel technique of biopsy that overcomes the issue of accuracy of biopsy.
Methods & Results: The technique is applicable to pregnancies undergoing first trimester surgical termination. Following cervical dilatation, a rigid hysteroscope is introduced into the cervical canal. The pressure of the saline distending medium shears the membranes of the gestation sac away from the decidua parietalis, leaving the pregnancy suspended at the site of the early placenta. Under direct vision a biopsy forceps is used to sample the decidua parietalis, and then the forceps in introduced beneath the gestation sac to sample the decidua basalis. Morphological and immunohistochemical studies with anti-cytokeratin antibodies have confirmed the accuracy, purity and adequacy of the samples, with a high (40%) myometrial spiral artery presence.
Conclusion: This is a simple and safe novel technique of decidual biopsy under direct vision which allows for high accuracy of biopsy material, and therefore has the potential to revolutionise research on trophoblast-decidua interactions during the critical early stages of human pregnancy.

131. Naim Slim

Mechanisms underlying pre-ischaemic conditioning in the caudate nucleus
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Fast cyclic voltammetry can be used to measure dopamine release after oxygen and glucose deprivation (OGD) induced anoxic depolarization in vitro. Here we measure dopamine efflux with 1 second time resolution, which is appropriate to measure OGD-evoked dopamine efflux accurately. In the present study, we show that OGD-evoked dopamine efflux could be used to show pre-ischaemic conditioning in the rat caudate brain slice and we further attempt to

clarify the receptor mechanisms involved in this phenomenon. Male adult rat caudate slices were exposed to 0 or 10 min OGD pre-ischaemic conditioning, then 60 min later exposed to a second OGD event of 15 min duration. We measured the OGD-evoked dopamine efflux. 10 min OGD pre-ischaemic conditioning resulted in a longer time to onset of OGD-evoked dopamine efflux on the second OGD event (475±31 and 287±30s for 10 Vs 0 min pre-ischaemic conditioning respectively). Further, 10 min OGD pre-ischaemic conditioning resulted in less dopamine efflux on the second OGD event (4.23±1.12 and 8.14±0.82 □M for 10 Vs 0 min pre-ischaemic conditioning respectively), despite these slices having similar tissue dopamine content and DOPAC/DA ratio, and the rate of dopamine release was slower in the main OGD event (21±5 and 74±8nM/s for 10 Vs 0 min pre-ischaemic conditioning respectively). These data suggest that 10 min OGD pre-ischaemic conditioning can evoke tolerance to a second OGD event. We further explored potential mechanisms underlying PIC by incubating the brain slice in antagonists for 1) adenosine A2 receptors (50 µM caffeine); 2) delta opioid receptor (1 µM naltrexone); dopamine D2 receptor (metoclopramide 1 µM) and the glutamate NMDA receptor (MK801 10 µM). Our data show that antagonists at the dopamine D2 and NMDA receptors attenuate the pre-ischaemic conditioning effect in the caudate nucleus. These data suggest that agonists at these receptors could evoke a chemical preconditioning that evokes tolerance to a future anoxic event and could be the basis for future pharmacotherapy for stroke.

132. Nidhi Sofat

What makes osteoarthritis painful? Evidence of central sensitization in a cohort of subjects with hand osteoarthritis

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Background: Hand osteoarthritis (OA) is a prevalent condition in which most patients report chronic pain despite current treatments. We hypothesised that local joint changes in OA enhance sensitivity and firing of peripheral nociceptors, thereby causing central sensitisation and chronic pain. Objective: To evaluate the characteristics of pain perception in a cohort of hand OA participants to assess if central sensitisation contributes to pain perception.
Methods: Participants with proximal and distal interphalangeal joint (PIP/DIP) hand OA and non-OA controls were recruited. VAS pain scores, HAQ, Kellgren-Lawrence scores for radiological severity and pain thresholds using algometers were assessed. Central brain pain processing was evaluated using functional brain neuroimaging (fMRI) whilst subjects performed a finger flexion-extension (FFE) task. Functional MRI data was analysed using FMRIB software.
Results: All hand OA participants reported pain despite 92% taking oral analgesic drugs. The mean VAS in hand OA participants was 59.31 mm +/- 8.19 compared with 4.00 mm +/- 1.89 in the control group. Hand OA participants also had HAQ scores 8-fold higher than controls, indicating high levels of functional impairment. Objective measures of pain using algometers on 30 hand joint regions per subject (15 joint regions per hand) showed lower pain thresholds in the OA group versus controls ($p<0.0001$). There was a global reduction in pain threshold in the hand OA group despite the main radiological changes being found in the proximal and distal interphalangeal joints. Pain threshold in the OA group did not vary significantly with increasing radiological severity. Brain functional MRI during the painful FFE task demonstrated increased activation of the thalamus, cingulate gyrus, frontal and somatosensory cortex in the hand OA group that was not observed in the control group ($p < 0.05$). The activated regions observed by fMRI in the hand OA group also showed a positive correlation with patient-reported VAS scores that mapped to pre-frontal brain regions.

Conclusions: Hand OA subjects are sensitised to pain due to increased firing of peripheral nociceptors. Our findings suggest that specific brain regions mediate central sensitisation. Such information could be used to develop novel therapeutic strategies for treating pain in hand OA.

133. Matthew Szarko

Ankle Position Affects the Exposure of the Dorsalis Pedis Artery in Ankle Arthroscopy
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Karia P, Szarko M. Department of Anatomy, Division of Biomedical Sciences, St. George's, University of London Nathdwarawala Y, Department of Orthopaedic Surgery, Nevill Hall Hospital
Anterior ankle arthroscopy currently provides the best chance of restricting local anatomy damage during ankle surgery. The anterior working area (AWA) of the ankle is restricted by the Dorsalis Pedis Artery (DPA) and the extensor muscle tendons when the procedure is conducted both in dorsiflexion and plantarflexion. During surgery, iatrogenic damage to the DPA can lead to the formation of a pseudoaneurysm, which can be difficult to identify intraoperatively. Our study investigates whether dorsiflexion or plantarflexion provides variability in the movement the DPA to determine the positions at which anterior ankle arthroscopy provides the greatest anterior working area (AWA) without causing vascular damage. The current study expects the distance of the DPA from the inferior border of the medial malleolus (IBMM) (ankle joint) to be greater on ankle dorsiflexion than in ankle plantarflexion.
Materials and Methods: Twelve cadaver ankles embalmed with a mixture of phenol and glycerol, allowing greater motion, were dissected to access the DPA. The ankles, while in a distracted position (in accordance with common surgical practice), were forced into dorsiflexion from a plantarflexion position at 5° intervals. The distance between the IBMM and the DPA was measured at the 5° intervals.
Results: The mean amount of ankle flexion achieved was 24.58° (Range = 20-35). All twelve ankles showed positive range of movement (ROM) anteriorly from the IBMM with a mean ROM of 3.58mm (SE = 0.29mm) dorsiflexion.
Discussion and Conclusion: Anterior movement of the Dorsalis Pedis Artery during dorsiflexion puts it at a lower risk of iatrogenic damage in a dorsiflexed position compared to plantarflexion. The increased AWA allows the surgeon more manoeuvrable space, possibly allowing the use of larger diameter surgical instruments.

134. Kinga Szewczyk

Visualisation of the Apical Surface of Activated Osteoclasts
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Bone resorption occurs at the substrate-apposed, apical surface of osteoclasts, a surface that has not been accessible to direct visualisation. We have recently shown that it is the high affinity of bone mineral for vitronectin-receptor ligands that endows bone with the ability to activate osteoclasts. Consistent with this, osteoclasts secrete acid hydrolases and develop a ruffled border, as they do on bone, when they are incubated on vitronectin-coated plastic. Therefore, osteoclasts can be activated for resorption by substrates other than bone. We have developed a novel approach to inspect the substrate-apposed surface of cells. To do this, cells are sedimented onto glass coverslips that have previously been coated with nail varnish. After incubation on such coverslips, the discs of nail varnish, with attached cells, are inverted onto a glass slide, and the nail varnish is removed with acetone. This exposes the underside of

the cells, which can be inspected in the scanning electron microscope. To analyse the substrate-apposed surface of activated osteoclasts, the nail varnish was coated with either vitronectin, or neonatal calf serum as a source of vitronectin. After incubation, the underside of osteoclasts showed a striking appearance. The whole undersurface was closely applied to the substrate, as it is on bone, in most osteoclasts. The central region was sometimes covered by fine, finger-like processes, often flattened against the substrate. More commonly the processes formed islands, or rings at the periphery of the central area. We also noted distinctive sucker-like structures of different sizes and shapes in almost all cells. Some of the central regions showed compartmentalisation, with compartmental walls similar in appearance to the stalks of 'suckers'. In some ruffled border regions we saw orifices, perhaps reflecting endocytic or exocytic processes. The circumferential region appeared flat and featureless. However, if osmium tetroxide was omitted from fixation, rings, crescents and ridges of nodular protrusions, corresponding in position with podosomes, were observed circumferentially. The surface between and adjacent to podosomes, overlying the peri-podosomal cytoskeletal network, was extensively pitted. This approach provides an exciting opportunity to characterise the morphological correlates of the resorptive process in osteoclasts.

135. Behnam Tahmassebypour

Characterization of prostate acinar-like structures in a 3D model that mimics the prostate microenvironment

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Most prostate adenocarcinomas have their origins in preneoplasic lesions in the acinar structures of the prostate. We have set up a morphogenesis assay in micro-wells filled with a matrix mimicking the basal membrane that allows monitoring the formation/disruption of prostate acinar-like structures from single prostate cancer cells by time-lapse and confocal microscopy. Our current results show that the highly metastatic prostate cancer cell line PC3 is able to organize in tubules resembling structurally the acini observed in prostate. We propose to use a series of antibodies and fluorescent reagents present in acinar structures – active caspase-3, b-catenin, DAPI, Dlg, GM130, Ki67, phospho-ERM, phospho-ERK and ZO-1 – to characterise them at the molecular level using an immunofluorescence approach. Subsequently, we will follow the same approach with 2 less metastatic prostate cancer cell lines-DU145, LNCaP–and a normal prostate cell line–RWPE1. We believe that characterising these acinar structures in these 4 cell lines will set us with a model to recapitulate the progression from normal prostate to adenocarcinoma. This will allow us to investigate the events that lead to the organization of prostate acini and also identify genes important for the disruption of these structures that could potentially lead to tumour formation and subsequent dissemination to other tissues.

136. Edward Tarelli

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Background: Polyclonal IgG and antiphospholipid (aPL) antibodies from patients with different clinical manifestations of the antiphospholipid syndrome (APS) have been shown to

exert differential effects on signalling pathways and tissue factor activity in target cells. Interestingly, these biological effects were not distinguished by their degree of aPL binding which did not differ significantly between the different APS subgroups. Given that glycosylation is known to influence the biological activity of IgG, and that changes in IgG glycosylation patterns have been shown to predict clinical manifestations for various autoimmune diseases, we examined whether differential glycosylation of IgG may be a factor in determining the observed differences in the mechanism of the effects of IgG from APS patients.

Method: The glycosylation profile of IgG N-glycans, enzymatically released, from protein G purified IgG from four sets of 8 patients; APS with pregnancy morbidity (PM) alone (aPL+ PM), vascular thromboses (VT) alone (aPL+ VT), aPL+ve patients without APS (aPL+ APS-) and healthy controls (aPL- HC) was examined using Matrix Assisted Laser Desorption Ionisation Time-Of-Flight Mass Spectrometry (MALDI-TOF MS). IgG glycans were divided into three main groups based on the number of galactose residues: G0, G1 and G2. These biantennary complex glycans may be further modified by the presence / absence of fucose (F) and / bisecting N-acetylglucosamine (bis).

Result: There were no significant differences in aPL binding between the different APS and aPL+ groups. In contrast, the glycosylation profile of IgG was found to be significantly different in the 4 groups examined (Table). IgG from the APS patients had significantly higher ratios of total G0:G2 compared with the aPL+ APS- patients ($p=0.038$) and those from aPL- HC ($p=0.002$). On further, more detailed, analysis, the IgG from patients with VT, which showed the most marked difference in total G0:G2 ratio, could be differentiated from the PM group based on significant differences in the levels of G1F, G2, G2F and G1Fbis ($p<0.05$).

Conclusion: Our findings show that IgG from patients with diverse clinical manifestations of APS and aPL positive healthy controls exhibit differential patterns of glycosylation that were not predicted by differences in aPL binding. Therefore, these glycosylation differences, which include the degree of galactosylation as well as fucosylation, could be used as a biomarker to discriminate between patients with VT and PM, and may provide a better insight into the different mechanistic action of IgG in these patients.

The degree of IgG galactosylation can be used to distinguish different clinical phenotypes of antiphospholipid antibody positivity.

Patient Groups	Total G0:G2 Ratio Mean±SEM
aPL+ PM	1.16±0.25
aPL+ VT	1.81±0.25
aPL+ APS-	0.89±0.15
aPL- HC	0.42±0.08

137. Laura Tennant

Effects of progesterone on adrenal glucocorticoid metabolism

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Adrenal physiology determines function of the ovary and is well documented on impacting ovarian function. Has implications for disorders such as PCOS/POF. Aim of the study is to assess impact of ovarian steriod secretion on glucocorticoid metabnolism at two defined stages of the ovine oestrous cycle. Radiometric conversion assays and protein assays were used to detect activity in adrenal samples on day 5 (high progesterone) and day 14 (low

progesterone). Activity was determined via the isoenzymes 11βHSD 1 and 2. WHich facilitate the conversion of cortisol to cortisone and vice versa. Adrenal has very high cortisol oxidation - predominantly NAD+-dependent 11βHSD2 activity. (Irrespective of the day of the luteal phase, significantly higher rate of NAD+- vs. NADP+-dependent cortisol metabolism; $P<0.001$)Adrenal 11βHSD2 activity significantly lower on day 14 vs. day 5 ($P<0.001$); no significance difference with NADP+ ($P=0.771$). The presence of progesterone decreases metabolism of cortisol, i.e. 11βHSD2 activity is inhibited. Day 5 of the ovine oestrous cycle is an animal model of PCOS/POF.

138. Neale Tillin

Training explosive muscle performance affects agonist activation and the mechanical properties of the muscle-tendon unit

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Aim: Training for purely explosive strength, may provide important functional and health benefits, with minimum discomfort and fatigue, but the neuromuscular adaptations to this type of training are not fully understood. The current study assessed changes in the rate of force development (RFD), and neural and mechanical adaptations, following short-term unilateral explosive isometric strength training.

Methods: Ten previously untrained young adults (18-24 yrs) completed 4 sets of 10 explosive isometric knee extensions (1-s contractions completed 'fast and hard'), 4 times a week for 4 weeks. Pre and post training measurement trials consisted of recording force and EMG of the three superficial quadriceps throughout a series of voluntary and involuntary contractions of the knee extensors. Participants completed 10 explosive voluntary contractions to determine force at 50 (F50) and 100 ms (F100) after force onset. Supramaximal octet contractions (8 pulses at 300 Hz) were evoked to determine the contractile properties of the muscle. Agonist activation was measured with the ratio of voluntary/octet force, and average EMG normalized to Mmax of the three superficial quadriceps. Participants also completed 3, 3-s maximal voluntary contractions (MVCs) to establish maximal voluntary force (MVF). Ultrasonic images of the vastus lateralis were recorded during ramped MVCs to measure muscle-tendon stiffness (slope of the force-aponeurosis displacement curve between 50-90% MVF).

Results: There was an increase in voluntary F50 (+54%), voluntary F100 (+15%), octet F50 (+7%) and octet F100 (+10%). Increases in voluntary F100, octet F50 and octet F100 were proportional to improved MVF (+11%). However, the increase in voluntary F50 was +37% even after normalisation to MVF, and coincided with a 42% increase in both voluntary/octet force and agonist normalised EMG over the first 50-ms. MTU stiffness between 50-90% MVF also increased (+34%).

Conclusion: Enhanced agonist neural drive and MVF accounted for improved explosive voluntary force production in the early and late phases of the contraction, respectively. The increases in explosive octet force and MTU stiffness provide novel evidence of peripheral adaptations within just four weeks of training for explosive force production.

139. In-Ae Tribe

Postnatal murine neural crest-like stem cell differentiation into smooth muscle

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Introduction/aims: Stem cells are of interest for their ability to self-renew and differentiate into diverse cell types. Neural crest-like stem cells (NCLSCs) found in postnatal murine skin, provide a unique population that have already demonstrated differentiation into Schwann cell precursors, melanocytes, chondrocytes and functional neurons (Sviderskaya et al., 2009). Our aim is to further explore the multi-lineage potential of the NCSLC NC-m6, inducing smooth muscle determination with a combination of growth factors: FGF2, TGFβ1, and BMP2. In addition, identify whether TPA, an essential growth factor for NC-m6 survival (Sviderskaya et al., 2009), is necessary for their differential growth.
Methods: Immortalised NC-m6 cells were incubated, with and without TPA (2nM), in DMEM with FCS (10%), FGF2 (8nM), TGFβ1 (24nM), and BMP2 (2μg/ml), in combination and separately. Cell proliferation was measured at 7 days and phase contrast images were used to analyse cell morphology. Immunocytochemistry for Phalloidin-FITC and α-SMA were used to identify protein markers at culture incubations of minimum 14 days. Contractile function was analysed with application of caffeine (10nM), KCl (30nM, 60nM) and NA.
Results: All growth factors stimulated differentiation of NC-m6 cells into smooth muscle, with a maximal 10% positive presence of α-SMA. Their survival did not require TPA (p-value>0.05), and FGF2 showed the most significant effect to proliferation (p-value=0.000279). Results suggest BMP2 had the most inductive effect, however, no statistical significance was established. Function assays did not reveal any contractions.
Conclusions: We demonstrate NC-m6 cells, under the appropriate conditions, can proliferate and differentiate into smooth muscle. Future investigations need further exploration into contractile analysis. Nonetheless, results show NC-m6 cells have a new expanded potential for stem cell research, and present as a suitable model to study neural crest cell biology and differentiation.

140. Fotini Tsofliou

Differences in metabolic profile between young obese woman with polycystic ovary syndrome (PCOS) and obese controls

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Introduction: Several studies point to increased cardiovascular risk among women with PCOS. The present study aimed to investigate whether metabolic disturbances in PCOS are an effect of obesity itself or associated to factors specific to the syndrome.
Methods: Eighty six obese women (BMI=31.4±1.7 kg/m^2, age=29.9±5.3 years) with PCOS and eighty six age and BMI matched control women (BMI =31.3±1.7 kg/m^2, age=31.2±6.3 years) recruited from local OB/GYN offices participated in the study. Diagnostic criteria for PCOS were clinical +/or biochemical evidence of hyperadrogenism and polycystic ovaries on ultrasound. Dietary intake data were collected from a 24-h recall diary. Fasting blood samples were collected for the measurement of glucose, HbA1c and lipids. Statistical analysis of the data was carried out using two-tailed independent two-sample t-tests and Pearson correlations.
Results: The PCOS group had a significantly greater waist/hip ratio (WHR) (0.87± 0.09 vs. 0.84±0.09, p = 0.047) than the control group. Fasting plasma concentrations of lipids, glucose and HbA1c were significantly higher in women with PCOS than the control group (Table). Consumption of total energy, protein and dietary fiber was similar between groups (p>0.05).

Significant correlations were found between total cholesterol levels and dietary intake in PCOS group only (total energy (kcal) r=0.41, p<0.001; fat (% energy) r=0.24, p=0.03; fat (g) r=0.39, p < 0.001; cho (g) r=0.26, p= 0.02; pro (g) r=0.27, p=0.01). Central fat deposition markers (waist circumference (WC), WHR, waist/height ratio (WHtR) correlated significantly with biochemical parameters (glucose, HbA1c, lipids) in PCOS group only.
Conclusions: Obese women with PCOS represent a group with increased prevalence of disturbed metabolic profile associated with cardiovascular risk. These disturbances might not be attributed to obesity per se, since obese women with PCOS had higher biochemical markers than their BMI matched controls. Furthermore, central fat deposition is more prevalent in PCOS group and it's correlation to the biochemical cardiovascular risk factors, found specifically in PCOS group, might suggest that it is not obesity but it is central fat deposition that affects the cardiovascular risk in PCOS.

Table

	PCOS mean±SD, n = 86	**Controls mean±SD, n = 86**	**p value**
Glucose (mmol/lt)	112.9 ± 18.4	105.4 ± 10.8	0.001
HbA1c (%)	6.0 ± 1.0 (n=85)	5.4 ± 1.5 (n=39)	0.010
Total cholesterol (mmol/lt)	272.2 ± 32.6	219.5 ± 27.0	<0.001
LDL-C (mmol/lt)	179.5 ± 15.1	125.2 ± 28.3	<0.001
HDL-C (mmol/lt)	57.5 ± 8.2	62.3 ± 10.8	0.001
Triglycerides (mmol/lt)	217.8 ± 37.0	157.6 ± 42.7	<0.001

141. Irene Tuffrey-Wijne

A new model for breaking bad news to people with intellectual disabilities
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Background: Current models for breaking bad news are inadequate in meeting the needs of people with learning disabilities (LD). People with LD are often not told of a life-limiting diagnosis. The task of breaking bad news is often left to carers who are poorly prepared and supported to cope with this. Health care professionals don't know how to communicate adequately with people with LD. A new model for breaking bad news to people with LD. The model was developed following a focus group/interview study involving 96 participants, including people with LD, family carers, LD professionals and health care professionals (see related abstract for oral presentation). The findings were combined with the literature. A preliminary model was developed; 60 stakeholders gave feedback on this, including a wide range of family carers, professionals and academics. The preliminary model was modified following this feedback. The poster will present the model in a visual format. The model has four components.
1. Building a Foundation of Knowledge is central to the model. Gradually and over time, the person with learning disabilities builds his/her understanding of the way his/her situation is changing because of the bad news. The people around him/her help with this, by giving small, singular chunks of information that make sense to the person. This does not have to done by talking: much of the information will be understood through experience. The other three components must be considered throughout:
2. Capacity and understanding, taking account of the Mental Capacity Act
3. The people involved, including family, partners, friends, paid carers and professionals

4. The support needed by everyone involved, including information, emotional, social, practical and spiritual support. The model now needs to be tested in practice.

142. Irene Tuffrey-Wijne

Supporting people with learning disabilities (LD) who are affected by a relative or friend with cancer

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Introduction: Around 2.5% of the population have LD. Most people with LD will be affected by cancer of family or friends at some point in their lives. Their support needs are insufficiently understood**.**

Aims:

- To explore the experiences and support needs of adults with LD who have a relative or friend with cancer
- To make recommendations for practice

Methods: Twenty-two people with LD took part in focus groups and face-to-face interviews. All participants had experience of having a close relative/friend with cancer. The groups were co-facilitated by two co-researchers with LD, using a range of methodologies (including story-telling, role play and Nominal Group Technique) to extract participants' experiences and views. Data were analysed using content analysis.

Results:

- Being protected from information (as many participants were) negatively affected their coping.
- Participants worried about their relative/friend's illness and the impact on both the patient and on themselves; but had not shared their worries or questions with others.
- Several participants had become carers themselves.
- The greatest need was for "someone to talk to"; this need was not met by either families or professionals.
- There was a lack of understanding about cancer, and a lack of access to cancer information; participants wanted such information. However, accessible information materials were viewed as less important than access to someone who could explain.

Discussion: The needs of people with LD who are a relative/friend of someone with cancer are often overlooked. This group does not ask for support, and therefore pro-active support is needed from professionals. This includes emotional support as well as informational support.

143. Ferran Valderrama

Radixin regulates cell migration and cell-cell adhesion through Rac1

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The ERM proteins ezrin, radixin and moesin are adaptor proteins that link plasma membrane receptors to the actin cytoskeleton. Ezrin and moesin have been implicated in cell polarization and cell migration, but little is known about the involvement of radixin in these processes. Here we show that radixin is required for migration of PC3 prostate cancer cells, and that radixin, but not ezrin or moesin, depletion by RNAi increases cell spread area and cell-cell adhesion mediated by adherens junctions. Radixin depletion also alters actin organization and distribution of active phosphorylated ezrin and moesin. Similar effects were observed in MDA-MB-231 breast cancer cells. The phenotype of radixin-depleted cells is similar to that induced by constitutively active Rac1, and indeed we observe that Rac1 is required for the radixin knockdown phenotype. Radixin depletion also increases the activity of Rac1 but not Cdc42 or RhoA. Analysis of Rac guanine nucleotide exchange factors (GEFs) suggests that radixin affects the activity of Vav GEFs and could thereby stimulate Rac1. Indeed, Vav depletion reverts the phenotype of radixin knockdown. Our results indicate that radixin plays an important role in promoting cell migration by regulating Rac1-mediated epithelial polarity and formation of adherens junctions through Vav GEFs.

144. Ferran Valderrama

Development and characterization of a multichannel micro-plate for the analysis of 3-Dimensional cell migration

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Our knowledge of cell migration has been mostly nurtured with data obtained through analysis of cell lines, typically grown in two-dimensional (2D) cultures. However, there is clear evidence that those cells lose most of the key components of their normal phenotypes, when grown in 2D. This phenotype change can in part be attributed to the absence of a proper substratum and/or loss of a normal three-dimensional (3D) architecture. In contrast, cells grown in a 3D microenvironment containing extracellular matrix (such as collagen or a reconstituted basement membrane) sustain most of their capacities. Moreover, if the complexity of this 3D microenvironment is increased by addition of growth factors or by co-culturing other cells usually found in their environment, we can mimic to a great extend the expected in vivo behaviour of these cells. Also, in contrast to in vivo models, they are more amenable to rapid experimental manipulations of hypothesis and more sensitive to real time and/or fixed imaging by microscopy.

There are two aspects that require further development to facilitate 3D cell migration studies:

- A lack of systems allowing analysis of several conditions in the same experiment (screen) through microscopy techniques (in particular live imaging).
- A need to reduce the high cost of the reagents needed to recreate and manipulate the environment where the cell will move (matrices, growth factors, drugs…). Based on a micro- slide from Integrated BioDiagnostics GmbH (IBIDI®) I proposed a new plate that would have the following characteristics:
- Keeps the advantages of the IBIDI® micro-slide:
  - Low amount of reagents needed (cost-effective).
  - Allows live imaging and subsequent sample fixation for further analysis.
- Incorporates all the capacities for 3D analysis of cells.
- Increases number of samples per experiment, making it amenable for screen purposes.
- The standard size of the micro-plate should also allow its use in plate readers. All these features should open studies of 3D cell migration to drug, growth factors, DNA over-expression or RNAi screens using microscopy live imaging in a cost-effective manner.

145. Elisabeth Julie Vargo

Towards “recreational” cocaine use? An explorative study on a sample of young adults.

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Abstract: The study aims to explore through ethnographic instruments, young adult (18-34 years old [EMCDDA, 2009]) illicit substance users, an increasing population throughout the Western world (Parker, 2005). The analysis of 31 Narrative interviews (Atkinson, 2002) together with quantitative data obtained through a semi-structured questionnaire, gave us access to the social representations, motivations, beliefs and consumption styles regarding recreational cocaine use in Rome. Data was conceptually organized using Atlas.ti and Grounded Theory methodology (Chiarolanza, DeGregorio, 2007). The scenario emerging from the results evidences how cocaine use is often part of a more general behavior that implies multiple-drug use; “experimentation” seems to be very diverse between individuals and groups and constantly changing, according to personal motivations and collective trends. What all of these patterns have in common is being part of “integrated” lifestyles and playing an important role in the identity formation of these individuals. The participants contextualize their consumption within private environments; important differences emerge between subgroups of consumers (males and females; opportunistic, occasional and habitual users; “selective” drug experimentation). It becomes consequently necessary to further explore the social constructions and complexities of this “hidden” population,

and consider how these new practices tied to drug use influence the individuals' overall wellbeing and health.

Keywords: recreational cocaine use, qualitative research, young adults, motivations, social representations. Bibliography: Atkinson R. (2002), L'intervista narrative (Narrative Interviewing), Raffaello Cortina Editore, Milano. Chiarolanza C., De Gregorio E. (2007), L'analisi dei processi psico-sociali: Lavorare con Atlas.ti (The analysis of psycho-social processes, working with Atlas.ti), Carocci Faber, Firenze. European Monitoring Centre for Drugs and Drug Addictions [EMCDDA] (2009), Comparative Analysis of Research into Illicit Drugs in the European Union, European Commission, Brussels. Parker H. (2005), "Normalization as a barometer: Recreational drug use and the consumption of leisure by younger Britons", in Addiction Research & Theory, Vol. 13, Issue 3, pp. 205-215.

146. Abdul Waheed

A novel steroidal saponin from *Fagonia indica* induces cell-selective apo-necrosis in cancer cells.

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Saponins are secondary metabolites of glycosides, widely distributed in higher plants and usually present in roots, tubers, leaves or seeds. Their diverse structural differences and surface-active properties, allow saponins to form colloidal solutions in aqueous phase and confer the ability to lyse erythrocytes and other cells. Steroidal glycosides with a pregnane skeleton have been shown to possess potential cytotoxic activity, which may represent new leads in the development of anti-cancer drugs. *Fagonia indica* (family Zygophyllaceae) is an important medicinal plant indigenous to Pakistan, India, United Arab Emirates, and a source of triterpenoid saponins. An aqueous decoction of the aerial parts of *Fagonia indica* is claimed to be a remedy for cancer in its early stages in folklore medicine (Chopra *et al.,* 1956). The aim of this study was to isolate, using an activity-guided fractionation approach, novel pregnane glycosides from *Fagonia indica* for testing on the growth of cultured breast cancer cells (MCF-7, MDA MB-468) and colorectal carcinoma cells (Caco-2).

A novel pregnane glycoside was isolated from potent fractions of *Fagonia* using repeated flash column chromatography. Structural elucidation was carried out through a series of spectroscopic experiments (1-D and 2-D NMR, GC-MS, LC-MS). The apparent IC_{50} of this steroidal saponin was estimated from serial dilutions of eight concentrations (0.78 – 100 μM), tested against breast and colon cancer cell lines, using two cell viability assays (MTT and neutral red uptake NRU) for 24 h and 48 h treatments. This pregnane glycoside, isolated for the first time from *Fagonia indica*, was found to be more potent in suppressing cell growth in oestrogen-negative breast cancer cells (MDA MB-468, IC_{50} 6.25 – 25 μM), compared to MCF-7 oestrogen-positive cancer cells (IC_{50} 50 – 100 μM)**.** A similar extent of cell suppression was also observed for Caco-2 cells, where a statistically significant activity was observed at 3.2 μM and higher concentrations (in MTT assay for 24 h), while in the NRU assay this effect was more pronounced at 1.56 μM and higher concentrations, after 24 h incubation.

PARP (Poly-ADP ribose polymerase, intact-116 kDa) is a key signaling enzyme involved in triggering the repair of single-strand DNA damage. Cleaved PARP (89 kDa), was detected in Western blots, suggesting apoptosis, as did decreased cell viability of cancer cells in a NRU assay pre-treated with a pan-caspase inhibitor (Z-VAD-FMK). However, cytomorphological alterations in a DAPI staining assay showed cell swelling suggestive of necrosis.

This study suggested that a novel steroidal saponin from *Fagonia indica* shows some selectivity between oestrogen-dependent (MCF-7) and oestrogen-independent (MDA MB-468) breast cancer cells and there is a possibility of the apo-necrosis phenomenon of cell death.

147. Alison Wallace

Gene Array Analysis of Extravillous Trophoblast - Spiral Artery Interactions Modelled Using 3-Dimensional Spheroid Culture

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Objectives: During successful placentation, fetal extravillous trophoblast (EVT) interact with decidual spiral arteries to transform them into large, non-vasoactive vessels capable of transporting nutritional and oxygen requirements to the fetus. To investigate the remodelling process, most in vitro approaches have used cell monolayers to study the endothelial cells (EC) and vascular smooth muscle cells (VSMC) which make up spiral arteries. Previous work has indicated that co-culturing EC with VSMC in 3-dimensional spheroids better represents the interactions occurring in an intact vessel in vivo, as EC and VSMC orientate to mimic a vessel lumen. This study utilised this EC/VSMC spheroid co-culture system to model the changes in gene expression in decidual vascular cells induced by invading EVT.
Methods: EC and VSMC cell lines were co-cultured in equal numbers in hanging droplets to form EC/VSMC spheroids. Control media or conditioned media which had been incubated with the EVT cell line, SGHPL-4, was added to spheroids for 24 hours. Spheroid RNA was then isolated and subjected to analysis by Illumina Sentrix BeadChip array and Genespring software. Array results were validated by quantitative RT-PCR and western blot, and immunohistochemistry was used to examine first-trimester decidual tissue for expression of proteins of interest.
Results: EC migrated within the spheroids to form a monolayer around a core of VSMC, representing the lumen of a vessel. EC/VSMC spheroids incubated with EVT conditioned media showed significant up or downregulation of 101 genes (>1.5 fold, $p<0.05$) and further analysis revealed a number of significantly enriched gene ontologies including vasculature development ($p<0.05$) and cell-cell signalling ($p<0.01$). Of these, 3 upregulated genes were chosen for further analysis: CD93 (2.59 fold, $p<0.001$), STC-1 (2.34 fold, $p<0.001$) and CXCL10 (2.24 fold, $p<0.001$). The localisation of these proteins to modified spiral arteries in first trimester decidua was demonstrated by immunohistochemistry.
Conclusion: Using 3-dimensional spheroid co-cultures presents an opportunity to study spiral artery interactions in vitro. EVT conditioned medium promotes changes in gene expression of decidual vascular cells. Expression of these genes may have implications for spiral artery remodelling due to their roles in apoptosis and chemoattraction of trophoblast and immune cells.

148. Adam Witney

MDR *P. aeruginosa* sensitive only to colistin: whole genome sequence analysis of outbreak isolates.

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Multidrug resistant (MDR) *Pseudomonas aeruginosa,* susceptible only to treatment with colistin, is an increasing clinical problem. Colistin is a toxic drug and *P. aeruginosa* infections would become untreatable if colistin resistance developed in these MDR strains. Outbreaks of MDR *P. aeruginosa* have been noted across the world and within UK hospitals, in which MDR *P. aeruginosa* has been found in both patients' samples and the hospital environment. Genome sequencing of these MDR isolates aims to reveal further insights that will benefit the treatment and control of this developing clinical problem.
Representative patient and environmental isolates of MDR *P. aeruginosa* from outbreaks at two UK hospitals have been sequenced using an Ion Torrent Personal Genome Machine. This next generation sequencing (NGS) platform enables rapid bacterial genome sequencing within a hospital setting for real-time clinical application. NGS analysis of the MDR *P. aeruginosa* isolates indicated the genetic basis of antimicrobial, disinfectant or heavy metal ion resistance and also determined the presence of key virulence genes, pathogenicity islands or mobile genetic elements.

Further comparative analysis of patient and environmental isolates from the two UK hospitals aims to provide clues to the MDR phenotype acquisition and potential source of the outbreaks. The clinical application of NGS technology in infectious disease outbreaks provides a forensic level of analysis, facilitating discrimination of isolates typed as identical by current phenotypic or genotypic methods. This detail enables potential transmission routes to be identified to help inform infection control measures and patient management. Furthermore, the real-time use of NGS in a clinical setting may also provide clues to alternative therapy options and reveal targets for improved diagnostics to monitor ongoing or future outbreaks.

149. Friederike Zunke

Kv7.4 channels contribute to β-adrenoceptor regulation of rat renal artery
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KCNQ-encoded voltage-dependent potassium channels (Kv7) have been identified in vascular smooth muscle cells and shown to regulate vascular tone (Greenwood and Ohya, 2009). Kv7.4 channels have been identified as one of the main functional contributors in vascular tissue. Although modulation of these channels with selective activators and blockers causes profound changes in vascular tone, it is not known to what extent Kv7.4 channels contribute to endogenous dilator mechanisms. The present study utilises Kv7 modulators and a selective Kv7.4 knockdown protocol to characterise the role of these channels in rat renal artery. In addition, the contribution of Kv7.4 channels to β-adrenoceptor-mediated vasodilatation is investigated. Renal arteries were isolated from male Wistar rats (200-225 g). Isometric tension recordings were performed on a myograph. In some vessels, Kv7.4 channel knockdown was achieved by transfecting with specific KCNQ4 siRNA using a reverse permeabilisation protocol. Subsequent experiments were conducted following 72 hrs incubation. Kv7 channel blocker (Linopirdine) and activator (S-1) were used to assess Kv7 channel functionality. Furthermore, responses to isoprenaline (β-adrenoceptor agonist) and forskolin (adenylyl cyclase activator) were determined in arteries preconstricted with 3 uM Methoxamine, in the presence and absence of various K+ channel blockers. Kv7.4 channel expression was assessed by Western blotting. Values are presented as mean ± s.e.m., compared by two-tailed Student's t-test. Exposure to Kv7 activator S-1 caused concentration-dependent relaxation in renal arteries, which was inhibited in the presence of linopirdine. In preconstricted arteries, isoprenaline-induced relaxation was inhibited in the presence of 10 μM linopirdine (Emax, 39.3±15.5 vs. 103.6±4.2 vehicle control; n=5-6; $p<0.01$). Relaxation caused by forskolin was also attenuated in the presence of linopirdine (pEC50, 6.3±0.1 vs. 7.2±0.1 vehicle control; n=4; $p<0.01$). In arteries transfected with KCNQ4 siRNA, S-1 relaxation was attenuated (1 μM S-1: 17.2% ± 5.8 vs. 43.5% ± 9.2 in control vessels; n=7; $P<0.05$). Moreover, in these arteries, relaxation in response to isoprenaline was significantly attenuated compared to control vessels (pEC50, 7.1±0.1 vs. 8.2±0.1 scrambled siRNA control; n=7; $p<0.001$). These results show that Kv7.4 channels are important regulators of vascular tone in the rat renal artery and appear to be functional contributors to the β-adrenoceptor-mediated relaxation.

150. Velislav Batchvarov

ECG Wavelet Analysis for the Detection of Gene Mutations in Patients with Brugada Syndrome
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Background: We applied time-frequency analysis using wavelet transform (WT) to ECGs acquired during positive diagnostic ajmaline test for Brugada syndrome (BS) in order to compare patients with and without identified gene mutations.
Methods: Digital electrocardiograms (ECG) were acquired during positive ajmaline test in 13 patients (6 men, age 46±16 years) with additional leads V1 to V3 from 3rd intercostal space (ics) (V13, V23, V33, group A) and 13 patients (7 men, age 37±19 years) with leads V1 and V2 from 3rd and 2nd ics (V13, V23, V12, V22, group B). During genetic testing, mutations for BS were identified in 4 patients in group A and 7 in group B whereas 9 patients in group A and 6 in group B had no mutations. Discrete WT was applied to the QRS complex and the ST-T wave (J-point – end of T wave) on leads V1, V2, V3, V13, V23 and V33 (group A) and leads V1, V2, V13, V23, V12 and V22 using…
Results: In group A, there was significantly higher energy within the QRS at scales corresponding to frequencies above 27 Hz in patients with mutations both at baseline (p=0.013 to p=0.040) and during maximum drug effect (p=0.012 to p=0.025). In group B, patients with mutations had higher QRS energy at 6.1 to 26 Hz at baseline (p=0.007 to p=0.045) and at 7.9 to 26 Hz and 1.7 to 3.4 Hz during drug effect (p=0.003 to p=0.045). Within the ST-T wave, there was higher energy at lower frequencies during baseline (p=0.018 to 0.045) and maximum drug effect (p=0.009 to p=0.046).
Conclusion: In patients with BS, WT of the QRS and ST-T wave seem to correlate with the presence of gene mutations. Superior results are achieved when WT is applied to a set of leads including V1 and V2 from the 2nd to 4th i.c. space.

151. S Reeves

Breakfast Habits and Health in a Nationaly Representative UK Sample
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INTRODUCTION: Breakfast eaters have been reported to have better cognitive function, be less depressed, have better nutrient intakes and a lower body mass index (BMI) than people who skip breakfast. However, there is limited information relating breakfasting habits to measures of health and wellbeing across the UK.

OBJECTIVES: To report on UK breakfasting habits and the relationship with health and wellbeing from a recently surveyed nationally representative sample.

METHOD: 1,068 adults completed a web-based survey. The survey included validated standardised scales to report wellbeing, physical activity and eating habits as well as original questions on breakfasting habits and frequency, and questions asking for height, weight and waist circumference.

RESULTS: 66% of the respondents consumed breakfast every day, with only 6% never eating breakfast. Cereal was the preferred choice for 50% of the sample. Frequency of breakfast consumption was found to correlate with conscientiousness (r=0.12), and wellbeing (r=0.16) (for all, p<0.001). Those eating breakfast every day get up earlier by 20 minutes during the week and 35 minutes during the weekend, compared to those eating breakfast less regularly (p<0.001). Correlations between breakfasting frequency and moderate and vigorous physical activity were 0.08 and 0.09 respectively (p<0.01). No correlation was observed between breakfast frequency and BMI or waist circumference (both r<0.05; p>0.3) and there were no differences in BMI and waist circumference of participants who never consumed breakfast those who always consumed breakfast(p>0.25).

CONCLUSIONS: The majority of UK adults have breakfast regularly and these individuals tend to be, slightly more conscientious and show slightly higher levels of wellbeing. They get up

earlier, and are marginally more active. In contrast breakfasting frequency is not correlated with BMI.