Session 3

Genetic Studies

Chair: H Asemota and J Anzinger

(O – 13)
Phenotypic analysis of mutants of the Rho-GTPase subfamily member Cdc42

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Objectives: To investigate the role of cell division control protein 42 homolog (Cdc42) in dendrite growth and the effectors necessary for Cdc42 mediated dendrite growth. This is important to understand the mechanisms and relevant pathological pathways underlying neurodegenerative diseases such as amyotrophic lateral sclerosis.
Methods: The morphology of class I sensory neurons were analysed by imaging the dorsal body wall of the third instar larvae of transgenic *Drosophila melanogaster* lines containingCdc42 mutants. Class I neurons were observed through confocal microscopy of cells labelled with a pan-neuronal driver of GFP. The sample sizes of the dissected *Drosophila* lines are as follows: wild type- 6, Cdc42^{V12}- 12, Cdc^{V12A37}- 11, Cdc^{V12C40}-13.
Results: A manual count of the number of branch points in each larva was performed. The average branch point counts in the class I sensory neurons in the wild type group was 24.5 ± 1.118. The average branch count for the Cdc42^{V12} group was 49.667 ± 1.578. The average branch count for the Cdc^{V12A37} group was 36.272 ± 1.342. The average branch count for the Cdc^{V12C40} group was 29.846 ± 1.270. The *p*-value for all groups was $p < 0.01$.
Conclusion: This experiment suggests that the Cdc42 effectors in the CRIB family, Jnk/MAP pathway, ROCK family and the IQGAP1 family are important for, and involved in regulating dendrite growth and guidance.

(O – 14)
Forensic use of DNA: knowledge of and attitudes to its use among law students in Jamaica

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Objective: To assess the knowledge of, and attitudes to the forensic use of DNA among law students in Jamaica.
Methods: Four hundred law students from four local universities were recruited by convenience sampling. They were each asked to complete a questionnaire.
Results: While 15% correctly defined DNA, 36.7% were unsure. Tele-media were the main sources of information and tertiary education was the least accessed source. Most students were able to identify the main methods of sample collection, but the majority did not know how DNA was stored in a databank. Forty-three per cent strongly agreed that DNA should always be kept. When asked about the concerns they had in regard to its use, 75.4% cited inappropriate handling by custodians and 65.7% cited their own insufficient knowledge. Seventy-eight per cent said that DNA profiles of convicted sexual offenders should be kept permanently. Fewer respondents said that profiles from all convicted, all arrested persons and newborns should be kept. Ninety-two per cent believed that profiles and samples of convicts who 'have done their time' should also be kept.
Conclusions: The participants favoured the advances in DNA technology and its use in fighting crime. Their perceptions were based on limited knowledge of its forensic use and they were not fully aware of the implications in regards to ethical norms and values. Since it is being argued that ethical rights and values are being compromised during the collection, storage and use of DNA, more should be done to educate the stakeholders before the establishment of a databank in Jamaica.

(O – 15)

Glut1 expression on intermediate monocytes is a potential marker of inflammation in HIV-1-positive subjects

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Background: Monocyte activation during HIV-1 infection is associated with elevated plasma levels of inflammatory markers and increased risk of age-related diseases. As activated monocytes primarily utilize glucose to support cellular metabolism, we hypothesise that chronic monocyte activation during HIV-1 infection induces a hypermetabolic response with an obligate requirement for glucose. We therefore evaluated the impact of HIV-1 infection on glucose transporter (Glut1) expression and glucose uptake in monocyte subsets.
Methods: HIV-1-positive treatment naïve patients (n = 15), HIV-1-positive patients on combined antiretroviral therapy (cART) with viral loads below detection (n = 17) and HIV-negative controls (n = 15) were studied. Surface expression of Glut1, surface expression of phenotypic markers (CD14 and CD16), and cellular uptake of the fluorescent glucose analogue 2-NBDG were analysed for monocyte subsets from fresh blood samples by flow cytometry. Linear and multivariable regression models were applied to assess the relationship between Glut1 expression on monocyte subsets and other covariates.
Results: Monocytes from patients with HIV-1 infection irrespective of cART treatment status had significantly increased levels of Glut1 compared with HIV-negative controls. Across the three subject groups, non-classical pro-inflammatory (CD14+CD16++) and intermediate monocyte subsets (CD14++CD16+) showed higher Glut1 expression than classical (CD14++CD16-) monocytes. Intermediate monocytes from HIV-1-positive treatment naïve patients and HIV-1-positive cART-treated patients showed significantly increased uptake levels of the fluorescent glucose analogue 2-NBDG compared with intermediate monocytes from HIV-negative controls. In multivariate analysis, the level of Glut1 on intermediate monocytes was independently-associated with the levels of plasma D-dimer (p = 0.003), low HDL cholesterol (p = 0.02) and treatment status (p = 0.01).
Conclusions: Glut1 expression on monocytes is a potential marker for hypermetabolic responses during HIV-1 infection. Further studies are required to determine if Glut1 expression on the intermediate monocyte subset may identify individuals at risk for HIV-1-related co-morbidities.

(O – 16)

Comparison (including cost comparison) of VITEK® 2 Compact, VITEK® 60, VITEK® Mass Spectrometry and API® 20E/NE for the identification of clinical gram negative bacteria

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Background: VITEK® 2 Compact (VTC), VITEK® 2 60 (VT60) and API® 20E/20NE (API) use biochemicals for bacterial identification (ID), VITEK® MS [mass spectrometry] (VMS) uses matrix-assisted laser desorption/ionization-time of flight mass spectrometry. This pilot study compared these methods in terms of agreement and cost of ID.
Methods: Eighty gram negative bacilli (GNB) from swab, blood and urine specimens submitted to the University Hospital of West Indies (UHWI) Microbiology Laboratory, Jamaica (September 2011–March 2013) were analysed by VTC, then shipped to Canada for ID by VT60, VMS and API20E/NE. Resolution of discrepant ID was by biochemicals, phylogeny or 16S sequencing. Direct and indirect costs were calculated in Canadian dollars.
Results: Of the 80 GNB, 92.5% (95% CI 86.5, 98.5) agreed by VTC, VT60, VMS and API: 16 *E coli*, 14 *A baumannii*, 11 *K pneumoniae*, 6 *P fluorescens*, 5 *E cloacae*, 5 *P mirabilis*, 5 *P aeruginosa*, 4 *K oxytoca*, 2 *M morganii*, 2 *S maltophilia*, and 1 each of *A faecalis*, *C freundii*, *E aerogenes*, and *S marcescens*. Reproducible ID disagreement in 6 [7.5% (95% CI 1.7, 13.3)] GNB were resolved in favour of VMS by 16S sequencing for *Rahnella aquatilis* and *Leclercia adecarboxylata* (*Pantoea* by VTC/VT60/API), while *Chryseobacterium defluvii* needed indole/motility (*Brevidomonas diminuta* by VT60) additionally and confirmation of *Morganella morganii* (*P mirabilis* by VT60) was by lack of swarming/H_2S-production. Costs for ID of 80 GNB were $850.28, $796 and $140.36 for VT60, API and VMS.
Conclusions: There was acceptable agreement between all tests for common organisms. VITEK® MS ID was accurate and 80% cheaper.

(O – 17)

Genetic diversity of *Toxoplasma gondii* in patients with neurotoxoplasmosis in Jamaica

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Objective: To identify and describe *Toxoplasma gondii* (*T gondii*) genotypes detected in patients presenting with neurotoxoplasmosis.
Method: *Toxoplasma gondii* antibody positive sera and corresponding cerebrospinal fluid (CSF) from patients presenting with suspected neurotoxoplasmosis at the University Hospital of the West Indies were analysed for evidence of *T gondii* DNA using a nested-polymerase chain reaction [PCR] (Biosearch Technologies, Novato, CA) and sequencing performed using primers specific to the PK1 genome region. Sequence alignment, phylogenetic relationships and genotypic analyses were generated using MegAlign™ DNASTAR® (Lasergene® version 8) and Geneious version 6.0 (Biomatters). Subsequent sequence comparison was done using the NCBI DNA BLAST® programme.
Results: *Toxoplasma gondii* DNA was detected in 18.7% (3/16) of CSF and 0% (0/16) of *T gondii* positive sera. Genomic analysis of DNA showed alignment to two previously documented *T gondii* isolates, eleven strains, and one protein gene. The first isolate had 98% alignment with *T gondii* genotype I strain RH, the second isolate had 96% alignment with the more divergent genotype I/III MAS and the third isolate had 99% alignment with the highly divergent atypical strain TgCkNg1. Phylogenic relationship analysis revealed that *T gondii* isolates identified as MAS and RH circulated from a common human ancestor identical to the *T gondii* protein serine/threonine kinase gene; while the third isolate was identical to *T gondii* isolates TgCkNg1 found in chickens.
Conclusion: *Toxoplasma gondii* isolates from a small group of patients presenting with neurotoxoplasmosis in Jamaica revealed genetic diversity. Further research is needed to evaluate possible associations between genomic variations and pathogenesis of *T gondii* in such patients.

(O – 18)

Putative epigenetic influence of DNA adenine methylase on P fimbriae expression and attachment among fluoroquinolone-resistant uropathogenic *Escherichia coli* to human urinary cell lines

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Objective: Uropathogenic *E coli* (EPEC) is a major cause of urinary tract infections among infants and adults. Since differential Dam methylation can regulate the pyelonephritis-associated pili (*pap*) operon essential to cellular adhesion in uropathogenic *E coli*, we sought to investigate the influence of the DNA adenine methylase (*dam*) gene on *pap* expression in uropathogenic fluoroquinolone-resistant *E coli*.
Methods: To monitor the influence of *dam* on *pap*, one uropathogenic *pap*-positive control *E coli* strain and three fluoroquinolone-resistant *E coli* clinical isolates were screened for *pap*, *qnr* and the quinolone resistance-determining region (QNDR) by polymerase chain reaction (PCR). DNA adenine methylase mutants were created *via* one-step allelic exchange and comparative attachment assays (relative to wild type) determined using human embryonic kidney (HEK-293) and urinary bladder cell lines (HTB-9). Additionally, plasmid-cured wild type strains were transformed using a control *qnr*-positive plasmid (pMG252) and assessed for antibiotic susceptibility to determine the influence of *dam* on quinolone resistance.
Results: The *dam* and *papEF* genes and *papIB* intergenic region were identified in all three clinical *E coli* strains and *pap* control strain; DNA methylation was absent among *dam* mutants. The percentage adherence ranged from 0.14–1.10% for HEK-293 cells and 0.5–1.69% for HTB-9 cells, with higher attachment rates among *dam* mutants. Two *qnr*-positive clinical strains had mutations within GyrA (gyrase) and ParC topoisomerase IV subunits while the remaining strain had a ParC subunit mutation. Successful transformation with pMG252 produced a one-fold (ciprofloxacin; gentamicin), eight-fold (ampicillin; ceftriaxone) and 32-fold (amoxicillin/clavulanic acid; carbenicillin) increase in resistance.
Conclusion: The *dam* gene was confirmed to play a vital role in the DNA methylation in local *qnr*-positive UPEC isolates. The low-level increase in attachment mutants to mammalian cells may be due to the expression of alternate adhesins sharing common regulatory features to *pap*. Further investigations are warranted to shed light into the post-transcriptional influence of *dam* on virulence genes and their associated regulatory networks vital to pathogenesis.

(PO – 02)

Priapism and overactive bladder symptoms in sickle cell disease: is there a link?

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Objective: We sought to see if there was an association between priapism and overactive bladder (OAB) symptoms in men with sickle cell disease (SCD).

Methods: Structured questionnaires were administered to 40 men with haemoglobin SS who attended the Sickle Cell Unit (SCU) of The University of the West Indies, Jamaica. A past history of enuresis and age of cessation and a current history of enuresis were determined. The OAB questionnaire-short form (OAB-q SF) was used to assess bothersome bladder symptoms and quality of life (QOL). A history of ischaemic priapism was determined.

Results: Forty men with homozygous SCD of mean (SD) age 29.7 ± 6.4 years were recruited. Twenty men (mean age 28.4 ± 6.8) gave a history suggestive of ischaemic priapism (stuttering and/or major) and 20 men did not have a history of priapism (mean age 31 ± 5.9 years). Twenty men had a positive history of enuresis, of which 11 also had history of priapism. Four men (10%) had a persistent history of enuresis. There was no association of history of priapism and history of enuresis. Mean age (SD) of cessation of enuresis was 12.6 ± 4.4 years. There was no difference in OAB-q SF bother and OAB-q SF QOL scores by priapism history status (median with IQR 10, 32 *vs* 8.3, 16.3) and (median with IQR 95.4, 4.6 *vs* 96.9, 9.2), respectively.

Conclusion: Priapism and enuresis are common in SCD. There is no association of priapism and voiding symptoms. Large scale studies will be required to further investigate this possible association.