Abstracts

S01
Genomic Medicine Programs of the National Human Genome Research Institute
T.A. Manolio
National Human Genome Research Institute, Bethesda, MD, USA

Genomic medicine is an emerging medical discipline involving the use of an individual patient’s genetic or genomic results in their clinical care. The National Human Genome Research Institute (NHGRI), in close collaboration with its research community, is pursuing an ambitious research and dissemination effort to facilitate and promote the implementation of genomics in clinical care. Major projects include the Undiagnosed Diseases Network (UDN), expanding NIH’s Undiagnosed Diseases Program for diagnosing rare and new diseases; the Newborn Sequencing in Genomic Medicine and Public Health (NSIGHT) program, exploring possible uses of genomic sequence information in the newborn period; the Clinical Sequencing Exploratory Research (CSER) program, exploring the infrastructure, methods, and obstacles to integrating genomic sequence into clinical care; the Electronic Medical Records and Genomics (eMERGE) Network, using biorepositories with electronic medical records and genome-wide association data to incorporate genomics into clinical research and care; the eMERGE-PGx project, using targeted sequencing of 84 pharmacogenes for discovery and clinical care in 9,000 patients; the Implementing Genomics in Practice (IGNITE) network, to facilitate development and disseminate methods for incorporating patients’ genomic findings into their clinical care; and the Clinical Genomics Resource (ClinGen), to create a centralized resource of clinically annotated genes and variants to optimize the use of genomic variation in medical care.

In addition, NHGRI has organized and led a series of genomic medicine symposia and meetings to shape these research initiatives and facilitate collaboration and sharing of materials. Several of these meetings have led to new, independent efforts such as the Inter-Society Coordinating Committee for Practitioner Education in Genomics (ISCC), to facilitate development and sharing of genomic education materials across professional societies; and the Global Genomic Medicine Collaborative (G2MC), an international genomic medicine community formed to catalyze the implementation of genomic tools and knowledge into health care delivery globally.

Most recently, President Barack Obama has announced a new initiative in Precision Medicine to accelerate progress in developing and implementing prevention and treatment strategies that take individual variability into account. Much of the groundwork for this initiative in the areas of genomics and electronic medical records has been laid by programs such as those described above. The field of pharmacogenomics in particular is expected to produce notable short-term successes for this new initiative, as we work to identify those at risk for adverse drug reactions and improve the effectiveness of therapy, and look to define rare loss-of-function mutations that protect against common diseases as attractive drug targets for broader patient populations. These efforts provide a valuable complement to the highly successful basic genomics research enterprise that has at last enabled the transition of genomics from the bench to the bedside.

S02
The Role of Genetic Polymorphisms of Drug Targets and Transporter Genes in Responsiveness to Antiepileptic Drugs
Z. Mohamed, H.M. Sha’ari, B.S. Haerian
Pharmacogenomics Laboratory, Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

Epilepsy is a common neurological disorder affecting over 50 million people worldwide. While seizures can be effectively controlled with anti-epileptic drugs (AEDs) in most, about 20–30 percent however, do not adequately respond thereby affecting patient’s health and quality of life. The objective of this study is to determine whether polymorphisms in the transporter and target genes can explain the biological mechanism underlying response to AED, with a potentially significant impact on clinical practice.

Genotyping was carried out using Sequenom MassARRAY PCR on numerous relevant single nucleotide polymorphisms (SNPs) of drug transporter genes, including \( ABCC2 \), \( ABCB1 \) and its regulator \( PXR \), and of drug targets, including \( SCN1A \), \( SCN2A \) and \( SCN3A \) in more than 1100 patients and 1500 controls.

Results showed that \( ABCB1 \) rs3789243 C>T, C1236T, G2677T/A, rs6949448 C>T, and C3435T polymorphisms and their haplotypes, \( PXR \) G7635A polymorphism and interaction between these genes do not contribute to response to the carbamazepine (CBZ) or sodium valproate (VPA) monotherapy. However, significant association was observed between \( ABCC2 \) rs2273697-rs3740066 GT haplotype with drug responsiveness in the Malaysian Chinese. Data analysis of 39 polymorphisms of the \( SCN1A \), \( SCN2A \) and \( SCN3A \) genes in Malaysian and Hong Kong epilepsy cohorts showed no significant association with AED responsiveness.

Our comprehensive study in epilepsy suggests that unlike the \( ABCC2 \) rs2273697-rs3740066 GT haplotype with drug responsiveness in the Malaysian Chinese, the common polymorphisms in \( ABCB1 \), \( PXR \), \( SCN1A \), \( SCN2A \), and \( SCN3A \) do not play significant roles in influencing response to AEDs.
Pharmacogenetics/genomics has been around for a long time: although this has led to many discoveries, i.e. associations between phenotypes (drug efficacy and safety) and genotypes, translation of these discoveries into clinical practice has been poor. Lack of robust evidence is cited as the main reason. In other clinical areas, the randomised controlled trial is often regarded as the top of the evidence hierarchy, but very few trials have been undertaken in pharmacogenomics. Undertaking a RCT for a pharmacogenomics phenotype is more complicated than undertaking a conventional RCT – apart from the usual factors such as design, sample size, clinical outcome measures, and follow-up, additional factors such as how patients will be genotyped, how genotype will affect drug dose/choice, whether it will be cost-effective, and how many patients will need to be screened to identify those that fit with the inclusion criteria, all need to be considered. These additional factors inevitably also make it much more expensive to undertake pharmacogenomics-based RCTs. An additional factor that needs to be considered is the frequency of the phenotype, especially in the case of rare adverse events, where it may be impossible to undertake a RCT even if a prospective cohort study. In order to improve the translation of laboratory findings into the clinic, we need to consider different forms of evidence in a more intelligent, rather than purely relying on a hierarchy of evidence.

Numerous genetic markers have been identified to associated drug induced adverse reactions. However, the use of pharmacogenetics in clinical practice has been limited mostly due to lack of understanding and experience with the use of pharmacogenetics by the physicians and the limited access to genotyping facilities.

One way to solve these hurdles is to conduct prospective clinical trials using Pharmacogenetics. Clinical studies will allow us to assess the clinical benefit of using Pharmacogenetics, raise awareness among physicians as well as setting up infrastructures for genotyping.

Here, I present two clinical studies conducted in Taiwan which incorporated clinical genetics in clinical use.

Warfarin is the mostly widely prescribed oral anticoagulant (blood thinner) in the world. It is used for the prevention of thromboembolism in patients with deep vein thrombosis, atrial fibrillation, or prosthetic heart valve replacement. The drug is, however, very challenging for physicians as the required dose for each patient varies widely and is hard to predict. Administration of the wrong dose can compromise a patient’s safety causing hemorrhaging if too much of the drug is prescribed and embolism if less than the ideal dose is given. Numerous reports have demonstrated that SNPs in VKORC1 and CYP2C9 are associated with warfarin dose and dosing algorithms have been developed to better predict warfarin doses. To determine whether genotype-guided dosing can improve warfarin treatment, a controlled-randomized trial using algorithms developed from Taiwan’s population, IWPC and standard of care was conducted. Patients with indication of warfarin and without prior history of warfarin use are recruited. These patients are monitored at regular basis to determine whether using pharmacogenetic dosing algorithms can help patients to achieve therapeutic INR (2–3) faster as well as maintain in the therapeutic range.

Allopurinol, the commonly prescribed medication for gout and hyperuricemia, is one of the most frequent causes of severe cutaneous adverse reactions (SCAR), which include the hypersensitivity syndrome, Stevens-Johnson syndrome, and toxic epidermal necrolysis. The HLA-B*58:01 allele has been shown to associate with Allopurinol-induced SCAR. The risk of allopurinol induced SCAR was significantly higher inpatients carrying the HLA-B*5801 allele than those without the allele. To determine whether by predetermining the presence of this allele can reduce the incidence of allopurinol induced SCAR, we conducted a prospective study by identifying individuals at risk using HLA-B*5801 genotyping. Due to ethical considerations, the study was designed as a nonrandomized trial which used historical incidence as control. More than 2000 patients were recruited from 14 medical centers in Taiwan. Patients testing positive for the allele were advised not to take allopurinol and were given alternative medications. The patients were then followed by telephone on weekly basis for three months to monitor for any adverse events.

Results from the above two studies will be presented and their clinical implication will also be discussed.
would be the next generation of pharmacogenomics card. The implementa-
tion may be highly dependent on the success of pharmacogenetic card.

### S06
**New Technologies in Pharmacogenomics Research**
**C.S. Ku**  
Karolinska Institute, Sweden

The rapidly evolving discipline of genomic medicine is devoted to utilizing genomic information to further the clinical care of patients and to improve health outcomes. Pharmacogenomics is that branch of genomic medicine which specializes in applying genomic information in the context of therapeutic decision-making. This includes the detection of somatic mutations in tumor tissue to inform the prescription of molecular targeted drugs. My talk will discuss the application of next-generation sequencing (NGS) to this field and the preconditions for bringing NGS tests into the diagnostic setting. Recent developments regarding the launch of NGS-based pharmacogenomic tests into the marketplace are highlighted.

### S07
**Are We Ready to Practice the Pharmacogenomics for Personalized Pharmacotherapy?**
**J.G. Shin**  
Department of Pharmacology and Pharmacogenomics Research Center, Inje University, Department of Clinical Pharmacology and Clinical Trial Center, Inje Univ. Busan Paik Hospital, Busan 614-735, Korea

Pharmacogenomics (PGx) biomarkers are considered to be good predictors of individual drug responses for the personalized pharmacotherapy in the given individual patient. Although it is limited that the genetic tests for the personalized pharmacotherapy become popular in the medical practice, several pharmacogenomics biomarkers already allowed to genotype based pharmacotherapy and the validated biomarkers are listed in the drug labels. Therefore, it seems to be already in the ear of personalized medicine in limited therapeutic drugs. However, it is long term process for a genetic biomarker to be applied in to clinical practice through such many steps of functional and clinical validation. In general, the biomarker for the personalized pharmacotherapy should be validated for their clinical application including following steps; from discovery of genetic biomarker, pre-clinical validation, clinical validation and clinical utility validation as well as analytical validation for the diagnostics.

In addition to this scientific evidence, many of other infrastructures should be established in the community for the practice of personalized medicine; i.e., regulatory approval and insurance coverage of the service, experienced prescribers with pharmacogenetic councilors, ethical, legal and social considerations etc. The presentation will also touch the issues on the application of pharmacogenomics knowledge in to clinical practice together with cases of genotype guided personalized pharmacotherapy which has been experienced in our center.

### S08
**Deciphering Next-Generation Pharmacogenomics: An Information Technology Perspective**
**G. Potamias**<sup>a</sup>, **K. Lakiotaki**<sup>b</sup>, **E. Kartsaki**<sup>b</sup>, **A. Kanterakis**<sup>a</sup>, **T. Katsila**<sup>b</sup>, **G.P. Patrinos**<sup>b</sup>

<sup>a</sup>Institute of Computer Science, Foundation for Research and Technology Hellas, Crete, Greece, <sup>b</sup>University of Patras, School of Health Sciences, Department of Pharmacy, University Campus, Rion, Patras, Greece

In the post-genomic era, the rapid evolution of high-throughput genotyping technologies and the increased pace of production of genetic research data, are continually prompting the development of appropriate informatics tools, systems and databases as we attempt to cope with the flood of incoming genetic information. This dictates the development of information systems that would contribute to the creation of a powerful knowledge environment for genotype-to-phenotype information in the context of translational medicine. In the area of pharmacogenomics and personalized medicine, it is evident that database applications providing important information on the occurrence and consequences of gene variants involved in pharmacokinetics, pharmacodynamics, drug efficacy and drug toxicity, will become an integral tool for researchers and medical practitioners. We will present advances and challenges in the field of pharmacogenomics information systems, in particular the electronic molecular diagnostics assistant (eMoDiA), designed to provide personalized drug recommendations based on linked genotype-to-phenotype pharmacogenomics data, as well as to support biomedical researchers in the identification of pharmacogenomic related gene variants. The provisioned services are tuned in the framework of a single-access pharmacogenomics portal.

### S09
**Pharmacogenomics of SCAR and DILI in Thailand**
**S. Mahasirimongkol**  
Medical Life Sciences Institute, Department of Medical Sciences, Ministry of Public Health, Thailand

Severe cutaneous adverse reactions (SCAR) and drug induced liver injury (DILI) are common causes of hospital admission due to drug adverse reactions in Thailand. SCAR including Stevens-Johnson Syndrome/Toxic Epidermal Necrolysis/Drug reaction with eosinophilia and systemic symptoms (DRESS) are disproportionately affected Southeast Asians. Some particular drugs are predominating...
in Thailand due to epidemic of some infectious diseases such as HIV infection and tuberculosis. Antiretroviral and sulfamethoxazole/trimethoprim are known to induce severe cutaneous adverse reactions and these drugs are predominated in inducing SCAR in Thais.

DILI associated with antituberculosis is the commonest DILI causing admission to the hospital. Recently, prevention of these unfortunate events became available through pharmacogenomics studies and implementation of pharmacogenomics testing in public health program.

Various research strategies have been done for supporting the public health implementation of pharmacogenomics tests. These included the discovery researches through pharmacogenomics study in case-control studies, cost-utility analysis of the pharmacogenomics test and implementation of pilot testing to inform policy development.

We discovered several novel findings for drug-induced SCARS in Thailand, including SCARS related to Nevirapine, sulfamethoxazole, and phenobarbital. With collaborations in among Thais pharmacogenomics researchers, we confirmed HLA-B*15:02 association with carbamazepine-induced SJS/TEN and provide the pilot implementation of preventive HLA-B*15:02 tests in Bangkok. The DILI from antituberculosis was confirmed to be associated with slow acetylator genotypes and potentially results in the revision of isoniazid usage in Thai population. These findings lead to the safer use of these drugs in Thais and might applicable to other populations with similar genetic background.

In the case of Indonesia, the study of NAT2 polymorphisms in association with AT-DILI has been conducted. In the bimodal distribution model, the frequency between fast and slow NAT2 acetylator in TB patients with AT-DILI and without AT-DILI are 34% vs 64.2% and 66% vs. 35.84%, respectively (p < 0.0001). The study confirmed the significance of NAT2 gene polymorphisms, particularly the slow acetylator type with increased risk to AT-DILI in the population. Further studies of the association between CYP2E1 and GSTs gene family polymorphisms and AT-DILI are worth done in the near future.

**S10**
**Pharmacogenomics of Drug Induced Liver Injury due to Anti Tuberculosis Drugs and Indonesia Experience**

*R. Yuliwulandari*
Genomic Medicine Research Group, Universitas YARSI, Jakarta, Indonesia

Tuberculosis (TB) as one of global public health problems needs to be treated and eradicated. According to the World Health Organization, the TB treatment needs combination of 4–5 drugs and has to be given during initial and continuation phases. The main disadvantages of drug combination and long duration of TB treatment are the possibility of over or under dosage, adverse drug reactions (ADRs) and the difficulties in determining which drug that causes the ADRs.

The most common ADRs is drug induced liver injury (DILI), as liver plays central role in biotransformation and excretion of most drugs. The risk for developing DILI is associated with genetic and other factors such as age, gender, concomitant drugs, etc. The involvement of genetic variation in anti-tuberculosis (AT) drugs biotransformation has been reported in many studies. The variation in Isoniazid (INH) metabolism involves the polymorphism in NAT2 gene, in which deficiency in the activity of N-Acetyltransferase 2 (NAT2) enzyme commonly lead to susceptibility to AT-DILI. Besides that, INH also can induce Cytochrome P450 enzyme, especially CYP2E1. INH-DILI also has been associated with the activity of Glutathione S-Transferase, even though the result is conflicting in some reports.

The past 20 years have seen the identification of genetic polymorphisms that can contribute to the inter-individual variability in the effects of treatment with antipsychotic drugs, both in the emergence of adverse effects and in symptom response. Candidate gene studies have hugely advanced our understanding of the pharmacogenetics of one of the most problematic side effects of antipsychotic drug treatment, weight gain, and the combined effects of several functional polymorphisms, particularly in genes important in the hormonal hypothalamic control of food intake, provide the potential for predictive genetic testing. Genetic findings differentiating treatment response of negative and positive symptoms in schizophrenia have indicated the mechanisms (respectively effects on dopamine and serotonin systems) underlying drug action in relieving, or not, these various symptoms of the disease. Many of these and other pharmacogenetic findings involve functional polymorphisms in regulatory regions of genes. Often these polymorphisms are within islands of CpG sequences, sites for DNA methylation that contributes to epigenetic control of transcription. This stimulated our recent studies of DNA methylation in schizophrenia and its response to treatment, and we have identified association of DNA methylation in the 5-HT1A promoter sequence that parallels our pharmacogenetic findings on negative symptom response.

**S12**
**Individualization of Anticancer Therapeutics Based on Pharmacogenomics**

*T. Mushiroda*
RIKEN Center for Integrative Medical Sciences, Japan

Neutropenia and/or leucopenia are common adverse drug reactions (ADRs) after treatment with chemotherapeutic agents, which often cause life-threatening infections and the delay to cure. Genome-wide association studies (GWAS) were carried out using DNA samples from Biobank Japan and revealed a shortlist of the suggestive
Intersectional Genomics, a new branch of genomics linking whole external factors. Interaction and understanding pharmacogenomic interpretation. Methods, mathematics, and AI means. Allowing simpler doctor/patient human and other genomic information to diet using state of the art those associated with disease, e.g., cystic fibrosis, but not generally very simple cases, e.g., eye colour. Predictable phenotypes include characteristic but cannot predict phenotype very well – the result except in chromosomes. Genotyping describes the genes for a particular character.

Intricate biochemical processes extract the energy and other useful components that enable us to grow and function, and many compounds, seemingly unimportant in the past, are now recognised as being beneficial. The problem, for scientists and consumers alike, is that the benefits appear not to be the same for everyone.

Throughout our and our relative’s lives, we choose to a complex mixture of foods, which can have a dramatic impact on our health. Genomics seeks to understand the structure and function of our entire DNA sequence – all three billion base-pairs across 23 pairs of chromosomes. Genotyping describes the genes for a particular characteristic but cannot predict phenotype very well – the result except in very simple cases, e.g., eye colour. Predictable phenotypes include those associated with disease, e.g., cystic fibrosis, but not generally diet or age-related diseases, which are controlled by many genes and external factors.

In this lecture, I will introduce a new method of analysis known as Intersectional Genomics, a new branch of genomics linking whole human and other genomic information to diet using state of the art methods, mathematics, and AI means. Allowing simpler doctor/patient interaction and understanding pharmacogenomic interpretation.

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S13 
Nutrigenetics: The Impact of Whole Human Genome (NGS) Analysis on Lifestyle and Healthcare

D. Baker
The Golden Helix Foundation, UK

Phenytoin (PHT) is a commonly used anticonvulsant. This drug has been reported as one of the most common culprit drugs for severe cutaneous adverse drug reactions (SCAR) including Stevens–Johnson syndrome (SJS), Toxic epidermal necrolysis (TEN) and Hypersensitivity syndrome (HSS) in several countries. Recent evidence suggests that HLA-B*1502 and CYP2C9 alleles may be used as good markers for prediction of SJS/TEN caused by PHT in Han Chinese. Since ethnic differences in the association between HLA genetic polymorphism and drug-induced SCAR is well recognized, we therefore conduct a case-control study in order to identify the HLA alleles and drug metabolizing enzyme gene that may be used as a valid genetic marker(s) for prediction of PHT-induced SCAR in Thai population. Sixty-nine PHT-induced SCAR and 93 PHT-tolerant patients were enrolled in the study. The HLA-A, B and C as well as CYP2C9 were genotyped from genomic DNA by the reverse sequence-specific oligonucleotide probes method and Taqman discrimination assay. Results reveal that HLA-B*1504 was strongly associated with PHT-induced SJS/TEN while HLA-B*5101 was strongly associated with PHT-induced HSS. Moreover, HLA-Cw*1402 was moderately associated with both SJS/TEN and HSS caused by PHT. No significance association between HLA-B*1502 and PHT-induced SJS/TEN was observed in our study population. Interestingly, the association between CYP2C9 genetic polymorphism and SCAR caused by PHT appear to be specific for certain cutaneous reactions.
**S15**

**Modeling the Functional Impact of Pharmacogenomic Variants in Genome-Edited Isogenic Human Cells**

A. Maillet, S.N. Sadananda, K. Tan, M.T. Toh, L.R. Brunham

Translational Laboratory in Genetic Medicine, Association for Science, Technology and Research (A*STAR) and National University of Singapore, Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

Genome-wide association studies (GWAS) have identified a large number of pharmacogenomic variants that are associated with drug efficacy and toxicity. However, the translation of these findings into clinical practice has been slow and in many cases the biological significance of variants identified by GWAS remains unclear. In part, this is due to a lack of appropriate model systems in which to validate the functional effect of these genetic variants. The development of genome-editing tools such as CRISPR-Cas9 has created the opportunity to precisely alter the genome of mammalian cells to allow for interrogation of the functional impact of specific sequence variants on an isogenic background. In addition, the availability of pluripotent stem cells, including both human embryonic stem cells and induced pluripotent stem cells, together with robust differentiation protocols for various cell types including cardiomyocytes, neurons and hepatocytes, has opened the door to using both patient-derived and genome-edited pluripotent stem cells as models for investigating drug-response in physiological relevant cell types. We have developed a platform to assess the functional significance of specific genomic variants on drug-response using genome-edited pluripotent stem cells. This platform will generate new approaches to interrogate genome function and genome variation and will enhance the discovery and translation of pharmacogenomic markers impacting drug-response.

**S16**

**Pharmacogenomics and Genetic Testing in the Malaysian Populations**

S.H. Gan

Human Genome Centre, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia

Generally, pharmacogenomics and genetic testing among the Malaysian populations are still in their infancies. Although there is variabilities in the ‘Asian gene’ when compared to Caucasian genes, most drug dosings in Malaysia are based on Caucasian data. In our previous study on CYP2D6 polymorphism and tramadol, we found that approximately 50% of patients possess the wild-type allele with the ‘Asian’ CYP2D6*10 allele contributing to another 40.22%. There was also a significant difference in the side effect profiles of the various genotype groups with the intermediate metabolisers (IMs) suffering more dizziness, headache, nausea, sweating and dry mouth with tramadol compared with the extensive metabolisers (EMs) and ultrarapid metabolisers (UMs). Generally, the mean total clearance of tramadol was lower (19.66 L/h) and the half-life longer (5.99 h) compared to that reported among the Western populations (28.0 L/h and 5.2 h respectively). The UM and EMs had 2.6 and 1.3 times faster total clearances compared to the IMs respectively. It was concluded that tramadol’s pharmacokinetics and adverse effects were influenced by CYP2D6 polymorphism. In another study, we found that subjects having the CYP3A4*1/*18 genotype had 34% longer elimination half-lives when compared to those with the normal genotypes (CYP3A4*1/*1). It is concluded that genetic polymorphisms of CYP3A4, specifically CYP3A4*18, play a major role in contributing to the inter-individual variability in repaglinide’s pharmacokinetics. In another study, we found that subjects having the CYP2A6*1B variants responsible for ultrarapid metabolism of artemisinine suffered a significantly higher incidence of adverse drug effects. In summary, our findings indicate that genotyping is important as it plays an important role in influencing the pharmacokinetics and pharmacodynamics of many drugs in Malaysia.

**S17**

**Pharmacogenomic Research in the Biobank Japan Project**

M. Kubo

RIKEN Center for Integrative Medical Sciences, Japan

The Biobank Japan project was started as a leading project of MEXT in 2003 [Project leader: Yusuke Nakamura (FY2003–2011), Michiaki Kubo (FY2011-current)]. Final goal of this project is the implementation of personalized medicine in Japan. To accomplish this goal, this project aims to 1) discover susceptibility genes of various common diseases and drug responses, 2) identify molecular targets for evidence-based drug development or diagnostic tools, 3) examine gene-environment interaction to find out clues for the prevention of diseases, and 4) identify important genetic and clinical information that can be applied for the establishment of personalized medicine and prevention.

In the first 5-year period of FY2003–2007, this project constructed the patient-oriented biobank, named as Biobank Japan, which collected DNA, serum and clinical information from 200,000 patients that suffered at least one of 47 target diseases. Using these samples, this project performed GWAS to identify susceptibility genes for various diseases as well as those for drug responses. Although several obstacles exist in the pharmacogenomic research, we could identify several susceptibility genes for pharmacogenomics with large effect. We applied some of these genomic variations to clinical intervention studies that examine clinical validity and utility of pharmacogenomic markers before prescribing drugs to patients. We hope this project will provide evidences for the implementation of personalized medicine in near future.
Abstracts

**S18**

**Translation of Opioid Pharmacogenomics into Pain Treatment: Commercial Reality versus Regulatory Oversight**

A.A. Somogyi  
Discipline of Pharmacology, Faculty of Health Sciences, University of Adelaide, Adelaide 5005, Australia

Many studies with opioids have been conducted in healthy subjects and patient populations with respect to pharmacokinetic (metabolic enzymes, transporters) and pharmacodynamic (receptor) genes. For opioids such as codeine requiring CYP2D6 to form active metabolites (eg. morphine), genotypic poor metabolisers have negligible active metabolite concentrations. In experimental pain models in healthy subjects, such CYP2D6 PMs have a substantially lower analgesic response but as soon as patients are investigated such ‘clean’ differences disappear and only for tramadol and not codeine, oxycodone or hydrocodone is the PM phenotype clinically important. However, patients with multiple gene copies (PMs) have high active metabolite concentrations and can experience opioid toxicity.

Opioid pharmacogenetic testing (exclusively CYP2D6) has become available in Australia through private pathology services, but is not reimbursed by the Government nor is there any national regulatory oversight. In Pain Clinics, patients are either requesting testing or bringing results with recommendations for their treatments, much to the frustration of the pain specialist and irritation to the patient. At issue is that the phenotype measurement for pain is imprecise and highly variable as analgesic response is controlled by many factors including psychological, social and cultural, and these may swamp any effect from pharmacogenomics. However the mu receptor variant (OPRM1 118A>G) has been consistently associated with increased opioid dose, but only in Asian patients.

The commercialization of current genetic tests for pain treatment efficacy with opioids is premature as the evidence is limited and regulation is necessary.

**S19**

**Health Regulatory Authority Perspective on Challenges of Clinical Adoption of Pharmacogenomic in Improving Drug Safety**

C. Sung\(^a,b\), D.S.L. Toh\(^a\), L.L. Tan\(^a\), C. Lake\(^a\), M. Limenta\(^a\), W.C. Tan-Kop\(^a\), C.L. Chan\(^a\)  
\(^a\)Vigilance and Compliance Branch, Health Sciences Authority, Singapore, \(^b\)Duke-NUS Graduate Medical School, Singapore

Pharmacogenomics studies have contributed considerable knowledge to identifying genetic variants that confer increased risk of experiencing an adverse drug reaction (ADR). However, clinical adoption of the knowledge has lagged behind. This may be due in part to genetic variability in different ethnic and geographic populations, which requires validation of such associations for specific populations. In addition, the positive and negative predictive values of the genetic test and the cost-effectiveness need to be estimated in order for healthcare systems to consider routine pharmacogenetic testing. Other factors such as convenience, cost and turnaround time of genetic test results as well as options for test-positive patients, such as availability of comparably effective and safe medication and/or alternate clinical strategies need to be addressed. The Singapore Health Sciences Authority will share a case example of its experience on validating a pharmacogenomic association in its multi-ethnic population and the challenges that had to be overcome in applying pharmacogenomic knowledge to reduce the risk of drug-induced serious skin rash. Extensive consultations with clinicians, genetic testing facilities, the Ministry of Health and other stakeholders, as well as communications to reach out to the larger clinical community were undertaken. Outcomes on uptake of genetic testing and ADR rates will also be presented.

**P001**

**Characterization of Multidrug-Resistant and Detection of Metallo-Beta-Lactamase-Producing among Nosocomial *Pseudomonas aeruginosa* Isolates at University of Malaya Medical Center, Malaysia**

A.M. Al-Kabsi\(^a\), M.Y. Yusof\(^b\), S.D. Sekaran\(^b\)  
\(^a\)Department of Medical Microbiology, Faculty of Medicine, Cyberjaya University College of Medical Sciences, Kuala Lumpur, Malaysia, \(^b\)Department of Medical Microbiology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

**Objective:** The emergence of *Pseudomonas aeruginosa* that produce Metallo-beta-lactamases (MBLs) and extended spectrum beta-lactamases (ESBLs) that are multidrug resistant (MDR), pose a serious therapeutic problem. In this study, we have investigated the contributions of various mechanisms of resistance to carbapenems among a collection of imipenem and meropenem-non-susceptible *P. aeruginosa* isolates.

**Material and Methods:** Sixty-five isolates of *P. aeruginosa* which showed resistance to meropenem and imipenem were evaluated for gene expression (*MexCD*, *OprM* and *MexEF*) using the RT-PCR and the ESBL production genes (*bla SHV*, TEM CTX-M, VEB and PER genes) by using the PCR amplification assay.

**Results:** The gene expression analysis of 65 MDR *P. aeruginosa* clinical isolates showed overexpression of efflux pumps MexCD and MexEF for most of the isolates and decreased OprM expression was observed.

Among the 65 isolates of *P. aeruginosa* that are resistant to imipenem and meropenem, 41 isolates were MBL producers. Out of 41 MBL gene PCR positive isolates were present in 20, 14, 4, 2 and only one for *blaTEM*, *blaVIM*, *blaGIM*, *blaNDM* and *blaPER* respectively. 33 out of 65 of imipenem and meropenem non-susceptible *P. aeruginosa* isolates used in this study tested positive for ESBL genes. Out of 33 ESBL genes, PCR-positive isolates were present in 25, 5 and 3 for *blaSIM*, *blaTEM* and *blaCTX-M* respectively. No *blaSIM* and *blaPER* genes were detected for imipenem and meropenem resistance.

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Conclusion: This study demonstrated that high efflux overproduction, MBL and ESBL production that confirm these resistance genes has the ability to increase the resistance to imipenem and meropenem among *P. aeruginosa*.

**P002**

**Association of HLA-B Alleles and Aromatic Anti-Epileptic Drugs Induced Stevens – Johnson Syndrome and Toxic Epidermal Necrolysis in Malaysian Population**

A.H.P. Khor, K.S. Lim, C.T. Tan, C.C. Ng

Division of Neurology, Department of Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia; Genetics and Molecular Biology Unit, Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia

**Objective:** Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) is a form of severe cutaneous adverse drug reactions, commonly associated with aromatic anti-epileptic drugs (AEDs), particularly carbamazepine (CBZ), phenytoin (PHT) and lamotrigine (LTG). Strong drug-specific association is reported in CBZ-SJS/TEN and HLA-B*15:02 in Han Chinese and some populations in Southeast Asia. In this study we aim to extend the investigation to phenytoin and lamotrigine to further explore the association between HLA-B alleles and SJS/TEN induced by aromatic AEDs in our multi-ethnic population.

**Material and Methods:** HLA-B alleles were genotyped in 26 AEDS-SJS/TEN patients, including 20 CBZ-SJS/TEN (6 Indian, 9 Malay, 5 Chinese), 3 PHT-SJS (1 Malay, 2 Chinese) and 3 LTG-SJS (1 Indian, 1 Malay, 1 Chinese), and 248 AEDs-tolerant controls (91 CBZ (22 Indian, 22 Malay, 47 Chinese), 83 PHT (23 Indian, 29 Malay, 31 Chinese) and 74 LTG (21 Indian, 23 Malay, 30 Chinese). The carrier frequency of HLA-B alleles was compared in cases and controls.

**Results:** HLA-B*15:02 was present in 77.8% (7/9) of Malay, 60% (3/5) of Chinese and 33.3% (2/6) of Indian CBZ-SJS/TEN patients. There was significant association between HLA-B*15:02 and CBZ-SJS/TEN in all three ethnic groups: Malay (77.8% versus 17.4%; \( p = 2.60 \times 10^{-5}; \) Odds ratio (OR) = 16.6; 95% confidence interval (CI) = 2.47–111.80), Chinese (60% versus 12.8%; \( p = 0.03; \) OR = 10.2; 95%CI = 1.41–74.52) and Indian (33.3% versus 0%; \( p = 0.04; \) OR = 25.0; 95%CI = 1.02–613.72). In PHT-SJS/TEN patients, HLA-B*15:02 was detected in 1 of 3, but not present in LTG-SJS patients. The carrier frequency differences of HLA-B alleles in PHT-SJS/TEN and LTG-SJS patients with PHT and LTG tolerant controls respectively, did not reach statistical significance \( (p > 0.05) \).

**Conclusion:** Our results found significant association of HLA-B*15:02 to CBZ-SJS/TEN in our Malaysian population. Due to limited PHT and LTG induced SJS/TEN cases, the association for PHT and LTG require replication in a bigger cohort for further confirmation.

**P003**

**Role of SYN2, TIMP4, and PPARG Gene Polymorphisms and Response to Anticonvulsants**


Pharmacogenomics Laboratory, Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia; Department of Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia; Department of Pediatrics, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia

**Objective:** Approximately one third of patients with epilepsy do not respond to antiepileptic drugs (AEDs). SYN2, TIMP4, and PPARG genes are highly expressed in the brain and potentially play a role in epilepsy. The objective was to investigate the association of SYN2, TIMP4, and PPARG gene loci and their linkage disequilibrium (LD) with response to treatment in the Malaysian epilepsy patients.

**Material and Methods:** SYN2 rs3773364, TIMP4 rs3755724, and PPARG rs2920502 loci were genotyped in 685 Chinese, Indians, and Malays subjects under carbamazepine (CBZ) or sodium valproate (VPA) monotherapy using the Sequenom MassARRAY iPLEX Gold platform.

**Results:** Our data revealed no allelic, genotypic, or haplotypic association of these loci with response to CBZ or VPA in each ethnicity.

**Conclusion:** This study failed to show any contribution of these loci with response to treatment in in Malaysian epilepsy patients.

**P004**

**Effect of MTHFR Polymorphisms on Methotrexate Efficacy in Acute Lymphocytic Leukemia Patient**

G. Mostafa-Hedeab, G.T.A. Ebid

Pharmacology Department–Aljouf Medical College–Al-Jouf, Kingdom of Saudia Arabia; Pharmacology Department–Beni Suef Medical College–Beni Suef–Egypt; Clinical Pathology Department–NCI–Cairo University–Egypt

**Objective:** Methotrexate commonly used in ALL treatment inhibits Methylene Dihydrofolate reductase (MDHFR) as a main target. MTHFR genes polymorphisms may affect methotrexate activity is widely varies by geographical origin. Based on that the present study was performed to assess the effect of MTHFR polymorphisms on the efficacy and toxicity of MTX therapy among ALL patient.

**Material and Methods:** 94 ALL Egyptian patients, receiving methotrexate in different protocols were included. MTHFR C677T (rs1801133) and A1298C (rs1801131) genotyping performed using RFLP.

**Results:** Frequency of rs1801133 42.6% CC, 46.8% TT and 10.6% CT, whereas rs1801131 62.8% AA, 24.5% and 12.8% CC.
There was no significant associations between rs1801133 and rs1801131 genotyping regarding hemoglobin (anemia) (1) (P = 0.4), Leukocyte count (0.6) (P = 1), platelets (P = 0.4) (P = 0.4), hepatic toxicity (P = 0.4) (P = 0.7), LDH levels (P = 0.3) (P = 0.9), relapse occurrence (P = 0.1) (P = 0.2) and 2ndry malignancy occurrence (P = 0.1) (P = 0.7) respectively. While there is significant association between uric acid (UA) level and rs1801131 genotype (P = 0.03), no significance regarding rs1801131 genotyping (P = 0.5). Wild-type plus heterozygous alleles versus homozygous alleles were compared. No significant association in either rs1801133 or rs1801131 regarding Hematopoietic toxicity (P = 0.7) (P = 0.2), platelet (p = 0.8) (p = 0.3), hepatotoxicity (P = 0.9) (P = 0.5), UA (p = 0.7) (p = 0.3), response (p = 0.7) (p = 0.2), relapse occurrence (p = 0.9) (p = 0.09) or 2ndry malignancy (p = 0.5) (p = 0.4) respectively.

**Conclusion:** rs1801133 genotyping may affect UA level. MTHFR polymorphism has no effect on Methotrexate efficacy or toxicity in ALL.

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**P006**

**High Genetic Variability in the 3’-UTR of CYP1A2, CYP2B6, CYP3A4, NR1I2 and UGT2B7 in Africans: Effects on Drug Metabolising Enzyme Regulation by microRNA**

M. Swart, C. Dandara

Pharmaco genetics and Cancer Research Group, Division of Human Genetics, Department of Clinical Laboratory Sciences and Institute of Infectious Disease and Molecular Medicine (IDM), Faculty of Health Sciences, University of Cape Town, Observatory, Cape Town, South Africa

**Objective:** It has been shown that DMEs are regulated by microRNAs through targeting the 3’-untranslated region (3’-UTR). Thus, genetic variation in this region may affect DME gene regulation and ultimate drug response by either creating or disrupting microRNA recognition sites. Together with variation in DME genes, genetic variation in the 3’-UTR could be used to explain a larger proportion of the inter-individual variability observed in drug response. We set out to investigate the extent of genetic variation in the 3’-UTR of DME genes and evaluate its possible pharmacogenomics significances.

**Material and Methods:** The 3’-UTR for CYP1A2, CYP2B6, CYP3A4, NR1I2 and UGT2B7 was sequenced in thirty Bantu-speaking South Africans. In Silico prediction tools were used to determine the effects of genetic variation on microRNA regulation. Six of the identified miRSNPs were selected for further genotyping among 300 Bantu-speaking South African HIV-infected patients receiving EFV-containing HAART.

**Results:** Previously reported and novel genetic variants were identified in the 3’-UTR for CYP1A2, CYP2B6, CYP3A4, NR1I2 and UGT2B7 supporting the high degree of genetic diversity among African individuals. Most of these variants were predicted to interfere with microRNA regulation potentially affecting response when patients are treated with commonly used therapeutic drugs including EFV-containing HAART. The CYP2B6 rs1042389CC (g.31950T>C) genotype was significantly associated with low EFV levels (P = 0.0272).

**Conclusion:** Results from the sequencing and further genotyping provide support for comprehensive studies of genetic variation in the 3’-UTR of genes coding for drug metabolising enzymes and their inclusion in predictive pharmacogenomics models to get a clearer picture on correlates of drug response.
Role of HLA-B*5801 Genetic Testing and Enhanced Safety Program When Initiating Allopurinol for Chronic Gout Management: A Cost-Effectiveness Analysis

D. Dong, W.C. Tan-Koi, G.G. Teng, E. Finkelstein, C. Sung

Objective: Allopurinol is an efficacious urate-lowering therapy (ULT), but on rare occasions, patients develop potentially fatal adverse reactions. The risk of reactions such as Stevens-Johnson Syndrome is significantly higher among HLA-B*5801 carriers. We assessed the cost-effectiveness of risk-mitigation strategies that use HLA-B*5801 genetic testing, a hypothetical enhanced safety program, or a combination of both.

Material and Methods: The model considered Singaporean patients with chronic gout, over a lifetime horizon, where allopurinol and probenecid are appropriate medications. The model incorporated response rates for different drugs, allele frequencies, drug prices, clinician and hospital costs, and mortality rates.

Results: Current standard of care with allopurinol as first-line treatment without genetic testing was the preferred strategy from a health systems perspective, based on a cost-effectiveness threshold of US$50,000/QALY. Avoidance of ULTs was the least preferred strategy which had significantly lower QALYs and higher costs.

Conclusion: Conditions under which genotyping or enhanced safety program would become cost-effective were identified.

Impact of Single Nucleotide Polymorphisms (SNPs) of Type 2 Diabetes (T2D) Genes on Patients’ Response to Sulphonylurea Therapy


Objective: Pharmacogenomic studies of sulphonylurea drugs used in the management of Type 2 Diabetes (T2D) are vital in understanding the influence of genetic markers on treatment outcome. This review compared the results of individual studies on the association between single nucleotide polymorphisms (SNPs) and T2D patients’ response to sulphonylurea therapy.

Material and Methods: All published T2D gene association studies on sulphonylurea treated patients between January 2003 and May 2014 which used glycosylated haemoglobin (HbA1c) or fasting plasma glucose (FPG) as outcome measures were included in this review.

Results: Among the different SNPs reviewed, rs7903146 polymorphism of the transcription factor 7 – like 2 (TCF7L2) gene was significantly associated with sulphonylurea failure in Caucasians (Scottish: OR 1.28, p = 0.014; Germans: OR 1.57, 95% CI 1.01–2.45, p = 0.046). The trend is however unclear for rs5219 (E23K) polymorphism.
Analyses using DAVID v6.7.

Target Scan Human v6.2 followed by gene-annotation enrichment.

These findings into the clinical setting.

document evidence on sulphonylurea pharmacogenomics to allow translation of these findings into the clinical setting.

Transfection with miR-210 inhibitors decreases the sensitivity and apoptosis upon treatment with ACA and CDDP by reducing cell proliferation and increasing apoptosis.

Conclusion: In conclusion, there is currently insufficient evidence on sulphonylurea pharmacogenomics to allow translation of these findings into the clinical setting.

**Abstracts**

**P010**

**Inhibition of miR-210 Enhances Sensitivity Towards 1’S-1’-Acetoxychavicol Acetate (ACA) and Cisplatin (CDDP) in Human Cervical Carcinoma Cells**

*N.H. Phuah*<sup>a</sup>, N.H. Nagoor<sup>a,b</sup>

*<sup>a</sup>Institute of Biological Science (Genetics & Molecular Biology), Faculty of Science, University Malaya, 50603 Kuala Lumpur, Malaysia, *<sup>b</sup>Centre for Research in Biotechnology for Agriculture (CEBAR), University of Malaya, 50603 Kuala Lumpur, Malaysia*

**Objective:** Cervical cancer remains the second most common cancer in women worldwide and third most common cancer among Malaysian women despite having good prognosis through early detection. The success of chemotherapy is often hindered by dose-limiting toxicities and development of drug resistance. Recent studies have shown that microRNAs (miRNAs) can regulate diverse biological processes, including response towards anti-cancer drugs. Hence, the aim of this study was to investigate the role of miR-210 in regulating response towards 1’S-1’-acetoxychavicol acetate (ACA), a natural compound, and a widely-used anti-cancer drug cisplatin (CDDP) in human cervical carcinoma cells.

**Material and Methods:** The levels of miR-210 in SiHa human cervical carcinoma cells following transfection with miR-210 inhibitors and negative control were analyzed using RT-qPCR. Cell viability and apoptosis upon treatment with ACA and CDDP in transfected cells were measured using MTT assay and Annexin V-FITC/PI staining, respectively. Gene targets of miR-210 were predicted using Target Scan Human v6.2 followed by gene-annotation enrichment analyses using DAVID v6.7.

**Results:** Transfection with miR-210 inhibitors decreases the levels of miR-210 in the cells, compared to cells transfected with negative control. Inhibition of miR-210 sensitizes these cells towards ACA and CDDP by reducing cell proliferation and increasing apoptosis. A pathway model illustrating the interaction of miR-210 with its predicted targets highlighted three key signaling pathways: MAPK, PI3K-AKT and TGF-β, which regulates cell proliferation and apoptosis.

**Conclusion:** These results suggest that miR-210 could play an important role in anti-cancer drug responses, hence providing a novel platform for potential therapeutic approaches in chemotherapy.

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**P011**

**Genetic Determinants of Antiplatelet Aggregation Response to Clopidogrel Under Steady State Condition**

*N. Nakkam<sup>a,b</sup>, S. Kanjanawar<sup>a,b</sup>, S. Tiamkao<sup>c</sup>, W. Tassaneeyakul<sup>a,b</sup>*

<sup>a</sup>Department of Pharmacology, Faculty of Medicine, Khon Kaen University, 40002, Thailand, *<sup>b</sup>Research and Diagnostic Center for Emerging Infectious Diseases, Khon Kaen University, 40002, Thailand, *<sup>c</sup>Division of Neurology, Department of Medicine, Faculty of Medicine, Khon Kaen University, 40002, Thailand

**Objective:** Clopidogrel is an antiplatelet agent. It requires two-step biotransformation via several cytochrome P450 (CYP) isozymes and paraoxonase-1 (PON1) enzyme to its active metabolite. Recent data demonstrated that genetic factors may affect clopidogrel response. The aim of this study was to evaluate the effect of CYP2C19, CYP3A5 and PON1 Q192R polymorphisms on antiplatelet effect of clopidogrel under steady state condition.

**Material and Methods:** Thirty-five healthy subjects were treated with clopidogrel (75 mg/day) for 7 days and blood samples were collected at pre-dose, 1, 2, 3, 4, 8, 12 and 24 hr after last dose administered. Platelet aggregation was determined using whole blood impedance aggregometry. Genotyping for CYP2C19<sup>*,*3</sup>, CYP3A5 and PON1 Q192R polymorphisms were performed by PCR-RFLP method. CYP3A5<sup>*3</sup> polymorphism was performed by custom Taqman single nucleotide polymorphism genotyping method.

**Results:** The maximal antiplatelet effect (*E<sub>max</sub>*<sup>2</sup>) and the areas under the time-antiplatelet effect curves (*AUEC<sub>0–24 hr</sub>*<sup>2</sup>) observed in CYP2C19<sup>*,*3</sup> carriers were significantly lower than those of wild-type carriers. There was no significant difference in *E<sub>max</sub>* and *AUEC<sub>0–24 hr</sub>* among subjects who carried different genotypes of both CYP2C19 and CYP3A5. Among the subgroup of subjects who are heterozygous of CYP2C19<sup>*,*3</sup> carriers, *E<sub>max</sub>* and *AUEC<sub>0–24 hr</sub>* of both CYP2C19<sup>*,*3</sup> and CYP3A5<sup>*3</sup> carriers were significantly different between subjects who carried different genotypes of both CYP2C19 and CYP3A5. Among the subgroup of subjects who are heterozygous of CYP2C19<sup>*,*3</sup> carriers, *E<sub>max</sub>* and *AUEC<sub>0–24 hr</sub>* of clopidogrel were not significantly different between subjects who carried different genotypes of CYP2C19 and CYP3A5. Among the subgroup of subjects who are heterozygous of CYP2C19<sup>*,*3</sup> carriers, *E<sub>max</sub>* and *AUEC<sub>0–24 hr</sub>* of clopidogrel were not significantly different between subjects who carried different genotypes of CYP3A5<sup>*3</sup> carriers.

**Conclusion:** CYP2C19<sup>*,*3</sup> is a major genetic determinant for clopidogrel response. CYP3A5<sup>*3</sup> genotype plays some roles in its antiplatelet effect. However, PON1 Q192R polymorphism does not appear to be a significant determinant of clopidogrel response.
**Population Differences in the Genetic Polymorphism of CYP2B6 May Impact on the Pharmacology of MMT**

M. Nurfadhina\(^a\), M. Nasir\(^b\), B. Darussalam\(^c\), M.Y.M. Azhar\(^d\), S.C. Tan\(^e\), R. Ismail\(^f\)

\(^a\)Pharmacogenetics & Novel Therapeutics Cluster, Institute for Research in Molecular Medicine (INFORMM), Universiti Sains Malaysia, Malaysia, \(^b\)Faculty of Medicine & Health Sciences, Universiti Sultan Zainal Abidin, Terengganu, Malaysia, \(^c\)Jabatan Penjara Malaysia, Bukit Wira, Kajang, Selangor, Malaysia, \(^d\)Department of Psychiatric, School of Medicine, Universiti Sains Malaysia, Malaysia, \(^e\)Centre of Excellence for Research in AIDS, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

**Objective:** Introduced by the German as a synthetic opioid analgesic, methadone is used worldwide as a substitution treatment for opioid dependence. It is a chiral compound and its pharmacology is complex. This can further be complicated by population differences in the genetic polymorphisms of enzymes metabolizing the drug and preferential metabolism of specific isomers. Administered as a chiral mixture, its active S-form is preferentially metabolized by the polymorphic CYP2B6, with the CYP2B6\(^*\)6 allele [SNPs 785A>G (rs2279343) and 516G>T (rs3745274)] associated with slow metabolism.

**Material and Methods:** We genotyped a well-characterized sample of 92 opiate dependent individuals in several prisons in Malaysia and treated with MMT. Daily doses ranged from 5 to 120 mg (mean 57.5±33 mg).

**Results:** Mean S-methadone serum concentrations corrected for dose in subjects homozygous for CYP2B6\(^*\)6 (4.44±1.87 ng/ml.mg) were significantly higher than for heterozygous (2.64±1.48) and non-carriers (2.70±1.83) \((P = 0.046)\). Similar patterns were observed for R to S-methadone ratios with CYP2B6\(^*\)6/\(^*\)6 having lower ratios (0.68±0.10) than heterozygous (1.02±0.39) and non-carriers (1.20±0.67) \((P = 0.000)\). No significant difference was found with R-methadone, the metabolites (EDDP), and the total R,S-methadone serum concentrations.

**Conclusion:** Preferential metabolism of active S-methadone is an important consideration for methadone when interpreting serum concentrations in diverse populations with different patterns of genetic polymorphism of CYP2B6 especially when it is administered as a racemate mixture.

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**Genetic Polymorphism of the Multidrug Resistance-1 in Once-Daily versus Twice-Daily Tacrolimus Formulations**

R.M. Guad\(^a\), N.L. Zaharan\(^b\), Z. Chik\(^a\), Z. Mohamed\(^d\), B.S. Haerian\(^e\), K.P. Ng\(^b\), W.A.H. Wan Md Adnan\(^b\)

\(^a\)Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia, \(^b\)Department of Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

**Objective:** A number of reports have been published on within-patient variability of tacrolimus formulations due to polymorphism in other countries. We aimed to clarify the impact of 2 common polymorphic sites in MDR1 (rs1045642 c. 3435C→T & rs1128503 c. 1236C→T) on tacrolimus dosages and dose-adjusted tacrolimus level in patients converted from Prograf to Advagraf in our population.

**Material and Methods:** We retrospectively reviewed 28 patients from whom a switch from Prograf to Advagraf was identified. Blood samples were collected for genotyping by using reverse transcriptase polymerase chain reaction (RT-PCR) and then correlated it with patients’ dose and dose-adjusted tacrolimus level for 1st month, 2nd month, 3rd month, 4th month and 6th months; both in pre- & post-conversion.

**Results:** The genotype distribution of studied patients demonstrated frequencies close to those expected from public databases: 3435C→T (genotype CC, CT & TT were 29%, 64% & 7%, respectively) and 1236C→T (genotype CC, CT & TT were 14%, 39% & 46% respectively). After conversion to Advagraf, mean daily dose was significantly lower, decreasing from 3.91±2.02 mg to 3.26±1.72 mg, corresponding to a 16.7% reduction \((p = 0.01)\). However, both ABCB1 3435C→T & ABCB1 1236C→T polymorphisms do not have a significant association on dosages and dose-adjusted tacrolimus levels in pre- and post-conversion tacrolimus formulations \((p > 0.05)\).

**Conclusion:** The results support that MDR-1 gene derived from polymorphisms on exon 26 c. 3435C→T & exon 12 c. 1236C→T do not lead to a significant association on dosing and dose-adjusted tacrolimus levels in patients converted from Prograf to Advagraf among Malaysian kidney transplant population.
P014
microRNA Expression Profiling of Methamphetamine Dependence in Rat Nucleus Accumbens Tissue
M.S. Sima, I.S. Parharb, T. Sogac, P. Vijayapandid, Z. Mohamede
aDepartment of Pharmacology, Faculty of Medicine, University of Malaya, bBrain Research Institute, School of Medicine and Health Sciences, Monash University, Sunway, Malaysia

Objective: Methamphetamine is a highly addictive psychostimulant that induces behavioral changes, the nucleus accumbens being the part of the brain that plays the important role in these changes, especially in drug addiction. However, little is known about the underlying mechanisms of methamphetamine effects on global miRNA expression. The objective of this study was to determine the global miRNA profiling of the methamphetamine dependence from the rat nucleus accumbens tissue and to identify the miRNAs which are associated with methamphetamine use and dependence.

Material and Methods: The study comprised of 18 male rats which were divided into 3 groups: 15 days continuous methamphetamine treatment (0.5,1,2,3,5,5.5 mg/kg), single dose acute methamphetamine treatment (5.5 mg/kg), and a control group. Addiction behavior was determined using Conditioned Place Preference task. The analysis of the miRNA profiling was performed using Affymetric microarray GeneChip® System.

Results: For behavior test, we found that the addiction behavior only occur with continuous treatment of methamphetamine, but not in acute treatment. Differential profiling of miRNAs indicated that 25 miRNAs were significantly up-regulated and 1 down-regulated when the acute treatment was given; 55 miRNAs were up-regulated and 15 down-regulated in the continuous methamphetamine treatment group. Comparing between acute treatment without addiction and the continuous treatment with addiction, 20 miRNAs were up-regulated with 3 down-regulated for addiction phenotype. The miRNAs were selected when there are more than 2 times fold changes, ANOVA test with \( p < 0.05 \) and FDR test with \( p > 0.05 \).

Conclusion: Our results suggest that dynamic changes occur in the expression of miRNAs, which may be associated with the methamphetamine use and dependence.

P015
The Association of MC4R and Metabolic Syndrome in Schizophrenia Patients on Long-Term Antipsychotics
S.N. Roffeera, G.P. Reynoldsb, N.Z. Zainalc, Z. Mohameda
aPharmacogenomics Laboratory, Department of Pharmacology, Faculty of Medicine, University of Malaya, Malaysia, bDepartment of Clinical Pharmacology, Second Medical School, University, Sheffield, UK, cDepartment of Psychological Medicine, Faculty of Medicine, University of Malaya, Malaysia

Objective: The melanocortin 4 receptor (MC4R) gene has been repeatedly associated with obesity. It was reported that the A-allele carrier of MC4R rs8087522 was associated with greater weight gain in European schizophrenia patients on clozapine. We want to investigate if rs8087522 was associated with metabolic syndrome in our schizophrenia patients on long-term antipsychotic treatment.

Materials and Methods: A total of 206 schizophrenia patients on at least a year of antipsychotic treatment were recruited. DNA was genotyped, while related anthropometric data (height, weight, etc.) and lipid profile (HDL levels, glucose levels, etc.) was recorded. Metabolic syndrome was identified by using the NCEP-ATPIII criteria.

Results: The patients have a total mean BMI of 27.89±5.06 kg/m², with 123 patients (59.7%) have MS while the rest 83 patients (40.3%) did not. MC4R rs8087522 was not associated with MS at allelic (G vs. A) and genotypic level (GG+GA vs. AA, GG vs. GA+AA) \((p > 0.05)\).

Conclusion: In conclusion, MC4R rs8087522 was not associated with the occurrence of metabolic syndrome in our Malaysian schizophrenia patients, which calls for further replication preferably within a larger sample size.

P016
The Effect of Genetic Polymorphism of CYP2C9 on Phenytoin Dosage Requirement in Thai Epileptic Patients
S. Vannaprasahta, P. Sirisawatat, P. Chiewthanakula, S. Tiamkaoa, W. Tassaneeyakulb
aDepartment of Pharmacology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand, bDepartment of Psychological Medicine, Khon Kaen Hospital, Khon Kaen, Thailand

Objective: Phenytoin is an epileptic drug which metabolized by CYP2C9 enzyme. Previous studies revealed polymorphism of CYP2C9 genes encoded the CYP2C9 enzyme activity. Patients who carried CYP2C9*3 mutant allele showed lower CYP2C9 activity than in wild type, resulted in increasing of phenytoin blood level. This study investigated the correlation between CYP2C9 polymorphism and phenytoin dosage regimen in Thai epileptic patients.
Material and Methods: One hundred and thirty patients who received phenytoin as single therapy were enrolled in this study from Srinagarind Hospital and Khon Kaen Hospital. Clinical and laboratory data were retrospectively reviewed. The blood sample was collected to analyze CYP2C9 genotype by using real-time PCR with TaqMan® probe.

Results: The genotype frequencies of CYP2C9*1/*1 and CYP2C9*1/*3 were 93.85% and 6.15%, respectively. There was no statistically difference between phenytoin dose and CYP2C9 genotypes (P > 0.05). CYP2C9*1/*3 patients had significantly higher free phenytoin blood level than CYP2C9*1/*1 patients (2.33±1.46 vs. 1.45±0.92 μg/mL, respectively, P < 0.05). Phenytoin dose to achieve therapeutic level for CYP2C9*1/*1 genotype significantly was higher than patients with CYP2C9*1/*3 genotype (330.36±44.70 and 275.00±50.00 mg/day, respectively, P < 0.05).

Conclusion: CYP2C9 polymorphism has significant effect on phenytoin treatment. Therefore, phenytoin dose adjusted by CYP2C9 genotype may increase efficacy and decrease adverse effect of phenytoin treatment.

P17
ABCC10 2759 C>T Allele Variant in Malaysian HIV Population and Its Association with Nevirapine Pharmacokinetic Parameters

S. Mustafa a,b, N.B. Hassan c, S. C. Tan d, M. Mustafa a, A.K. Abd Rahman e, L.L. Low a, W.N. Wan Yusuf a

aUniversiti Sains Malaysia, Kubang Kerian, Kelantan, bHospital Raja Perempuan Zainab II, Kota Bharu, Kelantan, Malaysia, cHospital Sultanah Nur Zaharah, Kuala Terengganu, Terengganu, Malaysia, dHospital Sultanah Bahiyah, Alor Star, Kedah, Malaysia

Objective: Nevirapine is reverse-transcriptase inhibitors widely used in a combination therapy to treat HIV infection. Recent work has suggested that nevirapine is a substrate for ABCC10. This study was conducted to explore ABCC10 2759 C>T allele frequency in Malaysian HIV patients and to identify the association of ABCC10 2759 variant with nevirapine pharmacokinetic parameters.

Material and Methods: 112 patients treated with nevirapine-based antiretroviral therapy (63 male and 49 female, aged 19-65 years old) were included in the study. Blood samples were drawn at 0, 0.5, 1, 1.5, 2, 3, 4 and 8 hours after morning dose. Plasma nevirapine concentrations were quantified using high performance liquid chromatography (HPLC) with UV detector method by liquid-liquid extraction. Real-time polymerase chain reaction was used to genotype ABCC10 2759 C>T.

Results: 97 patients were homozygous T allele, 14 were heterozygous T allele and only one patient with homozygous C allele was detected in the population, which met Hardy-Weinberg equilibrium. The allele frequencies observed in this study were 7.14% C and 92.86% T. Our study did not show significant difference between ABCC10 2759 C>T variant and pharmacokinetic parameters of nevirapine in Malaysian HIV patients.

Conclusion: In this study, we have determined allele frequencies of ABCC10 2759 in Malaysian HIV population, and our findings showed no association between ABCC10 2759 C>T variant and pharmacokinetic parameters of nevirapine. The small size of homozygous C allele carrier has undoubtedly influenced the findings.

P18
Pharmacogenomic and Pharmacokinetic Models of Warfarin and Its Application in Personalized Medicine

A. Pavan a, V.K. Kutala b, A.R. Malempati b, S.M. Naushad c

aDepartments of Clinical Pharmacology & Therapeutics, bCardio-Thoracic Surgery, Nizam’s Institute of Medical Sciences, Hyderabad, India, cSchool of Chemical & Biotechnology, SASTRA University, Tirumalaisamudram, Thanjavur, India

Objective: Wide inter individual variation in the dose of warfarin has become serious cause of concern of adverse events associated high or low dose of treatment.

Material and Methods: To address this issue, we have developed a population-specific pharmacogenomic algorithm by using multiple linear regression model by studying the cytochrome P450 IIC polypeptide9 (CYP2C9*2 and *3), vitamin K epoxide reductase complex 1 (VKORC1*3, *4, D36Y and -1639 G>A) CYP2C9*8, CYP4F2 V433M, GGCX G8016A polymorphisms. In addition, we evaluated the impact of CYP2C9*2 and CYP2C9*3 variants on binding and hydroxylation of warfarin by In silico approach.

Results: Our new algorithm explained 61% of the variability in warfarin dose. Higher percentage of INRs in therapeutic range and prolonged time in therapeutic range were observed. In the warfarin-resistant group, primary hypothyroidism was found to induce more resistance while in the warfarin-sensitive group, hyperthyroidism was found to increase sensitivity. In silico studies revealed that warfarin forms two hydrogen bonds with protein backbone i.e. I205 and S209, one hydrogen bond with protein side chain i.e. T301 and stacking interaction with F100 in CYP2C9*1. In CYP2C9*2 and CYP2C9*3 variants, two hydrogen bonds with protein backbone are disrupted. In double variant, all the hydrogen bonds are disrupted. Increase in warfarin-7-hydroxy warfarin ratio was observed with increase in variant alleles.

Conclusion: Our algorithm explains greater variability in warfarin dose requirement and its prolongs time in therapeutic range and minimizes out-of-range INRs. In silico studies revealed CYP2C9*2 and *3 variant result in disruption of hydrogen bonding interaction with warfarin thus impairing warfarin-7-hydroxylation.
**P019**

**Anti-Tuberculosis Drug Induced Liver Injury (AT-DILI): Preliminary Report of a Case-Control Study in a Malaysian Population**


*Department of Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia, The Pharmacogenomics Laboratory, Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia, Translational Laboratory in Genetic Medicine, A-Star, National University of Singapore, Singapore, Institute of Respiratory Medicine (IRM), Kuala Lumpur Malaysia*

**Objective:** Tuberculosis (TB) cases in Malaysia continue to rise leading to high rates of morbidity and mortality. Antitubercular medication is essential in curbing the spread but fatal adverse effect such as Anti-Tuberculosis Drug-induced Liver Injury (AT-DILI) is not uncommon. This study investigated the possible association of AT-DILI with genetic polymorphism of metabolising enzymes in the Malaysian population.

**Materials and Method:** Cases were identified from patients admitted to the National Institute of Respiratory Medicine (IRM) and the University of Malaya Medical Centre (UMMC) with AT-DILI diagnosis within 2–8 weeks of treatment initiation. Severity grading of liver dysfunction was based on the Common Toxicity Criteria for Adverse Events, National Institute of Health. Controls were age, sex and race-matched and comprised of patients undergoing the same anti-TB regimen without developing DILI. All samples were genotyped for certain candidate genes using microarray.

**Results:** A total of 27 cases and 68 controls were recruited. The patients consist of female 45% and male 55% with ethnicity breakdown of 58% Malays, 19% Chinese, 13% Indians and 10% others. The median age of the patients is 37, with age ranging from 18 to 86. Preliminary result indicated there is a strong association of N-acetyltransferase 2 (NAT2) genotype (p value of 0.024) with AT-DILI among the Malaysian population. This ongoing study will further assess the impact of other genetic risk factors for AT-DILI.

**Conclusion:** Polymorphism in NAT2 appears to have an influence on susceptibility to AT-DILI.

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**P020**

**Genetic Influences of UDP-Glucuronosyltransferase Genes on 20-HETE Glucuronidation in Human Liver Microsomes**


*Department of Pharmacology and Pharmacogenomics Research Center, Inje University College of Medicine, Inje University, Busan, South Korea, Department of Clinical Pharmacology, Inje University College of Medicine, Inje University, Busan, South Korea*

**Objective:** The 20-hydroxyicosatetraenoic acid (20-HETA), an arachidonic acid metabolite, is involved in many physiological functions, including blood pressure increase and platelet aggregation stimulation. The 20-HETA is glucuronidated by UDP-glucuronosyltransferases (UGTs) and it is eliminated through human urine mainly in the glucuronidated form.

**Material and Methods:** The present study identified major UGTs responsible for 20-HETA glucuronidation and investigated their genetic influence on the glucuronidation reaction using human livers (n = 44). Twelve recombinant UGTs were screened to identify the major contributors to 20-HETA glucuronidation.

**Results:** The results showed that UGT2B7, UGT1A9, and UGT1A3 were the major contributors to 20-HETA glucuronidation. Protein expression levels and genetic variants of UGT1A3, 1A9 and 2B7 were analyzed in human livers using Western blotting and genotyping, respectively. Glucuronidation of 20-HETA was significantly correlated with the protein levels of UGT2B7 (r^2 = 0.33, P < 0.001) and UGT1A9 (r^2 = 0.31, P < 0.001), but not UGT1A3 (r^2 = 0.02, P > 0.05). The genetic variations UGT2B7 802C>T, UGT1A9 –118T>A, and 1399T>C significantly altered 20-HETA-glucuronide formation (p < 0.05–0.001).

**Conclusion:** As altered levels of 20-HETA correlated with cardiovascular homeostasis and diseases, the present data may increase our understanding of 20-HETA metabolism and cardiovascular complications.
uptake of such technology. Findings of EEs are uncertain due to the limited evidence base of PGx’s clinical utility. In this review, quality of evidence and data sources used to derive input parameters – gene prevalence, analytical validity, clinical validity, and clinical utility were evaluated.

Material and Methods: PubMed, UK National Institute for Health and Clinical Excellence (NICE) database, Tufts CEA registry were searched for publications from 1 January 2008 to 30 September 2014. Inclusion criteria were: (i) article investigated a biomarker to target drug therapy (ii) article reported the economic analysis on a PGx. Data from all eligible articles were extracted using a standardized data collection form.

Results: Of 559 articles yielded, 37 articles were included. Diagnostic accuracy was explicitly examined in 8 studies where 6 were based on cohort or case-control studies and 2 were assumptions. The use of systematic review and/or meta-analysis (SR/MA) to derive input parameters was found in 10% (3/30) studies for gene prevalence, 30% (6/20) for clinical validity, 16% (4/25) for clinical utility. Data sources with the highest evidence level (SR/MA or large R) were used in less than half of the studies (49%, 18/37).

Conclusion: The utilization of data with low evidence level to derive input parameters may lead to less credible findings of EE studies. Evidence base generation in PGx is warranted to enable informed decision-making.

PO22
Multidrug Resistance-Associated Protein 2 (MRP2/ABCC2) Haplotypes Significantly Affect the Pharmacokinetics of Mycophenolic Acid in Renal Transplant Recipients

Z.I. Md Dom, I.S. Westley, J.K. Coller, A.A. Somogyi, B.C. Sallustio

Objective: Multidrug resistance-associated protein 2 (MRP2) plays an important role in the enterohepatic recirculation of mycophenolic acid (MPA) and its glucuronide metabolite (MPAG). However, it is unclear whether ABCC2 polymorphisms that result in altered MRP2 protein expression and/or functional activity influence MPA exposure. This retrospective study investigated the effect of recipient ABCC2 haplotypes on MPA and MPAG pharmacokinetics in the first two weeks following transplantation.

Material and Methods: Sixty patients gave informed consent and were prescribed mycophenolic acid loading, tacrolimus and prednisolone-based immunosuppression. Blood was collected for ABCC2 genotyping, and pharmacokinetic analysis was based on therapeutic drug monitoring data from recipients in whom abbreviated AUC (0–6 hr) monitoring had been carried out within 14 days of transplantation. ABCC2 genotyping (C-24T; G1249A; C3972T) was performed with PCR-RFLP. ABCC2 haplotypes were inferred using PHASE v2.1.1 and subjects were classified as low, wild-type or high expressers.

Results: Genotype frequencies conformed with Hardy-Weinberg equilibrium (P > 0.1). Although there was no effect of expressor status on average MPA and MPAG plasma concentrations, MPA trough (C0) concentrations were 2.3-fold higher in recipients with high- (n = 5) compared to low-expressor (n = 30) ABCC2 haplotypes (P = 0.04). A significant gene-dose effect was observed with an inverse step-wise relationship between ABCC2 expression and MPA C0 concentrations (Jonckheere-Terpstra test, P = 0.01). There was also a significant gene-dose effect between ABCC2 expression and MPAG/MPA C0 ratio (P = 0.04).

Conclusion: Determination of ABCC2 haplotypes may facilitate for more accurate MPA dosage adjustment.

PO23
mRNA Expression of CYP1B1 in Early Stage Malaysian Breast Cancer Patients – A Preliminary Report

A.A. Ahmad Aizat, M.D. Saleh M.S, V.M.K. Bhavaraju, D.Z. Andee, S.H. Gan, R. Ankathil

Objective: Early stage breast cancer patients are treated with adjuvant chemotherapy regimen after surgery. A significant number of breast cancer patients either fail to respond or acquire resistance to chemotherapy drugs resulting in cancer recurrence. The expression or activities of certain genes have been linked with the likelihood of cancer recurrence. CYP1B1 is a drug metabolizing enzyme involved in metabolic activity and response mediation of breast cancer chemotherapy drugs. Elevated expression CYP1B1 in tumour cells have been associated with host factor resistance against chemotherapeutic drugs. This study investigated the mRNA expression level of CYP1B1 in cancerous and non-cancerous adjacent normal tissues of breast cancer patients.

Material and Methods: Total RNA from 10 cancerous and 10 adjacent non-cancerous breast cancer tissues were isolated and CYP1B1 mRNA expression was quantified using quantitative real time PCR (qRT-PCR). Non-cancerous adjacent breast tissues were used as normalizing control and Beta-actin as reference gene.

Results: Five samples showed up regulation (mean value: 1.5108±0.3161) whereas the other five samples showed down regulation (mean value: 0.5144±0.3156) of CYP1B1 mRNA expression. The difference in mRNA expression level of CYP1B1 between up regulated and down regulated samples was significant (P = 0.001).

Conclusion: From this preliminary result, no firm conclusion could be derived. Additional studies on larger samples and further follow up are warranted to determine whether mRNA expression levels of CYP1B1 are associated with recurrence risk and resistance phenotype.
P024
Association of microRNA196A2 Gene Polymorphisms with Risk of Chronic Hepatitis B Infection in Malaysian Population
B. Riazalhosseini1, R. Mohamad2, Z. Mohameda
1The Pharmacogenomics Laboratory, Department of Pharmacology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia, 2Department of Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

Objective: Recent evidence has demonstrated that host genetic background plays an important role in susceptibility to infectious diseases such as hepatitis infection. Hepatitis B virus infection (HBV) is a major cause of chronic liver disease that affects more than 400 million people worldwide and is the most important cause of cirrhosis and hepatocellular carcinoma (HCC). The aim of our study is to define the effect of microRNA196A2 genetic variation on the consequences HBV infection patients.

Material and Methods: A total of 526 cases with hepatitis B infection and 720 healthy controls were enrolled in this study. The cases were divided to two groups, chronic hepatitis B with or without cirrhosis/HCC. We employed Sequenom MassARRAY® platform to genotype two single nucleotide polymorphisms (SNP) in the microRNA196A2 gene (rs11614913 C>T and rs12304647 A>C).

Results: The allele frequency of rs11614913 and rs12304647 were significantly different in HBV patients compared to controls (p = 0.007, OR = 1.239, CI = 1.060–1.448 and p = 0.042, OR = 0.828, CI = 0.690–0.994 respectively). However, no association was found when polymorphism in microRNA196A2 gene were compared in chronic HBV individuals with or without cirrhosis/HCC (p = 0.768, OR = 0.956, CI = 0.710–1.288 and p = 0.529, OR = 1.121, CI = 0.786–1.599 respectively).

Conclusion: Our results suggest that microRNA196A2 gene polymorphism predispose to HBV infection but not to cirrhosis and HCC.

P025
Prevalence of Intron 1 Inversion and Polymorphisms of Introns 18 and 19 in FVIII Gene of Hemophilia A in Sabah Populations
P.J. Cherry Prissiea, P.C. Leeb, A. Thiruchelvama, J.A.M.A. Tanc
aFaculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu Sabah, Malaysia, bFaculty of Sciences and Natural Resources, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu Sabah, Malaysia, cDepartment of Biomedical Science, Faculty of Medicine Building, University of Malaya, 50603 Kuala Lumpur, Malaysia

Objective: Hemophilia A is an x-linked recessive bleeding disorder caused by several mutations in the gene coding for Factor VIII (FVIII). Large gene alterations have been reported, including polymorphisms of introns 7, 8, 13, 19, XbaI in intron 22, BglII in intron 25 and exon 18. Inversions of introns 22 and 1 occurred in 50% and 5% of severe Hemophilia worldwide, respectively. Despite these studies, there is no report on the type of mutations that is common in Hemophilia A in Sabah. We report here the mutational analysis of 3 introns; introns 1, 18 and 19 and their distributions in Hemophilia A patients in Sabah, Malaysia.

Material and Methods: Eighty subjects consisting of 38 patients (36 severe, 1 moderate and 1 mild) and 42 carriers (25 obligate carriers and 17 possible carriers) were recruited for this study.

Results: Out of the 42 carriers, 15 carriers had low FVIII: C (<40%), which is similar to mild Hemophilia A patient. About 21.1% of the hemophilia A patients and 35.8% of the carriers had both Bcll (intron 18) and Hind III (intron 19) polymorphisms, respectively. The allelic frequency for both polymorphisms was 0.19 and it is similar to Japanese and Korean populations. We found that the intron 1 inversion of Hemophilia A in Sabah is not common in Sabah although it is commonly associated with severe hemophilia in Caucasian.

Conclusion: Therefore, we conclude that the distribution of intron 1 inversion is not common in Sabah. However, Bcll (intron 18) and Hind III (intron 19) are more prevalent compared to intron 1 inversion, but its polymorphisms is not associated with the Hemophilia A severity.

P026
Targeting Non-Structural 3 Protein of Dengue Virus Type 2 in Development of Antiviral Therapy: Bioactivity of Ethyl (1-Tert-Butoxycarbonyl-2-Butyl-4-Phenyl) Piperidinyl-3-Carboxylate
C.S. Gan1, Y.K. Lee2, R. Yusof3, S. Othman1
1Department of Molecular Medicine, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia, 2Department of Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia, 3Drug Design and Research Group, University of Malaya, 50603 Kuala Lumpur, Malaysia

Objective: The genome of dengue virus (DV) encodes for three structural protein and seven non-structural (NS) proteins. NS3 is a multifunctional protein with an N-terminal protease domain that is responsible for proteolytic processing of the viral polyprotein, and a C-terminal region that contains an RNA triphosphatase, RNA helicase and RNA-stimulated NTPase domain, which is essential for viral replication. This multifunctional protein has therefore gravitated ample interests as a target in antiviral research. By targeting DV2-NS3, we aimed to characterise the effects of antiviral compound, ethyl (1-tert-butoxycarbonyl-2-buty1-4-phenyl)-piperidinyl-3-carboxylate (YK51), on DV2 replication.

Material and Methods: The ability of YK51 in targeting DV2-NS3 was demonstrated by the sequestration of its serine protease catalytic activity. Human liver carcinoma cell line, HepG2, was used for the DV2 infection and YK51 was supplemented into the
medium for treatment. Cells were harvested 48 hours post-infection and assayed for the regulation of cellular translation factor, amplification of viral RNA, lipid metabolism and release of nascent virus.

**Results:** Upon inhibition of DV2-NS3, we demonstrated conquest of 4E-BP1 dephosphorylation along with the reduction of viral RNA replication. Nevertheless, the antiviral does not affect the increase of lipid metabolism in infected cells. At a concentration of 12.8 nM, the compound was shown to inhibit ~95% of viral replication.

**Conclusion:** Taken together, these results demonstrate that inhibition of DV2-NS3 by YK51 impedes the transcription and translational stages but not the packaging stage of viral life cycle. Further investigation involves the corroboration of the use of YK51 for the treatment of dengue diseases.

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**P028**

**Whole Exome Sequencing Approach to Identify Genetic Variants Causing Sudden Unexplained Death Syndrome within a Normal Population**

E.H.M. Mohameda,b, S.H. Kooa, P. Chui,c, C.S. Ku,a, Y. Pawitan,d, E.J. Leed

aPharmacogenomics Laboratory, Department of Pharmacology, Faculty of Medicine, University of Malaya, Malaysia, bPharmacogenomics Laboratory, Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, cForensic Medicine Division, Health Sciences Authority, Singapore, dDepartment of Medical Epidemiology and Biostatistics, Karolinska Institutet, Sweden

**Objectives:** Majority of sudden unexplained death syndrome (SUDS) are caused by channelopathies. Many genetic studies of SUDS have revealed that the proposed genetic causes have incomplete penetrance and variable expressivity. Thus it is postulated that SUDS may be a polygenic complex disease. However, to date, there is no direct evidence to support this. As such, it is hypothesized that the known causative variants should be present in the normal population as it would require multiple hits to exhibit its pathogenicity.

**Materials and Methods:** Whole exome sequencing (WES) approach was used to interrogate known genes which have been implicated in SUDS. DNA was extracted from 22 normal, healthy subjects. Subsequently, paired-end exome sequencing was performed using Illumina HiSeq2000 and TruSeq exome enrichment. Sequence alignment was carried out using BWA-SW, and variant calls using Genome Analysis Toolkit (GATK) HaplotypeCaller with annotations by Snpeff annotation software. The variants were then filtered using the standard 26 arrhythmia gene panel, out of which only high and moderate impact variants according to Snpeff were selected.

**Results:** A total of 54 variants from 15 known arrhythmia genes were finally left, 4 of which are insertion/deletion type and the rest were single nucleotide polymorphisms (SNPs). Ten of these variants were predicted as possibly damaging to damaging by PolyPhen-2 prediction tool.

**Conclusion:** As arrhythmias are presumed to be the event leading to SUDS, the presence of predicted pathological arrhythmia variants within the normal population supports the possibility of SUDS being polygenic in nature.
**P029**

**Association of BDNF Polymorphisms with the Risk of Epilepsy: A Multicenter Study**


*Pharmacogenomics Laboratory, Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia, School of Pharmacy, The Chinese University of Hong Kong, Shatin, Hong Kong, China, Department of Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia, Division of Neurology, General Hospital Kuala Lumpur, Kuala Lumpur, Malaysia, Division of Neurology, Department of Medicine and Therapeutics, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong, China, Department of Psychiatry, The University of Hong Kong, Pokfulam, Hong Kong, China, Department of Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia*

**Objective:** Epilepsy is a common neurological disease characterized by recurrent unprovoked seizures. Evidence suggested that abnormal activity of brain-derived neurotrophic factor (BDNF) contributes to the pathogenesis of epilepsy. Studies have shown association of epilepsy with genetic variants of BDNF. In the present study, association of BDNF polymorphisms with the risk of epilepsy has been examined in Hong Kong and tri-ethnic Malaysian (Malay, Chinese and Indian) populations.

**Material and Methods:** Genomic DNA of 6047 subjects (1640 patients with epilepsy and 4407 healthy individuals) was genotyped for rs6265 (V66M), rs11030104, rs7103411 and rs7127507 by using Sequenom MassArray and Illumina HumanHap 610-Quad or 550-Duo BeadChip arrays techniques.

**Results:** The rs6265 T, rs7103411 C, and rs7127507 T alleles and the TCT haplotype were associated with the risk of epilepsy in Malaysian Indians (p = 0.004, p = 1 × 10^−10, and p = 1 × 10^−10, respectively). No significant association was observed in other races either in subjects from Malaysia or from Hong Kong.

**Conclusion:** In conclusion, our data suggest that BDNF variants contribute to the risk of epilepsy.

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**P030**

**Genetic Findings and Molecular Characterization of Genetic Variants in Prostacyclin Synthase Gene (CYP8A1)**


*Department of Pharmacology and Pharmacogenomics Research Center, Inje University College of Medicine, Inje University, Busan, South Korea, Department of Clinical Pharmacology, Inje University College of Medicine, Inje University, Busan, South Korea, Department of Pharmacology and Toxicology, University of Alabama at Birmingham, Birmingham, Alabama, USA*

**Objective:** Prostacyclin synthase (CYP8A1) is an enzyme responsible for the biosynthesis of prostacyclin (PGI2) which inhibits platelet activation and exhibits anti-inflammatory effect. The objectives of this study were to identify CYP8A1 genetic variants and characterize functional consequences of CYP8A1 variants.

**Material and Methods:** In total, 27 variants including six previously unidentified single-nucleotide polymorphisms (SNPs) were identified by direct DNA sequencing in Koreans (n = 48). Among them, CYP8A1 A447T and E314Stop were newly assigned as CYP8A1*5 and CYP8A1*6 by the Human Cytochrome P450 Allele Nomenclature Committee, respectively. CYP8A1*5 was found in the heme binding area in three individuals as a heterozygous mutation. To investigate the functional change of CYP8A1*5, CYP8A1*5 and wild-type CYP8A1 protein were overexpressed in an Escherichia coli expression system and purified.

**Results:** Metabolism of PGI2 by the CYP8A1*5 protein exhibited significantly decreased activity, resulting in a 45% decrease in Vmax and a 1.8-fold decrease in intrinsic clearance compared to the wild-type. Based on the predicted crystal structure of CYP8A1*5 using the Molecular Operating Environment platform, the distance from CYP8A1 Cys441 to the heme was altered with a significantly changed binding free energy for the CYP8A1*5 protein.

**Conclusion:** Further studies would be needed to determine the effect of CYP8A1*5 on PGI2 levels in humans.

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**P031**

**microRNA Profiling in Women with Spontaneous Preterm and Term Delivery**

I.M. Langmia, Y.D. Apalasamy, S.Z. Omar, Z. Mohamed

*Pharmacogenomics Laboratory, Department of Pharmacology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia, Department of Obstetrics and Gynecology, University of Malaya, 50603 Kuala Lumpur, Malaysia*

**Objective:** Preterm birth (PTB), which is defined as child birth before completion of 37 weeks of gestation is the largest single cause of neonatal mortality in the world. PTB is a multifactorial condition involving more than one biological pathway and until now, the molecular determinants of PTB have not been fully elucidated.

**Abstracts**

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19
Highly conserved single-stranded non-coding RNAs such as microRNAs (miRNA) that play important roles in gene regulation have now been implicated in preterm birth complication. The objective of this study was to determine whether microRNA profiles in maternal blood are different in women who are destined to have PTB compared with term birth.

**Material and Methods:** The study involved a total of 20 women (10 preterm and 10 term) with spontaneous preterm and term delivery. The miRNA profiles were measured in 3 ml of maternal blood using Affymetrix® GeneChip® miRNA 3.0 Array. Data were analyzed using Expression console software, Transcriptome Analysis Console (TAC) and IPA software. A fold change of \(>2\) and a false-discovery rate of \(<20\%\) was used to determine the most differentially expressed miRNAs.

**Results:** Of the 2999 miRNAs that were analyzed on the array, thirty four were significantly different between the preterm and term groups.

**Conclusion:** The miRNAs profiles in maternal blood were significantly different in women destined to have a preterm as compared to term birth. This study suggests that specific microRNA regulators may have important roles for patient susceptibility to preterm.

**P032**

**Epigenetic Silencing Status of p16INK4a and MGMT Promoter Regions Identified Qualitatively in Diffuse Large B Cell Lymphoma**

L. Jalilah Mohd Ridah\(^a\), N.A. Talib\(^b\), F. Arbaeyah Hussain\(^c\), N. Zainuddin\(^b\)

\(^a\)Department of Biomedical Science, Kulliyyah of Allied Health Sciences, International Islamic University Malaysia, Kuantan, Pahang, Malaysia, \(^b\)Department of Basic Medical Sciences, Kulliyyah of Medicine, International Islamic University Malaysia, Kuantan, Pahang, Malaysia, \(^c\)Department of Histopathology, Hospital Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia

**Objective:** Apart from genetic mutation, DNA methylation, an epigenetic event, has emerged as an important genomic disorder which silences the gene through addition of methyl group to the cytosines especially in CpG islands located in promoter region. \(p16\), a tumor suppressor gene that inhibits cyclin-dependent kinase, is located within the protein, thus preventing mutagenic effects and lethal cross-replication. Methylation silencing of \(p16\) activates the Rb protein and blocks G1 phase in a normal cell cycle. A methylation status in DLBCL cases are different in women who are destined to have DLBCL significantly. We aimed to identify epigenetic event, has emerged as an important genomic disorder which links. Methylation silencing of tumor suppressor gene that inhibits cyclin-dependent kinase, in actinomycin D (Act) and IPA software. A fold change of \(>2\) and a false-discovery rate of \(<20\%\) was used to determine the most differentially expressed miRNAs.

**Results:** Of the 2999 miRNAs that were analyzed on the array, thirty four were significantly different between the preterm and term groups.

**Conclusion:** The miRNAs profiles in maternal blood were significantly different in women destined to have a preterm as compared to term birth. This study suggests that specific microRNA regulators may have important roles for patient susceptibility to preterm.

\(p16\) expression increases with aging. Yet to be noted, \(p16\) methylation is not the sole determinant of \(p16\) expression. MGMT methylation was reported to be strongly related with cancer patients who smoke, drink and are non-vegetarian. Thus, it is hypothesized that lifestyle might also trigger MGMT methylation in this study population.

**Conclusion:** Our findings indicate that \(p16\) and MGMT methylation were associated with DLBCL.

**P033**

**Gene Expression Profiling Reveals Underlying Molecular Mechanism of Hepatoprotective Effect of Orthosiphon stamineus and Morinda citrifolia on Thioacetamide-Induced Hepatotoxicity in Rats**

M.A. Alshawsh\(^a\), Z.A. Amin\(^b\), S. Ismail\(^c\), M.A. Abdulla\(^d\)

\(^a\)Department of Pharmacology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia, \(^b\)Department of Pharmacognosy, College of Pharmacy, Hawler Medical University, 44001 Erbil, Iraq, \(^c\)Institute of Biological Science, Faculty of Science, University of Malaya, Kuala Lumpur 50603, Malaysia, \(^d\)Department of Biomedical Science, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

**Objective:** Chronic liver cirrhosis and drug induced liver injury accounting the ninth leading cause of death by disease in western and developing countries. This study was carried out to assess the molecular mechanism of hepatoprotective effect of Orthosiphon stamineus and Morinda citrifolia against thioacetamide-induced hepatotoxicity in rats.

**Material and Methods:** Four groups of Sprague-Dawley rats were used in the hepatoprotective experiment. Group 1 served as normal control, while groups 2 to 4 were injected intraperitoneally with 200 mg/kg of thioacetamide (TAA) thrice weekly for two months. Groups 3 and 4 were orally administrated with O. stamineus and M. citrifolia (200 mg/kg) for two months. Liver homogenates were used for the estimation of the oxidative stress (MDA) and the liver fibrosis related genes namely; transforming growth factor (TGF\(\beta\)), matrix metalloproteinase-2 (MMP2), tissue inhibitor of matrix metalloproteinase-1 (TIMP1) and collagen \(\alpha\) (Coll \(\alpha\)). The real-time PCR reaction was performed in triplicate and gene expression values were calculated with the \(2^{\Delta\Delta C_T}\) method.

**Results:** Plant extracts significantly reduced the oxidative stress level and down-regulated the expression of the liver fibrosis genes. All four targeted genes i.e. TGF\(\beta\)1, MMP2, TIMP-1 and Coll \(\alpha\) were significantly up-regulated in TAA group compared to the normal group, whereas three of them namely; TGF\(\beta\)1, MMP2, and Coll \(\alpha\) were highly significant (\(P < 0.01\)) down-regulated in O. stamineus treatment group.

**Conclusion:** The possible mechanism of hepatoprotective and liver regeneration action of both plant extracts could be as consequence of a significant down-regulation of the liver fibrosis involved genes expression, as well as due to the anti-lipid peroxidation activities.
P034
Genetic Association of PDLIM5 and HTR2A Genes with Bipolar Disorder
M.A. Zaina, N.Z. Zainab, S. Kanagasundrama, Z. Mohamedb
aThe Pharmacogenomics Laboratory, Department of Pharmacology, University of Malaya, 50603 Kuala Lumpur, Malaysia, bDepartment of Psychological Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

Objective: Genetics has been implicated in an individual’s susceptibility to bipolar disorder (BPD). The PDLIM5 and HTR2A genes have been previously investigated in various populations for their association with BPD; however, the results have been conflicting. In this study, we investigate the association between BPD and the two genes of interest.

Material and Methods: We recruited 253 BPD patients and 505 control individuals in Malaysian population which we stratified into three ethnic groups (Malays, Chinese and Indians). We selected 3 SNPs of the PDLIM5 (rs2433320, rs2433322 and rs2438146) and 3 SNPs of the HTR2A (rs6313, rs2070040 and rs6311) and performed genotyping on the samples using RT-PCR.

Results: We observed significant associations between BPD and each of the 3 SNPs of PDLIM5 in Malays, Indians and pooled samples. However, only rs2438146 remains significant in the Malays under co-dominant (T/T vs. C/C, \( p = 0.004, \text{OR} = 0.128, 95\% \text{CI} = 0.031–0.524 \)) and recessive (T/T vs. T+T+C/C, \( p = 0.003, \text{OR} = 0.122, 95\% \text{CI} = 0.030–0.494 \)) models after applying conservative Bonferroni’s correction. Haplotype analysis of 3 SNPs of PDLIM5 also showed a significant association with BPD. No association was observed between BPD and each of the 3 SNPs of HTR2A in any of the ethnicities.

Conclusion: We conclude that PDLIM5 polymorphisms are associated with bipolar disorder in the Malaysian population.

P035
Is BASE a Putative Breast Cancer Marker?
N.A. Namc, L. Bingled, C.D. Binglec
aBiomedical Science Department, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia, bAcademic Unit of Oral and Maxillofacial Pathology, School of Clinical Dentistry, University of Sheffield, Sheffield, United Kingdom, cAcademic Unit of Respiratory Medicine, Department of Infection and Immunity, University of Sheffield Medical School, Sheffield, United Kingdom

Objectives: Breast cancer and Salivary gland Expression (BASE) gene, belongs to PLUNC genes family is located in chromosome 20q11.21. BASE is predicted to code for secreted protein normally expressed in salivary gland. Recently BASE expression had been detected in breast tumors and in a certain population of breast cancer cell lines. This study aimed to investigate BASE expression in a number of breast cancer tissue irrespective of tumour stages and histological types using RT-PCR and immunohistochemistry.

Materials and Methods: Twenty breast cancer tissues and 4 breast cancer cell lines MCF7, MDA-MB-231, MDA-MB-468 and ZR-75–1 were subjected to RT-PCR to study the expression of BASE mRNA. The expression of other putative breast cancer markers; HE4, mammoglobin A, MUC5B and small breast epithelial mucin (SBEM) were also performed for relative comparison to BASE gene expression.

Results: HE4 showed highest frequency of expression as it was detected in 17 out of 20 breast cancer tissue samples, followed by SBEM and BASE (10 out of 20), mammoglobin A (5 out of 20) and MUC5B (2 out of 20). IHC analysis done on breast cancer TMA consisting of 400 individual samples showed lower frequency of BASE protein expression in TMA samples studied (1%). A novel BASE splice variant, +20 has been successfully isolated from the tissue samples studied in addition to the existing splice variants, the wild type (214 bp), +17 (231 bp) and –40 (174 bp).

Conclusion: Our findings suggest potential application of BASE as a putative breast cancer marker however, further studies are needed to characterize its possible roles in breast cancer development.

P036
HLA-B*1502 Screening in Epileptic Patients Using a High Resolution Melting-Real Time PCR (HRM-QPCR) Method
aUKM Medical Molecular Biology Institute, Kuala Lumpur, Malaysia, bUniversity of Nottingham, Malaysian Campus, Semenyih, Selangor, Malaysia, cFaculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi, Selangor, Malaysia, dDepartment of Medicine, UKM Medical Centre, Kuala Lumpur, Malaysia

Objective: The HLA-B*1502 polymorphism in epileptic patients is known to be associated with carbamazepine-induced Stevens-Johnson syndrome (SJS). The prevalence of HLA-B*1502 polymorphism seemed to be ethnic-specific with a higher frequency of HLA-B*1502 in Asian patients compared to the Europeans. The incidence of HLA-B*1502 in patients treated with carbamazepine in Malaysia has not been extensively investigated. This study was performed to determine the frequency of the HLA-B*1502 polymorphism in Malaysian epileptic patients.

Material and Methods: We also developed a fast and effective in-house high resolution melting-real time polymerase chain reaction (HRM-QPCR) method and compared it with the conventional multiplex-PCR method.

Results: Using the conventional multiplex-PCR approach for screening, 25 out of 64 (39.1%) epileptic patients were positive for HLA-B*1502. However, using the HRM-QPCR technique, 24/64 (37.5%) of the patients were positive. The one patient who tested positive by the multiplex-PCR but negative using the HRM-QPCR turned out to be negative by DNA sequencing, thus suggesting that the HRM-QPCR is equally sensitive but more specific compared to the multiplex-PCR. The HRM-QPCR is also more cost-effective (~$16.40 USD per test) and less time-consuming when compared to...
the multiplex-PCR. The specificity and sensitivity of each test were also determined using DNA from saliva which is useful for paediatric patients as the test can be performed from as little as 10ng of DNA.

**Conclusion:** The HRM-QPCR method is more sensitive, robust and cost effective compared to the multiplex-PCR and can be used for HLA-B*1502 genotyping in epileptic patients.

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**P037**

**ADIPOQ rs266792 Polymorphisms and Obesity Related Parameters in Malaysian Adolescents Population**

*N.H. Muhamad, N.L. Zaharan, A. Hamidi, Z. Mohamed*

Department of Pharmacology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

**Objective:** Adiponectin (ADIPOQ) is involved in the regulations of glucose and fatty acid. Different population studies have shown that ADIPOQ gene polymorphisms are associated with obesity. We assessed the association between genetic variant in the ADIPOQ rs266729 with obesity traits in adolescents.

**Material and Methods:** 696 adolescents (13-year olds, 28% boys, 74% Malay, 15 Chinese and 10% Indian) were recruited from 23 randomly selected secondary schools in this cross sectional study. Anthropometric measures included were body mass index (BMI), waist (WC) and hip circumference and waist and hip ratio (WHR). Percentage of body fat (BF %) was assessed using portable bioelectrical impedance analysis (InBody 230). Fasting blood samples were taken for lipid profile (total cholesterol (TC), LDL, HDL and triglycerides) and insulin. A student with BMI ≥95th centile was classified as obese. Genotyping of rs266729 was performed using sequenom MassARRAY. Association of allele was performed using binary logistic regression adjusting for gender, ethnicity and puberty stage. Spearman’s correlation was used to examine the relationship between rs266729 and obesity parameters.

**Results:** The mean and standard deviations for obesity parameters were as follows: BMI 20.6±5.1, WC 68.9±12.2 cm, WHR 0.8±0.3 and BF% 29.6±10.7. 14% of students were classified as obese (44% boys, 77% Malay). ADIPOQ rs266729 was not associated with any of the obesity parameters measured in this cohort (p > 0.05). There was no correlation found between ADIPOQ rs266729 with BMI, WC, WHR, BF%, lipid profiles and insulin.

**Conclusion:** In conclusion, rs266729 ADIPOQ polymorphism was not significantly associated with obesity related parameters and metabolic markers in our Malaysian adolescent population.

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**P038**

**Up-Regulation of hsa-miR-608 Expression, in Response to Bcl-xL Silencing, Increases Cell Death in A549 and SK-LU1 Human Lung Adenocarcinoma Cells**

*N. Othman, N.H. Nagoor*

Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia, Centre for Research in Biotechnology for Agriculture (CEBAR), University of Malaya, 50603 Kuala Lumpur, Malaysia

**Objective:** Anti-apoptotic Bcl-xL is frequently overexpressed in non-small cell lung cancer, leading to inhibition of apoptosis and poor prognosis. microRNAs (miRNAs) play a role in regulating apoptosis and cell survival during tumorigenesis, with cancer cells showing perturbed expression of miRNAs. The aim of this study was to determine the change in hsa-miR-608 expression in lung adenocarcinoma cells in response to Bcl-xL silencing and to identify its targets.

**Material and Methods:** Overexpression and knockdown studies were performed via transfection of miRNA mimics and inhibitors and cell death was detected using the Annexin V-FITC detection kit. PCR products of fragments of 3'UTRs covering the predicted miRNA binding sites were cloned into the vector and transiently transfected into cells. Luciferase activity was assayed and measured in the presence of miR-608 mimic or negative control mimic and normalized using Renilla activity.

**Results:** Overexpression of hsa-miR-608 induced cell death in A549 and SK-LU1 cells and this effect was reversed when knockdown studies were performed. Combination of siRNA based silencing of bcl-xL (siBcl-xL) followed by inhibitor transfection led to a decrease in apoptotic population of A549 and SK-LU1 cells in comparison to cells only treated with siBcl-xL. Gene target prediction analysis implicated various signaling pathways as targets of bcl-xL induced miRNA alterations.

**Conclusion:** Bcl-xL silencing in A549 and SK-LU1 cells leads to the occurrence of cell death through the dysregulation of specific miRNAs, thus providing a platform for anti-sense gene therapy whereby miRNA expression can be exploited to increase the apoptotic properties in lung adenocarcinoma cells.
Modulation of Gene Expression Level by Thymoquinone and Thymoquinone-Rich Fraction in Response to Reactive Oxygen Species Generation on Differentiated Human SH-SY5Y Cells

N. Ismaila, M. Ismaila,b, N.H. Azmi, M.U. Imam, M.F. Abu Bakara, H. Basiric, M.A. Abdullah

aNutricosmeceuticals and Nutrigenomics Programme, Laboratory of Molecular Biomedicine, Institute of Bioscience, Universiti Putra Malaysia, Selangor, Malaysia, bDepartment of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Selangor, Malaysia, cDepartment of Medicine, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Selangor, Malaysia, dDepartment of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Selangor, Malaysia

Objective: The maintenance of health and the prevention and treatment of chronic diseases are influenced by naturally occurring chemicals in foods. In addition to supplying the substrates for producing energy, a large number of dietary chemicals are bioactive and they alter the regulation of biological processes including the expression of genetic information either directly or indirectly. In neurodegenerative diseases, this conception could be leveraged to augment neuroprotective pathways using the diet as well as through use of natural substances that can be more efficacious namely by induction of health-promoting genes and reduction of the expression of disease-promoting genes. Accordingly, this approach could be incorporated into the neuroprotective strategies of the future.

Material and Methods: We investigated the neuroprotective properties of thymoquinone-rich fraction (TQRF) extracted from Nigella sativa seed by supercritical fluid extraction system and commercial TQ towards reactive oxygen species (ROS), hydrogen peroxide (H2O2) on differentiated human SH-SY5Y cells. Gene expression study was carried out using multiplex GeXP system.

Results: Various antioxidants (SOD 1, SOD 2 and catalase), and signaling genes including JNK, p53, AKT1, ERK ½, p38 MAPK and NF-κB performed their respective cellular roles in responses to ROS generation. Indeed, TQ and TQRF exhibited neuroprotective properties against ROS insult partly through modulation of gene expression responsible for antioxidant defend, cell survival and cell death.

Conclusion: This suggests the potential use of TQ and TQRF for the prevention of neurodegenerative diseases due to oxidative injury, and is worth studying further.

Glucocerebrosidase Mutations in Malay Parkinson’s Disease Patients

N.F. Mohamad Pakarul Raza, N.A. Abdul Murad, S.E. Syafuddin, A. Ahmad-Annuar, S.Y. Lim, N.M. Ibrahim, R. Jama

aUKM Medical Molecular Biology Institute (UMBI), Jalan Ya’acob Latiff, Cheras, 56000 Kuala Lumpur, Malaysia, bDepartment of Biomedical Science, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia, cDivision of Neurology and the Mah Pooi Soo & Tan Chin Nam Centre for Parkinson’s & Related Disorders, University of Malaya, 50603 Kuala Lumpur, Malaysia, dDepartment of Medicine, UKM Medical Centre (UKMMC), Jalan Ya’acob Latiff, Cheras, 56000 Kuala Lumpur, Malaysia

Objective: Glucocerebrosidase (GBA) is the most common genetic risk factor in Parkinson’s disease (PD). Studies have shown that GBA mutations may modify age at onset of PD. The association between GBA and PD has not been reported in the Malay population. Therefore, the aim of this study is to screen for GBA gene mutations and determine their frequency in the Malay PD patients.

Material and Methods: GBA gene from 50 late onset PD (LOPD), 50 early onset PD (EOPD) patients and 50 ethnically and aged matched controls were amplified and subjected to library construction using the Ion Plus Fragment Library kit. Each library was barcoded using Ion Xpress™ Barcode Adapters. Pooled barcoded libraries were used in PCR emulsion and enrichment via the Ion One Touch™ System and sequenced using Ion Torrent Personal Genome Machine™. The Torrent Suite™ Software was used for data analysis. Variants were annotated using the ANNOVAR.

Results: Heterozygous GBA mutations that were identified in EOPD are p.L483R, p.L483P, p.R159Q and p.S146L. Meanwhile, p.R202Q (n = 2) was identified in LOPD and control cases. The frequency of GBA mutations were higher in PD cases (n = 5/100, 5%) compared to controls (n = 1/50, 2%) and higher in EOPD cases (n = 4/50, 8%) compared to LOPD cases (n = 1/50, 2%). However, the difference are not statically significant for both groups (PD vs Control: P = 0.664, OR = 2.58, 95% CI 0.293–22.690; EOPD vs LOPD: P = 0.362, OR = 4.26, 95% CI 0.459–39.546).

Conclusion: GBA mutations may be associated with PD and may modify age at onset in Malay but these results need further validation in a larger sample size.
Multiplex Gene Expression Approach for the Elucidation of Germinated Brown Rice’s Neuroprotective Mechanism of Action

N.H. Azmi\textsuperscript{a}, M. Ismail\textsuperscript{b}, N. Ismail\textsuperscript{a}, M.U. Imam\textsuperscript{a}, N.B. Mohamed Alitheen\textsuperscript{c}, M.A. Abdullah\textsuperscript{d}  
\textsuperscript{a}Nutricosmeceuticals and Nutrigenomics Programme, Laboratory of Molecular Biomedicine, Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia, \textsuperscript{b}Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia, \textsuperscript{c}Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia, \textsuperscript{d}Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

Objective: Oxidative stress is implicated in chronic neurodegenerative diseases such as Alzheimer’s disease (AD), and dietary components with high antioxidant properties emerge as potential therapeutic candidates. The pathogenesis of AD involves complex etiological factors, by which the deposition of beta-amyloid (Aβ) protein has been strongly implicated in cognitive impairment associated with the disease. Since hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) is the main source of highly reactive hydroxyl radical in the brain, we studied its effect on genes related to apoptosis and the processing of human amyloid precursor protein (APP) as well as the potential of germinated brown rice (GBR) to regulate such pathways.

Material and Methods: In the present study, differentiated human neuroblastoma SH-SY5Y cells were subjected to H\textsubscript{2}O\textsubscript{2}-induced oxidative stress, in the absence and presence of GBR. Gene expression level in response to H\textsubscript{2}O\textsubscript{2} was then determined using a multiplex GenomeLab Genetic Analysis System (GeXP).

Results: At mRNA level, H\textsubscript{2}O\textsubscript{2} upregulated apoptotic (p53, p38 MAPK and JNK) and downregulated anti-apoptotic (AKT, NFKB and ERK) genes while pretreatment of GBR showed reduction in the deleterious H\textsubscript{2}O\textsubscript{2} effects. Multiplex analyses also showed that the neuroprotective effects of GBR may partly be mediated through transcriptional regulation of the α-, β- and γ-secretases as well as regulation of Neprilysin and LRP genes.

Conclusion: Taken together, the transcriptional changes showed an inclination for the modulatory effect of GBR on apoptosis and APP metabolism with potential impact worth exploring further, especially for the management of AD.

The Role of NPY rs16147 and rs5574 Polymorphisms on Obesity Parameters and Cardiometabolic Risk Factors in Urban Malaysian Adolescents

N.L. Zaharan, S.M. Zain, A. Hamidi, Z. Mohamed  
Department of Pharmacology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

Objective: Neuropeptide Y (NPY) acts on the hypothalamus to influence dietary intake and directly on adipose tissue to store energy. This study was aimed to examine the association between genetic variants of NPY rs16147 and rs5574 with obesity traits in adolescents from Kuala Lumpur, Malaysia.

Material and Methods: 1,118 adolescents (thirteen-year-old) were recruited from 23 randomly selected secondary schools in this cross sectional study. Anthropometric measures included were body mass index (BMI), waist (WC) and hip circumference and waist and hip ratio (WHR). Percentage of body fat (BF\%) were assessed using portable bioelectrical impedance analysis (inBody230). Cardiometabolic profile included fasting insulin and lipid profile (total, LDL-, HDL-cholesterol and triglycerides). A student is classified as obese if BMI is more than 95\textsuperscript{th} centile. 698 students (29\% boys and 74\% Malay) were genotyped for rs16147 and rs5574 using Sequenom MassARRAY. Association of allele was performed using binary logistic regression adjusting for gender, ethnicity and puberty stage. The relationship between the SNPs and obesity parameter were examined with Spearman’s correlation. ANOVA and Kruskal Wallis test were used to compare means between different alleles.

Results: The mean and standard deviations for obesity parameters were as follows: BMI 20.3±5.1, WC 68.3±12.1, WHR 0.8±0.3 and BF\% 28.6%±10.3%. NPY rs5574 was not associated with obesity. Significantly negative correlation was found between NPY rs16147 and WC (r=-0.11, p < 0.001) WHR (r=-0.09, p = 0.02) and BF\% (r=-0.09, p = 0.02). The GG genotype of rs16147 was associated with decreased level of WC, BF\% and triglycerides (p < 0.05).

Conclusion: NPY rs16147 polymorphisms demonstrated trend towards reduced risk of obesity in our adolescents.

Genome Editing of Human Pluripotent Stem Cells (hPSCs) to Introduce the KCNH2 (G1681A) Mutation for Drug Testing

N.A.N.M. Yusof\textsuperscript{a,b}, V. Borgdorff\textsuperscript{a}, A. Kondrashov\textsuperscript{a}, C. Denning\textsuperscript{b}  
\textsuperscript{a}Wolfson Centre for Stem Cells, Tissue Engineering & Modelling, University of Nottingham, Nottingham NG7 2RD, United Kingdom, \textsuperscript{b}Stem Cell Laboratory, Cancer Research Centre, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia

Objective: Drug induced cardiotoxicity is one of major reason of early and late stage drugs withdrawal. It caused arrhythmias and disease like Long QT Syndrome (LQTS). Based on previous finding,
cells with KCNH2 G1681A mutation is in association with LQTS2. The KCNH2 (G1681A) mutation is known to encode blockade in potassium ion channel. In our research, we are trying to create efficient human editing for cardiotoxicity by creating KCNH2 mutations (G1681A) in human genome using Cas9/CRISPR system.

**Material and Methods:** First, we performed gene expression study for SNPs that involved in cardiotoxicity in undifferentiated (HUES 7), intermediate (Embryoid bodies) and mature cells (Heart cells). Then we have performed Cas9/CRISPR verification and gRNA KCNH2_G1681A ligation. Later, we transfected HUES7 with Cas9/ CRISPR and gRNA KCNH2_G1681A. Based on T7E1 assay we have detected enzymatic mismatch hence the disruption of endogenous human KCNH2 locus in HUES7.

**Results:** We demonstrated that hPSCs coupled with Cas9/ CRISPR with specific designed gRNA (KCNH2_G1681A gRNA) has successfully generated double strand breaks (DSB) at specific target site of KCNH2 in transfected human genome. The targeting could further be introduced within breaks that formed to create specific mutation needed.

**Conclusion:** This research demonstrate future pathway of generating cardiomyocytes with specific mutation for drug testing and disease modelling especially in cardiotoxicity which have not been available or because the background genetics vary so much from one patient to another that make determining the impact of a single mutation on drug response difficult.

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**P044**

**Functional Characterization of Cytochrome P450 2C19 Allelic Variants CYP2C19*23 and CYP2C19*24**

P.S. Lau\(^a\), K.V.G. Leong\(^a\), C.E. Ong\(^b\), Y. Pan\(^c\)

\(^a\)School of Medical Sciences, International Medical University, No.126, Jalan Jali Perkasa 19, Bukit Jalil, 57000 Kuala Lumpur, Malaysia; \(^b\)Jeffery Cheah School of Medicine and Health Sciences, Monash University Sunway Campus Malaysia, Jalan Lagoon Selantan, 46150 Bandar Sunway, Selangor, Malaysia; \(^c\)Department of Biomedical Science, The University of Nottingham Malaysia Campus, Jalan Broga, 43500 Semenyih, Selangor, Malaysia

**Objective:** CYP2C19 is essential for the metabolism of clinically used drugs including omeprazole, progamil, and S-mephentoin. This hepatic enzyme exhibits genetic polymorphism with inter-individual variability in catalytic activity. This study aimed to investigate the functional characterisation of two allelic variants of CYP2C19 (CYP2C19*23 and CYP2C19*24) in vitro.

**Material and Methods:** Mutations in CYP2C19 cDNA were introduced by site-directed mutagenesis. The CYP2C19 wild-type and variants were then expressed using *Escherichia coli* expression system, from which the CYP2C19 proteins were harvested. Catalytic activities of these proteins were determined using S-mephentoin as the substrate.

**Results:** The level of S-mephentoin 4′-hydroxylation activity of CYP2C19*23 protein relative to CYP2C19 wild-type protein was very similar with no significant difference in \(V_{\text{max}}\) and \(K_{m}\). In contrast, although the \(K_{m}\) values were not significantly different among CYP2C19 wild-type and variant CYP2C19*24 protein, the \(V_{\text{max}}\) of CYP2C19*24 protein was significantly lower than that of the wild-type protein. *In vitro* intrinsic clearance for CYP2C19*23 protein was 95.88% of that of CYP2C19 wild-type protein. The corresponding value for CYP2C19*24 protein was reduced to 12.98% of that of wild-type protein.

**Conclusion:** The findings suggest that catalytic activity of CYP2C19 was not affected by the corresponding amino acid substitutions in CYP2C19*23 protein; and the reverse is true for CYP2C19*24 protein. Carriers of CYP2C19*24 allele are potentially poor metabolisers of CYP2C19-mediated substrates.

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**P045**

**Genome-Wide Copy Number Analysis Reveals Candidate Gene Loci Associated with Disease Progression in Patients with Prostate Cancer**

P. Poniah\(^a\), S.M. Zain\(^b\), A.H.A. Razack\(^c\), Z. Mohamed\(^a,b,c\)

\(^a\)Department of Surgery, \(^b\)Department of Pharmacology, \(^c\)The Pharmacogenomics (PGx) Laboratory, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

**Objective:** Copy number variation (CNV) studies in the last two decades have identified several genomic alterations related to prostate cancer (PCa). We hypothesize that some of these CNVs could be involved in progression of PCa, thereby providing valuable mechanistic insights in identifying therapeutic gene targets.

**Materials and Method:** Genome-wide copy number changes in 38 subjects comprising patients with PCa and benign prostatic hyperplasia (BPH) were monitored using Agilent’s array comparative genomic hybridization (aCGH).

**Results:** A total of 339 CNVs were found to be unique to PCa subjects in this cohort. After a series of data segregation and filtering, six rare and/or novel CNV's loci were identified to be associated with pathogenesis of PCa. Five of these CNVs are copy number gains that are either rare or novel (1q21, 1q5q, 3q27.2, 7p12.1, and 12q23.1) encoding *ARNT*, *THBS1*, and *DCC* genes that are crucial in the p53 and cancer pathways. One more CNV was a copy number loss in the 8p11.21, harbouring the *SFRP1* gene from the Wnt signalling pathway. Gene ontology (GO) analysis in our study revealed significant CNVs involved in crucial biological processes that enunciate cancer pathogenesis via cytokine production (P value <0.003), disease progression through endothelial cell proliferation (P value <0.014) and xenobiotic metabolism.

**Conclusion:** With a stringent selection of subjects for this pilot study, indicative of moderately aggressive to aggressive cancers being normalized against BPH controls, findings of this study should be able to provide a clearer understanding of disease progression in PCa.
P046

Association of Genetic Polymorphisms of Dipeptidyl Peptidase-4 with Metabolic Syndrome Parameters in Malaysian Subjects


Department of Molecular Medicine, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia
Department of Biochemistry and Molecular Biology, Faculty of Medicine, Sana’a, Yemen, Department of Pharmacy, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

Objective: Dipeptidyl peptidase-4 (DPP-4) is a novel target in the management of metabolic syndrome (MetS) and Type 2 diabetes mellitus (T2DM). DPP-4 polymorphism may be genetic determinants of plasma DPP-4 activity. The aim of this study was to investigate the association of single nucleotide polymorphisms (SNPs) in the DPP4 gene with metabolic syndrome parameters in normal Malaysian subjects and to evaluate the impact of these polymorphisms on their plasma levels.

Material and Methods: Two DPP-4 loci were selected and genotyped in 164 normal subjects. In addition, the plasma DPP-4 level as well as levels of glucose, insulin, and the lipid profile at the fasting state was investigated.

Results: The general univariate analyses showed that the rs7608798 A–G genotype was associated with the BMI (P = 0.01), LDL-c (0.03). Moreover, the pairwise comparison showed that the subjects carrying the heterozygous genotype A–G had higher BMI (P = 0.006), LDL-c (0.014) than those with homozygous G–G genotype. General univariate analyses showed that rs1861978 G–T genotype was associated with the plasma DPP-4 levels (P = 0.034), total cholesterol (P = 0.006), triglyceride (P = 0.041). Additionally, the pairwise comparisons showed that the subjects with G-T had higher DPP-4 (P = 0.011) than those with T–T genotype, whereas the subjects with T–T had higher total cholesterol (P = 0.002) and triglyceride (P = 0.012) than those with G–T genotype.

Conclusion: Genetic variations in DPP-4 loci rs7608798 and rs1861978 were associated with variations in plasma DPP-4 levels and metabolic syndrome parameters (BMI, T-cholesterol, LDL-cholesterol, and triglyceride) in Malaysian subjects.

P047

Pharmacogenetics of Paraoxonase 1 Polymorphisms

R.Y.Y. Poh, J.B. Ng, J.A.M.A. Tan, V. Subrayan

Department of Biomedical Science, Faculty of Medicine, University of Malaya, Malaysia
Department of Ophthalmology, Faculty of Medicine, University of Malaya, Malaysia

Objective: Paraoxonase 1 (PON1) is an antioxidant enzyme that is physically attached to high-density lipoproteins. One of its many roles involves metabolizing a myriad of artificial substrates including statin adducts and glucocorticoids. Its activity is modulated by several factors; notable among these are genetic polymorphisms specifically at positions 55 and 192 of PON1 and possibly at -108, although recently the variation at -108 was found to affect PON1 level, rather than the activity.

Material and Methods: PON1 genotyping and activity assay were carried out in 109 Malaysian subjects comprising males and females of Malay, Chinese and Indian ethnicities to examine the influence of genetic variations at positions -108, 55 and 192 of PON1 on its activity. PON1 activity was measured spectrophotometrically using paraoxon as the substrate. Genotyping was performed using polymerase chain reaction followed by restriction digestion and agarose gel electrophoresis.

Results: Enzyme activity toward paraoxon increased in the genotype order of QQ<QR<RR, and MM=LM<LL (P < 0.01), after taking into consideration the difference that arises from ethnicity. However, activity across the T-108C polymorphism was similar. These findings bear similarity with other previous studies.

Conclusion: There is a need to consider the modulating role of polymorphisms in the analysis of PON1 activity due to its variability. In this way, the tendency of certain drugs to be metabolized by PON1 can be predicted, in order to provide effective dosage. However, the polymorphisms by themselves do not predict plasma PON1 activity accurately. In the final analysis, PON1 activity should be determined to gauge an individual’s pharmacological response.

P048

Evaluating Gene Expression of Chromosome 9p21.3 and Toll-Like Receptors Genomic Markers with Coronary Artery Disease among the Malays

S. Jamalpour, P. Ismail, N. Ismail, V. Ramachandran

Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Malaysia, Department of Medicine, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Malaysia, Institute of Gerontology, Universiti Putra Malaysia, Serdang, Malaysia

Objective: Coronary artery disease (CAD) is a complex disease that reduces or preoccupies blood flow in one or more of the arteries that encircle the heart. By 2020, it’s expected that CAD would be the largest cause of disease burden worldwide. The numbers of people, who die from CVD are mainly from CVD and stroke and estimated to reach 23.3 Million by 2030. The chromosome 9p21.3, of the CDKN2A and CDKN2B genes was selected based on their association with CAD. Toll-like receptors (TLR) are the most characterized innate immune receptors, have recently demonstrated to play a key role in coronary artery disease.

Material and Methods: This study was carried out on Malaysian Malay subjects, after ethical approval from the NMRR, 20 control subjects evaluated versus 26 cases. 3 to 5 ml of the subject’s blood was collected and extracted by commercial kits and amplified by reverse transcriptase-PCR (RT-PCR) method.

Results: The expression pattern of TLR 3, CDKN2A and CDKN2B mRNA were significantly different among the selected
groups ($P < 0.05$) which was down-regulated for the CAD subjects, on the other hand, TLR4 was significantly up-regulated in CAD patients compared with control group. The expression of CDKN2A, CDKN2B, TLR4 and TLR3 closely correlated with the severity of coronary artery disease as reflected by the number of coronary artery stenosis.

**Conclusion:** CDKN2A, CDKN2B, TLR3 and TLR4 have the potential to be a clinically useful biomarker of cardiovascular risk.

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**P049**

**A Duplication Copy Number Variation in the Exportin-4 (XPO4) is a Predictor for Histological Severity of Non-Alcoholic Fatty Liver Disease**

S.M. Zain$^a$, Z. Mohamed$^a$, M. Pirmohamed$^b$, H.L. Tan$^a$, R. Mohamed$^a$

$^a$The Pharmacogenomics Laboratory, Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia, $^b$The Wolfson Centre for Personalised Medicine, University of Liverpool, Liverpool, United Kingdom, $^c$Department of Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

**Objective:** Our recent genome-wide copy number (CNV) scan identified a 13q12.11 duplication in the exportin-4 (XPO4) gene to be associated with non-alcoholic steatohepatitis (NASH). In this study, we sought to confirm the finding in a larger cohort and to assess the serum XPO4 pattern in a broad spectrum of non-alcoholic fatty liver disease (NAFLD) cases.

**Material and Methods:** We analysed 249 patients with NAFLD and 232 matched controls using TaqMan PCR-based assay and serum XPO4 was measured.

**Results:** Copy number distribution was as follows: copy number neutral (NAFLD: 53.8%, controls: 68.6%), copy number losses (NAFLD: 13.3%, controls: 12.9%), copy number gains (NAFLD: 32.9%, controls: 18.5%). CNV gain was significantly associated with a greater risk of NAFLD (adjusted OR 2.22, 95% CI 1.42–3.46, $P = 0.004$) and NASH (adjusted OR 2.33, 95% CI 1.47–3.68, $P = 0.0003$) but not simple steatosis. Interestingly, subjects carrying extra copy number showed significantly higher serum ALT and triglyceride ($P < 0.05$). Serum XPO4 levels progressively declined ($P = 0.043$) from controls (24.6 ng/mL) to simple steatosis (20.8 ng/mL) to NASH (13.8 ng/mL).

**Conclusion:** XPO4 CNV duplication was associated with histological severity of NAFLD, and accompanied by changes in serum XPO4 levels providing insights into NAFLD pathogenesis, and has the potential for biomarker development.

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**P050**

**Association Study of Interleukin 10-1082 G/A Polymorphism and Interleukin-10 Levels with Occurrence of Spontaneous Preterm Birth in a Tri-Ethnic Malaysian Population**

S.Z. Suki$^a$, S.Z. Omar$^b$, Z. Mohamed$^a$

$^a$Pharmacogenomics Laboratory, Department of Pharmacology, Faculty of Medicine, University of Malaya, Malaysia, $^b$Department of Obstetrics and Gynaecology, Faculty of Medicine, University of Malaya, Malaysia

**Objective:** To examine the association between occurrence of spontaneous preterm birth (sPTB) with single nucleotide polymorphism in interleukin 10-1082 G/A (rs1800896) and with levels of interleukin-10 (IL-10) in three major ethnic sub-groups of Malaysia.

**Material and Methods:** Healthy women who had singleton pregnancy of a normal fetus, with spontaneous conception and delivery at either term or preterm at the University of Malaya Medical Centre (UMMC), Malaysia, from 2009 to 2011 ($n = 315$). A total of 315 women were enrolled and divided into those with sPTB ($n = 106$) as case group and those with term birth (TB) ($n = 209$) as control group. Real-time polymerase chain reaction method was used to genotype the samples while ELISA was used to determine the levels of IL-10 from the plasma samples.

**Results:** IL-10-1082 G/A polymorphism have been found to be associated with susceptibility to sPTB in the Malaysian cohort. We observed significantly higher A allele frequencies in cases compared to controls in all three ethnic subgroups ($p < 0.001$) and in pooled population ($p = 0.0001$). The AA and GA frequencies were significantly higher in cases compared to controls. The results of IL-10 levels in sPTB cases shows that the carrier of A allele has significantly higher IL-10 levels compared to the non-carrier ($p = 0.033$).

**Conclusion:** The study shows that IL-10-1082 G/A polymorphism is associated with spontaneous preterm birth as reflected by higher frequency of the A allele in sPTB cases compared to controls and by significantly higher levels of IL-10 in the AA genotypes and GA genotypes compared to the GG genotype.
Differential Expression of microRNAs in the Serum of Patients with Diffuse Large B-Cell Lymphoma
S. Suthandiram\textsuperscript{a}, G.G. Gan\textsuperscript{b}, S.M. Zain\textsuperscript{c}, P.C. Bee\textsuperscript{d}, L.H. Lian\textsuperscript{a}, K.M. Chang\textsuperscript{a}, T.C. Ong\textsuperscript{a}, Z. Mohamed\textsuperscript{d}

\textsuperscript{a}Pharmacogenomics Laboratory, Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia, \textsuperscript{b}Department of Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia, \textsuperscript{c}Department of Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia, \textsuperscript{d}Department of Hematology, Ampang Hospital, Kuala Lumpur, Malaysia

Objective: microRNAs (miRNAs) are a group of small noncoding RNAs, which can be isolated from blood serum samples and are mostly located at cancer-associated genomic regions. This suggests their potential role as biomarkers in multiple cancer types, including diffuse large B-cell lymphomas (DLBCLs). Several published reports have shown that expression levels of miRNAs in serum have been found to be reproducible and indicative of the disease. This study aimed to investigate the expression profiles of circulating miRNAs in the serum of patients with DLBCL and to analyze their potential as detection biomarkers.

Material and Methods: Global serum miRNA profiling was performed on 12 patients diagnosed with DLBCL and 12 demographically matched healthy controls using microarray analysis, followed by validation tests using real-time quantitative reverse-transcription polymerase chain reaction (qRT-PCR) in the same samples using five differentially expressed miRNAs.

Results: Our preliminary results indicated that miRNAs are present in human serum in a remarkably stable form. We found that 10 miRNAs were differentially expressed in the serum of DLBCL patients compared to healthy controls ($p < 0.05$). Six miRNAs were significantly up-regulated (miR-155-5p, miR-210-5p, miR-320a, miR-320b, miR-320d, and miR-320e), while four were significantly down-regulated (miR-15a-5p, miR-15b-5p, miR-21-5p, and miR34a-5p). The validation results are consistent with those obtained in the microarray method employed. Interestingly, five miRNAs (miR-155-5p, miR-210-5p, miR-15a-5p, miR-21-5p, and miR34a-5p) that are involved in DLBCL have also been found in our miRNA profiles.

Conclusion: We conclude that miRNA profiling in serum is a potentially useful tool as novel biomarkers for the diagnosis of DLBCL and possibly other cancers.
**P053**

**Lack of Association between CDKN1A c.93C>A Polymorphism and Cervical Cancer Risk**

S.C. Tan*, M.P. Ismail*, R. Ankathil*

*Human Genome Centre, School of Medical Sciences, Health Campus, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia, *Department of Obstetrics and Gynecology, School of Medical Sciences, Health Campus, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia

**Objective:** CDKN1A encodes for cyclin-dependent kinase inhibitor 1A, which plays an important role in cell cycle regulation and apoptosis. The c.93C>A polymorphism of CDKN1A causes a serine-to-arginine substitution in the protein product, which could potentially influence its functionality. In this study, we investigated the association between CDKN1A c.93C>A polymorphism and cervical cancer risk.

**Material and Methods:** The polymorphism was genotyped on 95 histopathologically confirmed cervical cancer patients and 95 cancer-free female controls by using PCR-RFLP technique. The risk association was evaluated by using logistic regression analysis. The finding was further stratified by self-reported ethnicity and human papillomavirus (HPV) type present in cervical smear of the subjects, as determined by using a flow-through hybridization method.

**Results:** By using the wild type genotype as reference, we found no significant association between the polymorphism and cervical cancer risk (heterozygous OR = 0.821, 95% CI = 0.418–1.612, \( P = 0.57 \); homozygous variant OR = 0.936, 95% CI = 0.406–2.156, \( P = 0.88 \)). A similar absence of significant association was observed when the results were stratified by the ethnicity of the study subjects (Malay, heterozygous \( P = 0.93 \), homozygous variant \( P = 0.78 \); Chinese, heterozygous \( P = 0.09 \), homozygous variant \( P = 0.31 \)). Likewise, lack of association was observed when the results were stratified by HPV types (HPV-16, heterozygous \( P = 0.16 \), homozygous variant \( P = 0.08 \); HPV-18, heterozygous \( P = 0.13 \), homozygous variant \( P = 0.79 \); other minor HPV types, heterozygous \( P = 0.41 \), homozygous variant \( P = 0.29 \); HPV negative, heterozygous \( P = 0.91 \), homozygous variant \( P = 0.47 \); multiple HPV types, heterozygous \( P = 0.24 \), homozygous variant \( P = 0.31 \)).

**Conclusion:** In conclusion, the present study suggests a lack of association between CDKN1A c.93C>A polymorphism and cervical cancer risk.

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**P054**

**Polymorphisms of the Resistin Gene and Their Association with Abdominal Obesity and Resistin Levels in Malaysian Malays**

Y.D. Apalasamy*, S. Rampal*, F.M. Moy*, A. Bulgiba*, Z. Mohamed*

*Pharmacogenomics Laboratory, Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia, *Julius Centre University of Malaya, Department of Social and Preventive Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

**Objectives:** Resistin is an adipocyte-secreted cytokine discovered and has been proposed as a link between obesity and diabetes. Single nucleotide polymorphisms (SNP) in the resistin gene (RETN) have been investigated and their implications with obesity, abdominal obesity and metabolic abnormalities are controversial. Therefore, this study aimed to investigate the association between resistin gene polymorphisms with its circulating levels and abdominal obesity in Malaysian Malay population.

**Material and Methods:** A total of 625 Malaysian Malay subjects were included in this study. RETN rs34861192, rs1862513 and rs3219175 SNPs were genotyped using Sequenom MassARRAY.

**Results:** There was no significant difference found in both allelic and genotype frequencies of each of the RETN SNPs between the obese and non-obese groups after Bonferroni correction. RETN rs34861192 and rs3219175 SNPs were significantly associated with log-resistin levels. The GG homozygotes were found to have higher levels of log-resistin compared to A allele carriers. It was found that haplotypes of the RETN gene were not associated with abdominal obesity. There were no significant correlation between resistin levels and abdominal obesity. Resistin level was not correlated to blood pressure and lipid levels.

**Conclusion:** In summary, RETN rs3219175 and rs34861192 SNPs are of apparent functional importance in the regulation of resistin levels but are not correlated with abdominal obesity and related parameters.
P055

**GABRA6 Polymorphism and Susceptibility to Epilepsy in Malaysian Population**


4Pharmacogenetics Laboratory, Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia
3Division of Neurology, Department of Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia
2Division of Neurology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

**Objective:** Epilepsy, a diverse set of chronic neurological disorders, is characterized by unprovoked seizures. Several polymorphisms in Gamma-aminobutyric acid A receptor, alpha 6 (GABRA6), a ligand-gated chloride channels, have been reported to be associated with susceptibility of childhood absence epilepsy and idiopathic generalized epilepsy. In this study, we evaluated the association of GABRA6 rs3811995, rs3811992, rs13184586, rs6865042 and rs6883758 with the risk of epilepsy in Malaysian population.

**Material and Methods:** Genomic DNA of 1789 Malaysian subjects (1088 healthy-declared population and 701 epilepsy patients consisting of 41% cryptogenic, 26% idiopathic and 33% symptomatic epilepsy) was genotyped by using Sequenom MassArray technique.

**Results:** Our results showed no significant association between the GABRA6 polymorphisms and susceptibility to epilepsy.

**Conclusion:** GABRA6 polymorphisms do not contribute to the risk of epilepsy in Malaysian populations.

P056

**Impact of Ethnicity on the Association of Plasminogen Activator Inhibitor-1 and Tissue Plasminogen Activator Polymorphisms with Type 2 Diabetes Mellitus**


2Department of Biochemistry and Molecular Biology, Faculty of Medicine, Sana’a, Yemen
3Department of Medicine, University of Malaya Medical Centre, University of Malaya
4Department of Molecular Medicine, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

**Objective:** The prevalence of type 2 diabetes mellitus (T2DM) differs widely across different ethnic groups and the genetic linkage of diabetes varies between populations. The aim of this study was to investigate the impact of ethnicity on the association of plasminogen activator inhibitor-1 (PAI-1) 4G/5G and tissue plasminogen activator (tPA) Alu repeat insertion/deletion (I/D) polymorphisms with T2DM in Malaysian subjects.

**Material and Methods:** PAI-1 4G/5G and tPA Alu repeat I/D were genotyped by allele specific PCR. Blood from 231 normal subjects without diabetes (114 Malays, 71 Chinese and 46 Indians) and 303 T2DM (149 Malays, 51 Chinese and 103 Indians) were recruited for analysis.

**Results:** The PAI-1 4G/5G polymorphism was a strong risk factor for T2DM among Chinese (the dominant genetic model odds ratio is 6.45, P = 0.005), whereas the tPA Alu repeat I/D polymorphism was a risk factor for T2DM among Indian subjects (dominant genetic model odds ratio is 3.27, P = 0.02).

**Conclusion:** The ethnicity significantly moderated the association of PAI-1 4G/5G and tPA Alu repeat I/D polymorphisms with T2DM.
Conclusion: DRD2 polymorphisms do not have impact on cold pressor pain threshold, pain tolerance and pain intensity. The present results also do not support the notion that DRD2 polymorphisms play a major role in cold pressor pain sensitivity.

P058
Possible Mechanism of Antiulcer Activity of Methanol Extract of Melastoma malabathricum Leaves in Rats
A.A. Seyedan, Z.A. Zakaria
Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Objective: Melastoma malabathricum L. belonging to Melastomaceae family is traditionally used to treat various disorders including gastric ulcers. In view of its application in the management of gastric ulcers, present investigation was carried out to assess the involvement of endogenous nitric oxide and sulfhydryls to elucidate the mechanism of action of methanol extract of Melastoma malabathricum (MEMM) in its gastroprotective effect against ethanol-induced gastric ulcer.

Material and Methods: The 24 hours fasted rats (3 groups) pretreated with N-ethylmaleimide (NEM) (10 mg/kg), and thirty minutes later, animals received an oral dose of the 10% DMSO, carbonoxalone (100 mg/kg) or MEMM (500 mg/kg). After 1 hour, all groups were orally treated with absolute ethanol (1 ml/200 g) for gastric-ulcer induction. Besides, three groups of animals previously had been pretreated intraperitoneally with N-nitro-L-arginine methyl ester (L-NAME) (70 mg/kg) and 30 min later, each groups of animals received an oral dose of the 10% DMSO, carbonoxalone (100 mg/kg) or MEMM (500 mg/kg). After 1 hour, ethanol was induced orally.

Results: The administration of NEM (a SH-blocker) significantly increased the effects of ethanol on gastric mucosa injury, reversing the gastroprotective effect of MEMM (500 mg/kg). The previous administration of L-NAME did not interfere with the gastro protection of MEMM, indicating that the NO system is probably not involved with the antiulcer effect of MEMM.

Conclusion: The MEMM exerts potential gastroprotective activity that could be partly attributed to the presence of SH group. The results supported the ethnomedicinal uses of the plant in the treatment of gastric ulcer.

P059
The Effect of Palm Oil Derived Tocotrienol Rich Fraction on Glutathione S-Transferases Expression in Mice Liver
A. Abdullah, N. Alrawaiq, A. Atia
Department of Pharmacology, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Kuala Lumpur, Malaysia

Objective: Tocotrienol is a hydrophobic fat soluble compound belonging to vitamin E family and has been reported to possess potent antioxidant activity. The aim of this study was to determine the effects of different doses of palm oil derived tocotrienol rich fraction (TRF) supplementation on glutathione S-transferases (GSTs) gene expression in mice livers.

Material and Methods: Fifteen male ICR white mice (25–30 g) were divided into five groups; three groups were administered palm TRF orally at doses of 200, 500 and 1000 mg/kg respectively (n = 3 for each group), a positive control group administered butylated hydroxyanisole (BHA) orally at a dose of 100 mg/kg (n = 3), and the last group (n = 3), which comprise control mice, were only administered vehicle which is corn oil. The expression levels of several GST genes were determined by quantitative real-time PCR.

Results: It was found that TRF at concentrations of 200, 500, and 1000 mg/kg caused a significant concentration-dependent increase in fold change of GSTs (Gst1, Gst3, Gstm1, Gstm3, and Gstp) gene levels in mice livers, compared to controls. Mice treated with BHA showed the highest increase in fold change in GSTs expression as compared to controls.

Conclusion: In the present study, we have demonstrated, for the first time that palm TRF treatment increased GSTs expression in mice liver dose dependently, with the highest expression seen with mice treated with 1000 mg/kg TRF, followed by 500 and 200 mg/kg respectively.

P060
Gongronema latifolium Lowers Blood Glucose via Pancreatic Islet Cell Regeneration and Insulin Sensitization
B. Al-Hindi1, I.J. Atangwho1, N.A. Yusoff2, M. Ahmad1, M.Z. Asmawi1, M.F. Yam2
1School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang, Malaysia, 2Advanced Medical and Dental Institute, Penang, Malaysia

Objective: Gongronema latifolium is one of the African folk medicines for diabetes mellitus. Previous studies justified its use based on its antioxidant and glucose lowering potential. This study evaluated the effect of Gongronema latifolium ethanolic extract on blood glucose tolerance, β-cell effect, and in vitro glucose uptake.

Materials and Methods: Whole dried G. latifolium was extracted using a soxhlet apparatus. Diabetes was induced by intra-
peritoneal injection of streptozotocin (55 mg/kg). The diabetic rats were used for a 14-day study. Effects on abdominal muscle glucose uptake and intestinal glucose transport were investigated in vitro.

Results: A 14-day oral administration of the extract (1 mg/kg b.w.) to diabetic rats significantly attenuated the elevated blood glucose levels starting from day-10 till end of study ($P < 0.05$). Unlike metformin, the 14-day oral treatment with – 1 g/kg and 0.5 g/kg of the extract caused 44% and 50% increases in the average area of the islets of Langerhans, respectively. The extract was also found to increase muscle glucose uptake significantly in the presence of insulin, indicating an insulin-sensitizing potential, which conformed to results related to serum insulin levels.

Conclusion: Overall, data from this study, besides validation of the antidiabetic action of Gongronema latifolium suggest that the anti-diabetic activity is established via combined pancreatic and extra-pancreatic mechanisms mediated probably by the Sitostenone and fatty acid esters predominant in the extract.

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P061

Triclosan Demonstrates Synergistic Effect with Amphotericin B and Fluconazole against Cryptococcus Neoformans

E. Movahed

Department of Medical Microbiology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

Objective: Conference In recent years, widespread use of antimicrobial drugs in the treatment of fungal infection has led to the global emergence of resistant fungal strains. Many fungal pathogens including C. neoformans demonstrated increasing resistance to common antifungal drugs such as amphotericin B and fluconazole. Therefore, studies to discover new drugs or combinations of several anti-microbial drugs are urgently needed to find a new treatment option for these emerging strains. Triclosan is a chlorinated compound that demonstrates broad-spectrum anti-microbial properties against various species of bacteria and fungi. It is widely used for personal care products due to its safety efficiency and a long-lasting effect.

Material and Methods: The effect of triclosan against Cryptococcus neoformans H99 was evaluated by agar diffusion and microbroth dilution assays. In vitro interactions between triclosan and standard anti-fungal drugs (amphotericin B and fluconazole) were evaluated by the microdilution synergy checkerboard and disk diffusion assays. Mechanism of fungicidal activity was investigated by examining the morphology under transmission electron microscope.

Results: Our data showed that triclosan demonstrate inhibitory effect on C.neoformans by causing cell death. Besides, addition of triclosan markedly enhanced the anti-fungal effect of amphotericin B and fluconazole to generate a larger inhibition zone.

Conclusion: Therefore, we reported that triclosan demonstrated synergistic effect with amphotericin B and fluconazole to inhibit the growth of C. neoformans.

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P062

Behavioral and Histological Effects of Lipopolysaccharide Preconditioning in a Pentylenetetrazol Rat Model of Epilepsy

E. Amini\textsuperscript{a}, M. Golpich\textsuperscript{b}, Z. Mohamed\textsuperscript{c}, M.I. Norlinah\textsuperscript{a}, A.A. Raymond\textsuperscript{d}, A. Ahmadiani\textsuperscript{c}

\textsuperscript{a}Department of Medicine, Faculty of Medicine, UKM Medical Centre, 56000 Cheras, Kuala Lumpur, Malaysia, \textsuperscript{b}Department of Pharmacology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia, \textsuperscript{c}Neuroscience research center (NRC), Shahid Beheshty Medical University, Tehran, Iran

Objective: Epilepsy is a most common neurological disorder characterized by recurrent unprovoked seizures. Prolonged seizures contribute to neuronal death in the hippocampus that is considered to initiate epileptogenesis. Lipopolysaccharide (LPS) preconditioning can promise to serve as an alternative therapeutic approach, because this strategy has demonstrated the capability to attenuate damage induced by seizures in rodent models. The aim of this study was to evaluate the effect of LPS Preconditioning on Pentylenetetrazol (PTZ)-induced seizure in rat.

Material and Methods: Four days before PTZ administration, male SD rats were induced by either low dose of LPS or normal saline as treated and control groups respectively. For investigating the anticonvulsant effect of LPS against seizures, animals were evaluated for behavioral and histological deficits, after PTZ administration. In behavioral test, development and severity of the PTZ-induced seizures were scored according to Racine. Finally, all animals were sacrificed to confirm the neuroprotective phenotype by histological analyses of CA1, CA3 and dentate gyrus (DG) hippocampal regions.

Results: Based on the behavioral observations, it was found that the preconditioning treatment used decreased the seizure excitability in epileptic rats. Likewise, the histological analyses of brain sections in the LPS-preconditioned rats showed decreased intensity of neurodegenerative changes in the regions of interest comparing with control and model groups.

Conclusion: Our findings suggest that LPS preconditioning may affects animal behavior and induces effective neuroprotection in the hippocampal sections. Moreover, in the LPS preconditioning-mediated neuroprotective events, the role of gene reprogramming is crucial which should be considering in future surveys.
Abstracts

P063
Dichloromethane Curcuma purpurascens BI Rhizome Extract Prevents Thioacetamide-Induced Liver Cirrhosis in Rats
aDepartment of Pharmacology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia, bDepartment of Biomedical Science, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

Objective: This study was designed to investigate the hepatoprotective effects of dichloromethane Curcuma purpurascens BI rhizome extract (DECPR) on thioacetamide-induced hepatotoxicity in rats.

Material and Methods: Sprague Dawley male rats were given intraperitoneal injections of vehicle 10% Tween-20, 5 mL/kg (normal control) or 200 mg/kg TAA thioacetamide (to induce liver cirrhosis) three times per week to assess the cytotoxicity. Three additional groups were treated with thioacetamide plus daily oral silymarin (50 mg/kg) or DECPR (250 or 500 mg/kg). Biochemical tests, macroscopic and microscopic tissue analysis, histopathology, and immunohistochemistry were employed to assess liver injury. Cytotoxicity of DECPR was tested in HepG2 and WRL-68 cells.

Results: Results revealed that rats treated with DECPR exhibited significantly lower liver/body weight ratios and more normal liver along with Masson’s Trichrome stain exhibited minimal disruption of cells, but possessed anti-proliferative activity on HepG2 cells, which were confirmed by a significant elevation of cytochrome c, and caspase-8, -9, and -3/7 activity in HepG2 cells.

Conclusion: The hepatoprotective effect of 500 mg/kg of DECPR is proposed to result from the reduction of thioacetamide-induced toxicity, normalizing reactive oxygen species levels, inhibiting cellular proliferation, and inducing apoptosis in HepG2 cells.

P064
Effects of Supercritical Fluid Extraction Conditions on Yield of Protein from Defatted Rice Bran
L.C. Foonga, S. Iqbalb, M.F. Abu Bakarc, M. Ismaila,c
aFaculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia, bDepartment of Chemistry, University of Sargodha, Sargodha-100, Pakistan, cNutrigenomics Program, Laboratory of Molecular Biomedicine, Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

Objective: Defatted rice bran (DRB) is a by-product of oil extraction from rice bran by supercritical fluid extraction (SFE). In the present study, effect of different supercritical fluid extractor (SFE) operational conditions i.e. pressure (300, 450, 600 bars), flow rate (10.0, 17.5, 25.0 g/min), and intervals of extraction (30, 90, 150 min) at 60°C on protein content of defatted rice bran (DRB) samples, has been evaluated.

Material and Methods: The DRB samples were collected from SFE vessel. Their moisture, residual fat and protein contents were analysed by drying method, Soxhlet method, and Kjeldahl method, respectively. Efforts were made to correlate the effects of parameters with the yield of proteins. Furthermore, residual oil content and moisture content from DRB samples were also correlated with the yield of protein.

Results: The protein content was ranged over 15.59 to 17.44%, while moisture content was ranged from 7.84 to 9.88%, and residual fat content was varied over 4.67 to 18.94%. Highest yield of protein was obtained at 450 bars, 90 min extraction time, and 17.5 g/min flow rate, while the lowest at 300 bars, 30 min of extraction time, and 10.0 g/min flow rate. Negative correlation with p-value ≤0.05 was found between moisture content and residual fat content of DRB. However, no correlation could be observed among protein, moisture and residual fat contents of DRB, respectively.

Conclusion: The study revealed that SFE could be used for the extraction of oil from rice bran while conserving the protein content in defatted residues.

P065
Biotransformation of Ferulic Acid to 4-Vinyl Guaiacol
A.A. Hadizaa, C. Kim Waia, I. Shahidb, I. Maznahab
aLaboratory of Molecular Biomedicine, Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia, bDepartment of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, UPM Serdang 43400 Serdang, Selangor, Malaysia

Objective: The use of phenolics as a starting material for the production of functional food, nutraceuticals and drugs, by extraction, isolation and purification has generated business opportunities
and given economic incentives to the industries. In this study, we investigated the potential of *Lactobacillus farciminis* ATCC 29644 for biotransformation of ferulic acid to 4-vinyl guaiacol (4VG). It is a volatile phenol with anti-inflammatory properties and is most extensively used in food and alcoholic beverages as a flavouring substance. It has been reported to have 40-fold higher economic value than ferulic acid and is biotransformable to acetonailone, ethylguaiacol and vanillin. Vanillin which is employed in pharmaceutical and medical industries possesses antimicrobial, antioxidant and antimutagenic properties.

**Materials and Methods:** Biotransformation was observed after 5 h incubation of *L. farciminis* with ferulic acid in Man Regosa and Sharpe (MRS) broth at 37°C under 5% CO₂ and production rate was at its peak after 48 h. Identification and of 4VG was done using GC/MS and subsequent quantification of yield by HPLC.

**Results:** The impact of initial concentrations of ferulic acid and bacteria on the production of 4VG was studied and yield of product was found to be three-fold than the initial ferulic acid concentration. The results showed that the production of 4VG was significantly influenced by the initial concentration of ferulic acid, and empirically 0.1, 1.45 and 3 mg/l of ferulic acid yielded 0, 3.34 and 10.26 mg/l of 4VG, respectively.

**Conclusion:** The findings are a milestone towards safe high yielding means of biotransforming some common agro-industrial wastes to a value added product.
P068
Influence of Cytochrome P450 Enzymes on Mitragynine-Induced Hepatotoxicity
W.M. Kong, Z. Chik, M.A. Alshawsh, Z. Mohamed
Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

Objective: Mitragynine is the major active alkaloid in Mitragyna speciosa Korth, the extracts of which is known to have opium-like sedative and coca-like stimulant activity. Although reports have indicated that mitragynine is hepatotoxic in vivo, its mechanism of hepatotoxicity still remains unclear. In this study, we attempted to determine the roles of the cytochrome P450 enzymes, namely, CYP3A4, CYP1A2, CYP2D6, CYP2C19 and CYP2C9 on mitragynine-induced hepatotoxicity.

Materials and Methods: Normal hepatic cell line (WRL-68) was co-incubated with mitragynine and CYP specific inhibitors such as ketoconazole, quinidine, furafylline, tranylcypromine and sulfa-phenazole for 72 h. Cell survival after the 72 h treatment was measured by XTT proliferation assay. Pure CYP supersomes were then used for further clarification of CYP function in mitragynine metabolism and hepatotoxicity.

Results: Our data showed that mitragynine is a potent hepatotoxic agent with IC50 of 16.17 µM. The inhibition of CYP2D6 by quinidine and of CYP1A2 by furafylline significantly reduced the toxicity of mitragynine, whereas inhibition of CYP3A4, 2C9 and 2C19 did not affect the toxicity of mitragynine. This finding has been strengthened by placement of 20 pmole/well of CYP2D6 and CYP1A2 supersomes in the presence of NADPH cofactor. CYP2D6 and CYP1A2-treated cells showed significant increase in cell survival.

Conclusion: Our report suggested that both CYP2D6 and CYP1A2 play important roles in mitragynine metabolism and enhancement of their activities can reduce mitragynine-induced hepatotoxicity.

P069
Water Extract of Clinacanthus Nutans Leaves has Chemopreventive Potential against Colon Cancer in vivo
M.N. Kuraa, N.M. Esaab, M. Ismailb
aLaboratory of Molecular Biomedicine, Institute of Bioscience, Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia; bDepartment of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia

Objective: Chemoprevention is a promising strategy in controlling the occurrence of colon cancer and is defined as the use of natural or pharmacological agents to suppress, reverse or arrest carcinogenesis at its early stage. In a colon cancer model induced through a combination of 1,2-dimethylhydrazine (DMH) administration and feeding with a high fat diet (HFD) and cholic acid, the chemopreventive effect of water and 80% methanolic extract of leaves of Clinacanthus nutans (Cn) was observed.

Materials and Methods: Leaves of Cn were sequentially extracted with water and 80% methanol using sonication. The formation of aberrant crypt foci (ACF) was observed after administration of 100 mg/kg DMH by gavage. Extracts were administered daily to Sprague-Dawley rats for eight weeks. After sacrifice, full blood count, histology and gene expression analysis of colons and livers were then carried out. Extracts were also analysed qualitatively for the presence of phytochemicals.

Results: There was significant decrease in the number of aberrant crypt foci and crypt multiplicity in the colon of rats treated with 500 mg/kg water extract. The 500 mg/kg water extract also decreased hepatic steatosis and increased mRNA expression of Gpx1 and Sod1 antioxidant genes. Phytochemical screening of the water and 80% methanol extracts revealed the presence of saponins, tannins, alkaloids and diterpenes.

Conclusion: Our results show that the water extract of Cn demonstrated potential in arresting abnormal colon cell growth and the possibility of halting progressive liver damage in chemically induced rat model of colon cancer.

P070
Comparative Efficacy of Ivermectin, Fenbendazole and Albendazole against Gastrointestinal Nematodiasis in Goats
M. Aktaruzzaman, M.M. Islam, Z. Mohamed
Pharmacogenomics Laboratory, Department of Pharmacology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

Objectives: This study aimed to evaluate the efficacy of Ivermectin, Fenbendazole and Albendazole against gastrointestinal nematodes in naturally infected Goats of Government Goat Development Farm, Sylhet, Bangladesh.

Material and Methods: The study included 50 Black Bengal breed of which 30 were naturally infected and randomly selected 20 on the basis of their weight and egg count. Goats were divided into 4 groups of 5 animals each. Group ‘D’ served as the untreated control, whereas groups ‘A’, ‘B’ and ‘C’ were treated with ivermectin, fenbendazole and albendazole respectively. The therapeutic efficacy was evaluated through determination of parasitic prevalence, body weight gain/loss and hematological findings. Faecal and blood samples were collected before treatment on day 0, and on post-treatment days 7, 14, 21 and 28 of study.

Results: The results showed that the efficacy of ivermectin was 100%, followed by fenbendazole 95.33% and albendazole 90.11%. Coprocultures from all pre and post-treatment samples showed a predominance of Haemonchus spp. (15.38%), with Trichostrongylus spp., Strongyloides spp., and Cooperia spp. also present. The body weight of the treated animals were slightly increased which were significant (p < 0.05). Of the haematological parameters, TEC, Hb and PCV values were lower on day 0 but turned to increase (p < 0.01) on day 28 of the study. On the other hand, ESR and TLC were higher before treatment (day 0) but decreased significantly (p < 0.01) on day 28.
**P071**

**Dihydroorotate Dehydrogenase (DHODH) Inhibitor Suppress Breast Cancer Cells Proliferation**

A.K. Mohamad Fairus, B. Choudhary, O. Shatrah, S. Hosahali, N. Kavitha

&Diacritical Marksb, *C. Aurigene Discovery Technologies (M) Sdn. Bhd., Level 2, Research Management and Innovation Complex, 50603 University of Malaya, Kuala Lumpur, Malaysia,

&Diacritical Marksac, &Diacritical Marksbd, *Department of Molecular Medicine, Faculty of Medicine, 50603 University of Malaya, Kuala Lumpur, Malaysia,

&Diacritical Marksbe, &Diacritical Marksbc, *Aurigene Discovery Technologies Limited, 39-40, KIADB Industrial Area, Phase II Electronic City, Hosur Road, Bangalore – 560100 Karnataka, India

**Objective:** Dihydroorotate dehydrogenase (DHODH) is a key enzyme in de novo pyrimidine biosynthesis pathway which is established as a drug target in inflammatory diseases but with no clinical success in cancer treatment. Various pre-clinical studies suggest that it can be a potential target for cancer therapy. In the present study, we have investigated the correlation between DHODH protein expression, proliferation rate and cell sensitivity to DHODH inhibitors in breast cancer (T-47D, MDAMB-436 and MDAMB-231) and normal cells.

**Material and Methods:** We compared the proliferation speed, DHODH expression and cell proliferation inhibition of different breast cell lines using Trypan blue exclusion, Western blot analysis and either XTT or Alamar blue assay respectively.

**Results:** Our study shows that fast-proliferating T-47D and MDAMB-231 cells express more DHODH enzyme and are more sensitive to DHODH inhibitors (brequinar, leflunomide and 4SC-101) in comparison to slow-proliferating MDAMB-436 and W3.006 cells. T-47D and MDAMB-231 cells are more sensitive to brequinar (BQR) with EC50 of 445±1.89 nM and 184±7.0 nM respectively in comparison to other two inhibitors. MDAMB-436 and W3.006 cells demonstrated EC50 of >30 μM and >100 μM respectively with all the DHODH inhibitors.

**Conclusion:** In conclusion, cancer cells expressing more DHODH enzyme, show high sensitivity to DHODH inhibitors. High DHODH expressing cell were associated with high proliferation rate. Our findings suggest that DHODH could be a potential target in breast cancer treatment. The current data proposed that cancer patient expressing high DHODH in tumor may respond better to DHODH inhibitor mediated therapy.

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**P072**

**Immunoinformatics and Pharmacoinformatics Elucidation to Design a Potential Epitope Based Vaccine and Drug Candidates Library for the Complete Treatment of West Nile Virus**


&Diacritical Marksaa, &Diacritical Marksab, &Diacritical Marksac, *Department of Biotechnology and Genetic Engineering, Life Science Faculty, Mawlana Bhashani Science and Technology University, Santosh, Tangail-1902, Bangladesh,

&Diacritical Marksad, *Department of Science and Humanities, Military Institute of Science and Technology (MIST), Mirpur Cantonment, Dhaka-1216, Bangladesh,

&Diacritical Marksae, &Diacritical Marksaf, *Department of Genetic Engineering and Biotechnology, Life Science Faculty, Shahjalal University of Science and Technology, Kumargaon, Sylhet-3114, Bangladesh,

&Diacritical Marksag, &Diacritical Marksah, *Department of Biochemistry, Gono University, Mirzanagar, Savar, Dhaka-1344, Bangladesh,

&Diacritical Marksai, *Molecular Biotechnology Division, National Institute of Biotechnology, Ganakbari, Ashulia, Savar, Dhaka-1349, Bangladesh

**Objective:** An outbreak of West Nile Virus (WNV) like the recent outbreak of Ebola virus can be more epidemic and fatal to public health throughout the world. WNV possesses utmost threat as no vaccine or drug is currently available for its treatment. At present, mosquito control is the only way to prevent this infection.

**Material and Methods:** In this study, we have used combined approach of immunoinformatics and pharmacoinformatics to design a potential epitope-based vaccine and drug candidates against the envelope glycoprotein of WNV.

**Results:** By analyzing the whole proteome containing 3157 proteins the envelope glycoprotein E was selected as it possesses the highest antigenic score, 0.772. After proper assessment KSFLVHREW and ITPSAPSYT were found to be the most potential T and B-cell epitopes. Predicted peptide could interact with at least 13 MHC alleles and demonstrated highest population coverage 91.53%. Besides, for drug development, we adopted a novel computational approach based on sequence, 3D structure building, pharmacophore, ADME/Tox and QSAR studies along with two most convenient docking simulation analysis. Furthermore, we have designed some novel drugs by producing analogue from a known inhibitor, AP30451. Further toxicity assessment, ADME and drug score of these designed drugs confirm us as effective drug candidates against WNV.

**Conclusion:** *In silico* analysis suggested that the predicted epitopes, either KSFLVHREW or ITPSAPSYT could be a better choice as universal vaccine component against WNV that can trigger significant immune response as pre-therapy and further analyses on target site with a designed drug candidates could assist as post-therapy for the complete treatment against WNV.
Abstracts

P073

Behavioral and Histological Effects of Lipopolysaccharide Preconditioning in a 6-Hydroxydopamine Rat Model of Parkinson’s Disease

M. Golpich, E. Amini, Z. Mohamed, A.A. Raymond, M.I. Norlinah, A. Ahmadian

Objective: Parkinson’s disease (PD) is the second most common neurodegenerative disorder linked to unclear etiology. Several lines of evidences have demonstrated that Lipopolysaccharide (LPS) Preconditioning as a potential strategy may reduce the neural deficits associated with neurodegenerative diseases. The aim of this study was to evaluate the effect of LPS Preconditioning on 6-hydroxydopamine (6-OHDA)-induced Parkinson model in rat.

Material and Methods: Four days before 6-OHDA injection, male SD rats were induced by low dose of LPS or normal saline as treated and control groups respectively. To investigate the neuroprotective effect of LPS Preconditioning on a 6-OHDA model of PD, animals were evaluated for neurobehavioral and histological deficits, 7 days after 6-OHDA lesioning. Development and severity of the 6-OHDA-induced Parkinsonism were assessed using behavioral tests including apomorphine-induced rotational, rotaor, and cylinder tests. Finally, animals were sacrificed to confirm the neuroprotective phenotype by histological analyses of substantia nigra and striatum regions.

Results: In comparison with control and model groups, preconditioned animals performed significantly better on the behavioral tests. These results were confirmed by the data obtained from histological analyses.

Conclusion: Our findings show that the LPS Preconditioning attenuates the behavioral symptoms and histological damage of 6-OHDA-induced Parkinsonism in rat. This report presents the new finding that LPS Preconditioning provides neuroprotection in a 6-OHDA rat model of PD which resulted by reprogramming of gene transcription in the brain and is crucial to be considering in future studies. Understanding the mechanisms involved in LPS Preconditioning-mediated protection may stimulate an alternative therapeutic approach in the near future.

P074

White Rice Promotes, while Brown Rice and Germinated Brown Rice Attenuate Hypercholesterolemia-Induced Cardiometabolic Disease in Rats through Nutrigenomic Regulation of Oxidative Stress and Cholesterol Metabolism Genes

M.U. Imam, M. Ismail

Objective: Brown rice (BR) has higher nutritional value than white rice (WR), which is the commonly consumed form of rice. When germinated, the bioactive compounds responsible for the bioactivity of BR are potentiated. In this study, we tested the hypothesis that germinated brown rice (GBR) will produce better regulation of cardiometabolic indices than WR and BR, in hypercholesterolemic rats.

Material and Methods: Hypercholesterolmia was induced by feeding high fat + cholesterol diet to male Sprague dawley rats for 6 weeks, after which rats were managed with semi-purified diets containing WR, BR or GBR, and compared with untreated control and simvastatin treated groups. Weight, lipid profile, plasma oxidized low-density lipoprotein and F₂-isoprostane, and hepatic mRNA levels of oxidative stress and cholesterol metabolism genes were evaluated.

Results: WR worsened cardiometabolic disease markers partly through modulation of hepatic oxidative stress and cholesterol metabolism genes, while BR and GBR improved cardiometabolic indices. Specifically, GBR reduced weight gain and improved cardiometabolic indices including nutrigenomic regulation of hepatic lipoprotein lipase, peroxisome proliferator-activated receptor gamma, adiponectin, ATP-binding cassette, sub family A, and v-akt murine thymoma viral oncogene homolog 1 and homolog 3 genes. The improved bioactivity of GBR over BR is attributable to the higher amounts of GBR bioactives.

Conclusion: The results suggest that WR consumption will promote risk of cardiometabolic diseases likely due to high glycemic index while BR and GBR can be used as a functional foods to lower the risk of such diseases.

P075

Alternanthera sessilis Extracts Lowered Serum Lipid Profile through Some Mechanisms in Hyperlipidemic Sprague Dawley Rats

N.S. Usman, I. Bello, S.Y. Hor, N.Y. Adlin, A.M. Zaini, M.F. Yam

Objective: Hyperlipidemia is a metabolic disorder characterized by increase in serum lipid profile level. The mechanisms by which this disorder can be reduced or inhibited are quite crucial in the treatment
of hyperlipidemia. The objective of this study is to determine the lipid lowering effect of Alternanthera sessilis extracts and mechanisms.

**Material and Methods:** The plant was serially macerated with petroleum ether, chloroform, methanol and water. 1 g/kg of each extract and a reference drug, atorvastatin (30 mg/kg) were given orally for 4 weeks with high fat diet (1% cholesterol, 0.5% cholic acid, and 15% margarine) and 3 days in poloxamer induced hyperlipidemic rat models.

**Results:** In both models, water and methanol extracts significantly reduced serum total cholesterol, triglyceride, and LDL cholesterol levels but did not significantly improve the HDL cholesterol level compared to the hyperlipidemic control with water extract being more potent. In high fat diet rat model, water extract (1000 mg/kg) significantly increased the fecal cholesterol (P < 0.05) and bile acid (P < 0.01) compared to the high fat diet control. Water and methanol extracts lowered hepatic cholesterol (P < 0.01), (P < 0.01) and triglyceride (P < 0.01), (P < 0.001) respectively compared to the high fat diet control.

**Conclusion:** These findings denote that the lipid lowering activity of the extracts is conceivably by improving the hepatic depletion and fecal excretion of lipids via inhibition of bile acid reabsorption.

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**P076**

**Sodium Benzoate as a Solvent for the Novel Hydrophobic 5-Phenylaminouracil Derivatives: Evaluation of Cytotoxic Effect in Vero 76 Cell Culture**


**Objective:** 5-Phenylaminouracil derivatives have recently shown potential antiviral effect. The high lipophilicity of the compound significantly limits their ability to be dissolved in aqueous media for further in vitro studies. Currently available solvents such as DMSO and methanol solubilise the 5-phenylaminouracil compound at cytotoxic dose. Sodium benzoate is widely used as a preservative in pharmaceutical and food industry, and could be used as potential solvent for 5-phenylaminouracil derivatives. However the cytotoxic concentration of sodium benzoate on Vero cells is unknown. This study aimed to evaluate cytotoxic effect of sodium benzoate on Vero cells.

**Material and Methods:** Vero cells 1×10^5 well were seeded into 96 well plates and incubated for 24 hours. After that, sodium benzoate was added into each well in dose ranged from 0.625 mM to 80 mM in triplicates and incubated for 72 hours. MTS assay (Promega) was then performed to each well in accordance to the manufacturer’s protocol. Absorbance was read at 490 nm and cell viability was calculated.

**Results:** Sodium benzoate at dose 0.625 mM did not produce cytotoxic effect and cell viability was 96.8%. However, at higher doses the cell viability significantly reduced and was less than 5% (2.2%) at dose 80 mM.

**Conclusion:** This study suggested that sodium benzoate at a concentration of 0.625 mM and below is safe to be used as a solvent for novel 5-phenylaminouracil derivatives.

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**P077**

**Aqueous Extract of Nypa fruticans Wurmb. Vinegar Alleviates Postprandial Hyperglycaemia in Normoglycemic Rats**

*N.A. Yusoff*, B. Al Hindi, T. Widyawati, M.F. Yam, M. Ahmad, R. Mahmod, K.N. Abdul Razak, M.Z. Asmawi

**Objective:** Nypa fruticans Wurmb. vinegar, commonly known as nipa palm vinegar (NPV) has been used as a folklore medicine among the Malay community for the treatment of diabetes. Early work has shown an aqueous extract (AE) dose of 500 mg/kg to exert a potent antihyperglycemic effect. This study has thus evaluated AE effect on intestinal glucose absorption and carbohydrate-metabolizing enzymes.

**Material and Methods:** AE was obtained through liquid-liquid extraction of NPV, and tested in vitro using intestinal glucose absorption, carbohydrate tolerance and spectrophotometric enzyme inhibition assays.

**Results:** 1 mg/ml of AE showed a comparable outcome to the use of chlorizidin dihydrate (1 mM) in vitro as it delayed glucose absorption through the jejunal sac, more effectively than acarbose (1 mg/ml). Further in vivo confirmatory tests showed AE (500 mg/kg) to cause significant suppression in postprandial hyperglycaemia 30 min after respective glucose (2 g/kg), sucrose (4 g/kg) and starch (3 g/kg) loadings in normal rats, as compared to the control group. Conversely, AE possessed rather weak inhibitory activities against both α-glucosidase and α-amylase in spectrophotometric enzymatic assays when compared with acarbose (IC50 value; 1.11±0.02 mg/ml and 0.45±0.07 mg/ml, respectively), as AE’s respective IC50 values were 28.92±0.5 mg/ml and 60.62±0.32 mg/ml only.

**Conclusion:** The findings suggest that NPV exerts its anti-diabetic effect by delaying the absorption of carbohydrates in the small intestine through selective inhibition of intestinal glucose transporters– a process which suppresses postprandial hyperglycaemia.
**Effect of trans-Resveratrol Treatment on Human Trabecular Meshwork Cell Viability**

N. Razali\(^a\), R. Agarwal\(^b,c\), P. Agarwal\(^a\), G.A. Froemming\(^a,b\), N.M. Ismail\(^a\)

\(^a\)Institute of Medical Molecular Biotechnology (IMMB), \(^b\)Faculty of Medicine, Universiti Teknologi MARA (UiTM), Sungai Buloh Campus, Selangor, \(^c\)Faculty of Medicine, Brain and Neuroscience Communities of Research, Universiti Teknologi MARA (UiTM), Shah Alam, Selangor.

**Objective:** Intraocular pressure (IOP) elevation is recognized as one of the main risk factors for primary open angle glaucoma (POAG). The accumulation of extracellular matrix (ECM) at the trabecular meshwork (TM) and Schlemm’s canal (SC) has been shown to be associated with ocular hypertension. We have earlier demonstrated that topical treatment with trans-resveratrol can reduce IOP in animals with oculohypertension and this oculohypotensive effect was found to be significant as early as half hour post treatment, peaking at 1.5 hours (H). In order to study the mechanisms of IOP lowering effect of trans-resveratrol, use of human trabecular meshwork cell (HTMC) seems most appropriate. Hence, in this study, we evaluated the in-vitro effects of trans-resveratrol treatment on primary HTMC viability.

**Materials and Methods:** HTMC from passage four were cultured with serum free media containing 25 μM trans-resveratrol or 0.1% DMSO for 0.5, 1, 1.5, 2H corresponding to in-vivo findings. Cells were then harvested and stained with trypan blue to differentiate between live and dead cells. The trypan-cell mixtures were loaded and counted using Countess Automated Cell Counter.

**Results:** There was no significant difference in the cell count and viability between trans-resveratrol and DMSO-treated groups with viability of the former ranging from 87 to 95 percent and the latter ranging from 89 to 93% at all time points.

**Conclusion:** Treatment of HTMC with trans-resveratrol for a period of 2 hours does not produce any cytotoxic effects.

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**Antioxidative Effects of Germinated Brown Rice-Derived Extracts on H\(_2\)O\(_2\)-Induced Oxidative Stress in HepG2 Cells**

N.D. Md Zamri\(^a\), M.U. Imam\(^a\), S.A. Abd Ghafar\(^b\), M. Ismail\(^a\)

\(^a\)Laboratory of Molecular Biomedicine, Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia, \(^b\)Faculty of Dentistry, Islamic Science University of Malaysia, Level 15, Tower B, Persiaran MPJ, Jalan Pandan Utama, 55100 Pandan Indah, Kuala Lumpur, Malaysia, \(^c\)Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

**Objective:** We extracted the bioactive compounds from germinated brown rice (GBR) (oryzanol, phenolics, gamma aminobutyric acid [GABA] and acylated steryl glycoside [ASG], determined their roles in improvement of antioxidant status by GBR and what transcriptional mechanisms they regulate, using HepG2 cells.

**Materials and Methods:** To test the hypothesis, HepG2 cells pre-treated with GBR extracts, were incubated with hydrogen peroxide (H\(_2\)O\(_2\)) and their hydroxyl radical (OH\(^-\)) scavenging capacities and thiobarbituric acid-reactive substances (TBARS) generation were evaluated.

**Results:** GBR-extracts increased OH\(^-\) scavenging activities in both cell-free medium and treatment culture media. The levels of TBARS in the culture medium after treatment were also reduced by all the extracts. H\(_2\)O\(_2\)-produced transcriptional changes in p53, JNK, p38 MAPK, AKT, BAX, and CDK4, GBR-extracts showed upregulation of BAX and p53 that suggested an inclination for apoptosis although upregulation of antioxidant genes, AKT, JNK, and p38 MAPK suggested that GBR-extracts preferred survival of the HepG2 cells.

**Conclusions:** GBR bioactive extracts decrease oxidative stress through enhancement in antioxidant capacity, partly mediated through transcriptional regulation of antioxidant and pro-survival genes.

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**The Lipid Lowering Effect of the Aqueous Root Extract of Morinda lucida in Albino Rats Fed on a High Cholesterol Diet**

O.J. Owolabi\(^a\), S.O. Innih\(^b\), U. Igbinadolor\(^c\)

\(^a\)Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Nigeria, \(^b\)Department of Anatomy, College of Basic Medical Sciences, University of Benin, Nigeria

**Objective:** Morinda lucida (Benth), Rubiaceae has been reported in folk medicine to be useful for the treatment of diabetes mellitus (DM) and hyperlipidemia. Previous studies have identified the hypoglycemic effect of this herb, but data on its lipid lowering effect is lacking. Considering the high incidence of hyperlipidemia with DM, this study was carried out to evaluate the effect of Morinda lucida on hyperlipidemia.
Material and Methods: The extracted Morinda lucinda roots through cold maceration by using distilled water was administered to the rats (n = 5 per group) on a high cholesterol diet for 14 days (40 mg/ rat/day). Five groups of rats were orally given the extract (100, 200 and 400 mg/kg), atorvastatin (5 mg/kg), and distilled water (2 ml/kg) for 14 days, whereas a positive control group received cholesterol and distilled water and a negative control group received only distilled water. After the 14th day, the rats were sacrificed via a cardiac puncture and blood samples were withdrawn via the abdominal aorta. Then, the separated plasma from the rat’s whole blood was tested for HDL, LDL, triglycerides and total cholesterol.

Results: Subacute treatment with the Morinda lucinda extract at 100, 200 and 400 mg/kg doses significantly reduced the levels of LDL (p < 0.0001 at all doses), whereas it increased the HDL levels (p < 0.0001 for all doses) in the hyperlipidemic rats as compared with positive control. Similarly, administered extract at 100, 200 and 400 mg/kg doses significantly reduced the total cholesterol (p < 0.0001 at all doses) and triglycerides (p = 0.001, p = 0.0005, and p < 0.0001, respectively) in comparison with the positive control. The effect of extract on total cholesterol and LDL were most prominent and it is as effective as atorvastatin.

Conclusion: Our results suggest that the aqueous root extract of Morinda lucinda as a useful remedy lowers the lipid levels in DM patients with hyperlipidemia.

P081
Investigation of Malaysian Phyllanthus niruri Anti-Angiogenesis Effect
R. Al Zarzour, M. Asmawi, M. Ahmad, G. Chatar Singh, A. Shah Abdul Majid, M. Asif, M. Yam

Objective: Angiogenesis, formation of new blood vessels from pre-existing ones, is a dynamic and complicated multistep process that plays an essential role in the pathogenesis of many major ailments, such as cancer and arthritis. Generally, compounds with anti-angiogenic properties are believed to be able to suppress tumor growth. The present study aimed to investigate the anti-angiogenic activity of Phyllanthus niruri L. (Euphorbiaceae), a well-known traditional herb in Malaysia, which possesses considerable and unique pharmacological activities – a fact which enticed many researchers to focus on it over the years.

Results: In this study, following a ring assay, a 50% methanolic extract of Phyllanthus niruri caused the highest observed rate of inhibition of the growth of blood vessels (IC50 = 14.42 μg/ml). The mechanism of action of the extract was investigated further using other tests.

Conclusion: Overall, the 50% methanolic extract of Phyllanthus niruri managed to significantly (P < 0.05) inhibit the formation of endothelial cells in vitro, cell migration, and colony formation, as compared with the untreated control. Results from this study may serve to confirm the anticancer potential of Malaysia-native Phyllanthus niruri.

P082
Effect of White Rice Consumption on Insulin Resistance in Rats Offspring
M.U. Imam, M. Ismail, D.J. Ooi, N. Sarega, A. Ishaka

Objective: White rice (WR) is a major staple food for people in Asia and it has been linked with the development of type 2 diabetes. Interestingly, the incidence and prevalence of the disease in developing countries are projected to increase. Direct links have not been made, but excessive consumption of polished rice may be a factor in these rising diabetes incidents.

Materials and Methods: WR and brown rice (BR) were fed to non-pregnant female rats for 8 weeks before mating them with age-matched male rats fed on normal pellet. Feeding of respective rice diets continued throughout pregnancy. At 4 weeks post-delivery, mothers were sacrificed and the offsprings maintained on normal pellet until 8 weeks post-delivery when they were sacrificed. Weekly weight measurements starting from 4 weeks post-delivery and oral glucose tolerance testing at 8 weeks were performed. Plasma and tissue samples (liver, muscle and adipose) were taken at sacrifice to measure markers of insulin sensitivity in both mothers and offsprings.

Results: WR groups (mothers and offsprings) showed signs of increased insulin resistance (hyperleptinemia, hypoadiponectinemia, worsened glycemic response, high homeostatic measurement assessment-insulin resistance and plasma retinol binding protein 4, and lower levels of plasma adiponectin) in comparison with BR groups.

Conclusion: The results demonstrate for the first time that in addition to the risk of insulin resistance in rats due to consumption of WR, in utero exposure of their offsprings to WR but not BR increases risk of insulin resistance in the offsprings after birth. This phenomenon may be underlying the growing diabetes incidents. Epigenetic factors may be responsible for the differences observed, and further studies are needed to evaluate the translational trajectory of these findings.

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**P083**

**Induction of Apoptosis by Hexane Extract of Curcuma zedoaria on Metastatic Ovarian Cancer Cells via DNA Fragmentation and Cell Cycle Arrest**

S.L. Khaing, S.Z. Omar, C.Y. Loo, M.A. Mohd

*Department of Obstetrics & Gynaecology, 2Department of Pharmacology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia*

**Objective:** Ovarian cancer is the deadliest gynaecological cancer worldwide. Our previous work proved that hexane extract of Curcuma zedoaria (CZ) inhibits growth of SKOV3 (metastatic ovarian cancer cells) and HUVEC cells (Human Umbilical Vein Endothelial cells). AOPI assay and Annexin-V staining assay showed that this extract induced apoptosis in SKOV3 after 48 hr and 72 hr treatment. In this study, we aimed to determine the anti-cancer mechanism of CZ hexane extract in human ovarian cancer-cell-line.

**Material and Methods:** SKOV3 cells were grown in RPMI media. Cells were seeded and incubated for 24 hours. Then, cells were treated with IC50 concentration of hexane extract of CZ. DNA from treated and control cells were extracted and electrophorized on 1.5% agarose gel. DNA fragments were analysed with UV-illuminated camera after 48 hr and 72 hr of treatment. SKOV3 cells were again treated with IC50 concentration of hexane extract of CZ, and samples were tested with flow cytometry for cell cycle analysis at 24 hours and 48 hours of exposure.

**Results:** SKOV3 cells treated with hexane extract of CZ showed characteristic DNA laddering with IC50 dose. The arrest of SKOV3 cells at G1 phase of cell cycle were observed consistently at 24 hr and 48 hr of incubation with hexane extract of CZ.

**Conclusion:** Our study revealed that hexane extract of CZ induces apoptosis of metastatic ovarian cancer cells (SKOV3) by DNA fragmentation and also cell cycle arrest at G1 phase. This apoptotic activity of CZ may be useful for new drug discovery in the treatment of metastatic ovarian cancer.

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**P084**

**Anti-Cancer Activities of a Synthetic Organotin Compound on MCF-7 Cells**

S. Fani, K.M. Chow, K.M. Lo, B. Kamalideghan

*Department of Pharmacy, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia, 2Department of Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia*

**Objective:** Breast cancer patients suffer from side effects of chemotherapy drugs; therefore investigation for discovery of new anti-cancer agents is focused by different research groups. Organotin derivatives are one of the many non-platinum metal-based antitumor agents that appear to be very promising as potential drug candidates.

**Material and Methods:** Organotin derivatives are made by the reactions of the Schiff base ligand, (E)-4-chloro-N’-(2-hydroxy-3,5-dichlorobenzylidene) benzohydrazide (L) with substituted dibenzyltin dichloride.

**Results:** The selective anticancer characteristic of Organotin compound 1 was established with IC50 of 2.5 and >30 μg/ml for MCF7 and normal hepatic cells, WRL-68, cell lines respectively. Morphological changes of necrosed, early and late apoptosis stages were observed in treated cells after staining with AOPI. A significant increase was observed in early apoptotic population after annexin v staining. Treatment of MCF7 cells with Organotin complex encouraged apoptosis with cell death-transducing signals, through MMP regulation and subsequent up-regulation of Bax and down-regulation of Bcl2. Cytochrome c release from mitochondria to cytosol boosted significantly at 20 μg/ml concentration. In addition, treated cells showed significant increase of ROS and LDH release at IC50 concentration. The DNA fragmentation results clearly show that these complexes can induce apoptosis in MCF7, a breast cancer cell line.

**Conclusion:** The results demonstrated that Organotin derivatives suppressed the proliferation of MCF-7 breast cancer cells therefore they could be promising agents for the treatment of breast cancer.

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**P085**

**Incidence, Severity Patterns and Predictability of Adverse Drug Reactions Associated with Highly Active Antiretroviral Therapy Regimen**

S. Vijayakumar, S. Anreer, C.H. Uma, G. Ashwin

*aDepartment of Pharmaceutical Sciences, Department of Pharmacy Practice, Vels University, Chennai, Tamil Nadu, India, 2Department of Pharmacy Practice, VCOP, MGM Hospital, Warangal, India, 3ART Centre, MGM Hospital, Warangal, India*

**Objective:** The aims of the present study was to assess the incidence, severity patterns, and predictability of adverse drug reactions (ADRs) to highly active antiretroviral therapy (HAART), as well as to examine the antiretroviral potency and tolerability of HAART regimen as initiation therapy for HIV.

**Material and Methods:** The study was carried out in HIV referral clinic of antiretroviral therapy (ART) centre, Mahatma Gandhi Memorial Hospital, Warangal, India from November 2012 to August 2013. ART centre serves a large population of Warangal district along with bonded districts Karimnagar, Khammam, Medak, Adilabad and Narsampet. All HIV-positive cases who were already on ART and who were newly started on ART were followed prospectively for the development of any ADRs. A detailed history of every patient was taken including past history of ART. The information obtained was tabulated and all data analysis was performed by using Graph pad prison version.5.

**Results:** Our results showed that 353 patients enrolled in the study, 227 (64.3%) were reported with ADRs to HAART regimen. The most common ADRs were experienced by 227 patients were, cutaneous reactions 102 (45%), gastrointestinal 45 (19.8%), hematologic 44 (19.4%), neurologic 32 (14%) & metabolic disorders 04 (1.7%).
Conclusion: To reflect the finding, the health authority at the centre should establish a program with effective follow up to monitor the ADRs among the HIV patients using HAART regimen and create awareness program in the society to decrease the prevalence rate of diseases.

P086
Antidopaminergic Effect of Scopoletin in Mice: An in vivo Study

V. Pandy, K. Vijeepallam, M. Narasingam, Z. Mohamed
Department of Pharmacology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

Objective: This study was undertaken to assess the potential effect of scopoletin on apomorphine-induced climbing behaviour and methamphetamine-induced stereotypy (licking, biting, gnawing and sniffing) in swiss albino mice.

Material and Methods: Experiments were performed using swiss albino male mice (bodyweight 25–30 g). In acute study, scopoletin at different doses 0.05, 0.1, 0.5, 1.0, 30 mg/kg, were administered orally one hour prior to apomorphine (5 mg/kg, i.p) and methamphetamine (5 mg/kg, i.p) injections respectively. Immediately after injection of apomorphine/methamphetamine, the mice were placed into cylindrical individual cages (12 cm in diameter and 17 cm in height) with the floor and wall consisting of vertical metal bars and recorded for climbing time and climbing behaviour/stereotypy.

Results: Acute oral treatment of scopoletin at 0.05, 0.1, 0.5, 1.0, 30, exhibited inverted bell-shaped dose-response relationship in cage climbing behaviour. Scopoletin at 0.1 mg/kg significantly decreased the apomorphine-induced cage climbing behaviour and climbing time and also significantly inhibited methamphetamine-induced stereotypy behaviour in mice.

Conclusion: Overall, the present study revealed the antidopaminergic activity of scopoletin; thereby scopoletin exhibited the antipsychotic-like activity in mice. However, further neurochemical studies are warranted to explore the actual mechanism of action of scopoletin as a promising novel antipsychotic agent.

P087
Quality of Prescribing: Is Prescribing in Patients who are on Oral Anticoagulant Evidence-Based?

S.H. Aziz, S. Alwi, K.S. Law, W.A.W. Ahmad

Objective: Evidence has showed that quality of oral anticoagulant therapy influences the risk of patients having undesirable outcomes. Thus, guidelines on the use of warfarin have been established to improve quality of prescribing by reducing variations in practice and outcomes. This study aimed to assess the quality of warfarin prescribing using a validated assessment tool based on guideline criteria.

Material and Methods: It was a retrospective survey of cases notes of patients who received warfarin treatment for at least two years and have a complete two years INR record in UMMC. Once patients were recruited, data collection forms were filled and subsequently, a 13-criteria assessment tool was applied to the data collected. Findings were reported as percentage level of adherence to specific criteria.

Results: Three hundred and two patients’ notes were included in the survey. The overall level of adherence was 54.1%. Criteria with high level of adherence include recognised indication (98.8%), appropriate duration of therapy (100%) and no contraindication (96.7 %) whilst criteria such as no intervals between INR measurements are more than 12 weeks, INR measured within one week after discharged and INR measured within 0.75 INR units of target range were measured at 19.5%, 30.4% and 44.7% respectively. The long interval between INR monitoring could be due to increasing patients’ number at the clinic and consequently, it will take longer to bring an out-of-range INR into range.

Conclusion: A few areas mainly patients’ INR monitoring services need to be improved in order to prevent undesirable outcome.

P088
Rice Bran (Oryza sativa) Extract Inhibits Platelet Aggregation, Adhesion, and Protein Secretion

W.T. Wong, M. Ismail

Objective: In pace with the tremendous growing of cardiovascular disease, one of the top leading causes of fatality worldwide, finding of natural substitutes for anti-thrombotic drugs is greatly important.
The platelets play a central role in the pathogenesis of atherosclerosis and anti-platelet therapy is effective in the secondary prevention of cardiovascular events. Brown rice was long believed to have health benefit in cardiovascular diseases. However, there were no research studies on the effect of rice bran extracts on platelet functions. In this study, rice bran extract was tested in vitro for its effect on platelet functions including aggregation, adhesion, and protein secretion.

**Material and Methods:** Crude rice bran extract was used due to high level of bio-actives. Platelet aggregation was studied using microplate reader. Rice bran extract was shown to inhibit platelet aggregation induced by different agonists in a dose dependent manner. Rice bran extract was shown to inhibit platelet adhesion to major components of basal lamina in the presence of different agonists respectively by using acid phosphatase assay. Granular secretion of protein was studied using modified Lowry method.

**Results:** Rice bran extract was shown to inhibit protein secretion from activated platelets. These results clearly established a correlation between platelet aggregation, adhesion to adhesive proteins and protein secretion from platelet granules. Low protein secretion is associated with lower adhesion and platelet aggregates formation.

**Conclusion:** The study support the hypothesis that dietary intake of brown rice may be beneficial in the nutritional prevention of vascular diseases and are potentially contributing in the development of new prevention alternatives.

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**P090**

**Mitochondrial Oxidative Stress in Coronary Collateral Growth**

Y.F. Pung, W.M. Chilian

School of Biomedical Science, University of Nottingham (Malaysia Campus), Selangor 43500, Malaysia, Integrative Medical Sciences, Northeast Ohio Medical University, Rootstown, OH 44272, USA

**Objective:** Studies have shown that patients with metabolic syndrome fail to develop coronary collaterals, leading to poor clinical outcome during cardiovascular events. We thus proposed to understand the role(s) of mitochondrial oxidative stress and bioenergetics on impaired growth of coronary collaterals using rat model that mimic human metabolic syndrome.

**Material and Methods:** Accordingly, Zucker obese fatty rat model was subjected to repetitive ischemia (RI) protocol for 10 days. Lean rats were infused with mitochondrial complex I inhibitor, rotenone (rot), through Alzet pumps at 1.5 mg/kg subcutaneously to induce mitochondrial oxidative stress. Obese rats, on the other hand, were given mitochondrial targeted antioxidant, MitoQ, at 50 μM in drinking water to scavenge mitochondrial oxidative stress for the entire experiment lasted for ~12 to 14 days. Aortae were collected from RI, RI+Rot and RI+Rot+MitoQ groups for functional and molecular analyses.

**Results:** Firstly, our findings showed that amelioration of mitochondrial oxidative burden using MitoQ partially restored the defect, indicating that mitochondrial oxidative stress was one of the major culprits leading to the aberrant growth of coronary collateral in the obese rats. In the subsequent study, the underlying mechanism by which mitochondrial dysfunction produced this impairment was investigated. Our findings revealed that activation of AMPK inhibited tube formation and silencing of AMPK promoted tube formation. Thus we believed AMPK linked mitochondrial dysfunction to impaired coronary collateral growth.

**Conclusion:** Findings from these two studies lay the groundwork to target AMPK as potential therapeutics to enhance the coronary collateral growth in patients with metabolic syndrome.
**P091**

Pharmacovigilance Study: Awareness among Medical Students of a New Medical School of Malaysia

A.R. Abubakar1, N.B. Simbak2, M. Haque3

1Unit of Pharmacology, Faculty of Medicine and Health Sciences (FPSK), Universiti Sultan Zainal Abidin (UniSZA), 20400 Kuala Terengganu, Terengganu, Malaysia, 2Dean of Faculty of Medicine and Health Sciences, Universiti Sultan Zainal Abidin (UniSZA), 20400 Kuala Terengganu, Malaysia

**Objectives:** Patients take medicine for the treatment of their illness. Drug is a double edge sword thus causes harm beside their therapeutic benefit. Pharmacovigilance program was designed to monitor and report the harmful effects of drugs after they are marketed for use by the larger society. This research was aimed at assessing the medical students’ knowledge, attitude and practice with respect to adverse drug reaction (ADRs) and its surveillance.

**Material and Methods:** The questionnaire was adopted, modified and validated from previous studies. It comprised of 25 questions (Knowledge-10, Attitude-7 and Practice-8). It was administered Year-IV and V medical students of UniSZA. The data collected was coded and analyzed using the SPSS version 20.

**Results:** The response rate was 74%. Sixty-eight percent knows the definition of ADRs correctly; 59% of them recognized the right functions of pharmacovigilance. In contrast, only 21% know the method commonly used in adverse drug reactions reporting and only 15% are aware of any nearby pharmacovigilance center. Eighty-seven percent strongly agree that ADRs reporting is professional obligation; 98% aware of any nearby pharmacovigilance center. Eighty-seven percent commonly used in adverse drug reactions reporting and only 15% are functions of pharmacovigilance. In contrast, only 21% know the method coded and analyzed using the SPSS version 20.

**Conclusion:** Our data show that spousal donation does not associated with inferior transplant outcomes, compared to living related donation. Spousal donation appears to be a viable option in circumstances of organ shortage in Malaysia.

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**P092**

Long-Term Outcomes of Renal Transplants from Spousal, Living-Related and Living-Related Donors

R.M. Guad1, H.S. Eng2, Z. Mohamed2, B.S. Haerian3, K.P. Ng2, S.K. Lim2, W.A.H. Wan Md Adnan2

1Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia, 2Department of Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

**Objective:** Deceased donor organ shortage has made living donors major source for renal transplantation (RTx) in Malaysia. Spouses represent an important source of allograft. We carried out a retrospective study of spousal RTx vs. other LDRTx to compare long term results.

**Material and Methods:** 145 living donor renal transplantation were performed between 1996 and 2012. Transplantations were performed with grafts donated by spouses in 33 cases (Group 1), living related donors (LRD) (n = 108) (Group 2), and living unrelated donors (LUD) (n = 4) (Group 3). Due to the small sample size, no further analysis was performed for the living unrelated donors group.

**Results:** Mean recipient age (years±SD) was 38.38±13.68 for Group 1 and 33.21±12.34 for Group 2 (p = 0.04). Group 1 had a slightly lower patient survival rate at 1 year post-transplant compared to Group 2 (90.1% vs. 100%), but the difference was not significant (p = 0.12). Graft survival for 6 month, 1 year and 3 years were 100%, 90.91% and 87.88% in Group 1 versus 98.15%, 97.22% and 93.52% in Group 2 (p = 0.4, p = 0.14 & p = 0.28 respectively). Risk of acute rejection was comparable between the groups (p = 0.14).

**Conclusion:** Our data show that spousal donation does not associated with inferior transplant outcomes, compared to living related donation. Spousal donation appears to be a viable option in circumstances of organ shortage in Malaysia.

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**P093**

Identifying Key Drivers that Influence the Cost-Effectiveness of Pharmacogenetics: A Case Study of the UGT1A1 Pharmacogenetic Test

F.H. Shabaruddin1, P. Tappenden2, R.A. Elliott3, K. Payne4

1Department of Pharmacy, Faculty of Medicine, University of Malaya, Malaysia, 2School of Health and Related Research, University of Sheffield, UK, 3School of Pharmacy, University of Nottingham, UK, 4Manchester Centre for Health Economics, University of Manchester, UK

**Objective:** Pharmacogenetic testing can potentially improve patient outcomes and direct more effective use of healthcare resources. Assessments of cost-effectiveness are needed to support their adoption within clinical practice. An economic evaluation of UGT1A1 testing to inform dose selection of irinotecan chemotherapy to reduce adverse events is presented as a case study to identify key drivers influencing the cost-effectiveness of pharmacogenetics.

**Material and Methods:** The economic evaluation for UGT1A1 testing compared to standard care was informed by NHS clinical practice and populated with data relevant to the UK NHS, using the healthcare provider perspective with 2013/2014 price year.

**Results:** UGT1A1 testing resulted in lower costs, fewer adverse events and better health outcomes, with cost savings of £157,600, 44 neutropenic episodes avoided, as well as 0.64 life-years and 0.45 quality-adjusted life-years gained for every 1000 patients tested. This analysis identified several key drivers influencing the cost-effectiveness of pharmacogenetics, including variations in the unit cost of a pharmacogenetic test, practice-relevant clinical pathways applied in the evaluation and the clinical outcomes of pharmacogenetic-guided dosing. The selection of adverse event parameters to include in economic evaluations also influences the cost-effectiveness of interventions that aim to reduce adverse events. Expected value of perfect information analysis showed that the estimated
place of a pharmacogenetic test within clinical pathways and uptake of a test within routine practice were the main factors influencing the value of future research investments to reduce parameter uncertainty.

**Conclusion:** The key drivers influencing the cost-effectiveness of pharmacogenetics identified in this study warrant further assessment in future economic evaluations of pharmacogenetics.

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**P094**

**Knowledge, Attitude and Practice Towards the Influence of Pharmacogenomics in Drug Safety among Future Healthcare Professionals**

A. Yau, R. Husain, M. Haque

Faculty of Medicine and Health Sciences, Universiti Sultan Zainal Abidin, 20400 Kuala Terengganu, Terengganu, Malaysia

**Objective:** Pharmacogenomics aims at understanding the roles of genetic variations in drug safety. Rational application of genomics into the cycle of patients care would leads to safer medication through individualized medicine. Healthcare professionals are increasingly expected to integrate pharmacogenomics into practice, but this integration has been below expectation and the increasing trends of pharmacogenetics in developed countries pose challenges to presence and future healthcare professionals not only in Malaysia. Therefore, this research, aimed to assess knowledge, attitude and practice of final-year future healthcare professionals towards pharmacogenomics in order to evaluate their readiness towards these pharmacogenomic based challenges for the first time in Malaysia.

**Material and Methods:** A cross-sectional study was conducted by administering self-completed questionnaire (Cronbach Alpha 0.822) to 247 respondents.

**Results:** A final 68.4% responded. Of 169 responses, 69.8% were females and 63.3% were medical students with mean age of 22.98±SD1.03. The total mean knowledge scores in percentage was 57.57±SD20.15 with statistical significant difference between the two professions (p-value = 0.002 at α = 0.05). A positive attitude, but low level of practice ware observed with a significant differences between pharmacy and medicine students with P < 0.05 respectively. Association between knowledge and practice, attitude and practice were investigated.

**Conclusion:** Majority of Malaysian future doctors and pharmacist demonstrated good knowledge and attitude toward pharmacogenomics but with very low level of practice and researches are required with large sample and to investigate the barriers to application of pharmacogenomics in to practice.

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**P095**

**Factors Affecting Pharmacists’ Level of Confidence Towards Pharmaceutical Care and Pharmacovigilance Skills in Aden & Hadhramout, Yemen: A Cross-Sectional Study**

M. Alshakka, A. Batis, M. Alshagga

Faculty of Pharmacy, Aden University, Aden, Yemen, School of Biomedical Sciences, Faculty of Science, University of Nottingham Malaysia Campus, Semenyih, Malaysia, Department of Pharmacology, Faculty of Medicine, University Malaya

**Objective:** The main purpose of the study was to determine factors associated with pharmacists’ level of confidence toward professional skills.

**Material and Methods:** This cross-sectional study was conducted among 200 Yemeni pharmacists in Aden and Hadhramout states, Yemen. Participants were selected from the main tertiary care hospital in each state and the surrounding pharmacies to the hospital. An anonymous questionnaire composed of demographic data and sections related to the level of confidence towards: patient care, communication, managerial and administrative, and pharmacovigilance skills. Statistical analysis was done using descriptive and student t-test.

**Results:** Two hundred community (86%) and hospital (14%) pharmacists responded to the questionnaire. Mean age was 28.4±4.7 years. The majority were male (66.5%) with experience of 5 years or less (69%). Pharmacists holding bachelor or higher degree were (53.2%). Pharmacovigilance skills mean was higher among hospital pharmacist (19.0±5.2) compared to community pharmacists (16.4±5.4) (p = 0.023). Pharmacists holding bachelor degree or higher associated with higher confidence, but not significant, to pharmacovigilance skills (17.4±4.8 vs 16.1±5.9) (p = 0.09) and statistically significant higher confidence for performing patient care skills (15.3±5.6 vs 13.8±3.9) (p = 0.034), communication skills (10.8±3.0 vs 9.4±3.0) (p = 0.002) and managerial and administrative skills (18.9±4.1 vs 16.8±3.8) (p=<0.01). Female gender showed statistically significant higher confidence for performing patient care skills compared to male gender (15.9±6.1 vs 14.1±3.8) (p = 0.045).

**Conclusion:** The study concluded that proper training is needed to improve pharmacists with low qualification, particularly with their high percentage, to perform pharmaceutical skills as well as community pharmacists to improve pharmacovigilance skills.
P096
An Indian Polyvalent Antivenom Protects Against the Naja kaouthia (Monocellate cobra) Venom-Induced Neuromuscular Blockade and Reverses the Cardio-Depressant Effect in Isolated Tissue Preparations
PT. Tan\textsuperscript{a}, S.M. Sim\textsuperscript{a}, N.H. Tan\textsuperscript{b}
\textsuperscript{a}Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia, \textsuperscript{b}Department of Molecular Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

Objective: Naja kaouthia is one of two common species of cobras in Malaysia. It is a category I medically important snake. In this study, the capabilities of an Indian polyvalent antivenom (Vins Bioproduct) to reverse Naja kaouthia venom-induced cardio-depressant effect and to protect against venom-induced neuromuscular blockade were investigated.

Material and Methods: The N. kaouthia venom used was obtained from species in Malaysia and has a much higher cardiotoxin but lower neurotoxic content than that in the venom from Thai N. kaouthia.

Results: At a dose of 8 μg/ml, the venom caused a 40% reduction of contractility in the isolated spontaneously beating rat atria after 1 h incubation but did not affect the heart rate. Addition of the antivenom (2 μl/μg venom) at 10 min post-venom exposure caused a rapid and complete reversal of the venom-induced cardio-depressant effect. The neurotoxicity of the venom was examined using chick biventer cervicis nerve-muscle preparation, and the median effective concentration (EC\textsubscript{50}) was determined to be 1.33 μg/ml. In the presence of the antivenom (27 μl), the EC\textsubscript{50} was increased to 4.07 μg/ml. In a separate study, when an EC\textsubscript{50} dose of the venom was added to the organ bath, the time to produce 50% and 90% inhibition (t\textsubscript{50} and t\textsubscript{90}) were 22.7 and 57.3 min, respectively. Preincubation with the antivenom (2 μl/μg venom) appeared to delay the progress of venom-induced neuromuscular blockade by about 70–80%.

Conclusion: Our results confirmed that the amount of antivenom used could effectively reverse the venom-induced cardiotoxic and delay the neurotoxic effect of N. kaouthia venom.

P098
Immunosuppressive Effects of Mesenchymal Stem Cells (MSC) Is Accompanied through T Cell Transcriptome Regulation
S. Veelasamy\textsuperscript{a}, T.C. Kong\textsuperscript{b}, A. Azhar\textsuperscript{c}, C.S. Choy\textsuperscript{d}, A. Veerakumarasivam\textsuperscript{b, c, d}, R. Ramasamy\textsuperscript{b}
\textsuperscript{a}Department of Biomedical Science, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia, \textsuperscript{b}Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia, \textsuperscript{c}Perdana University Graduate School of Medicine, Block D Level 1, MAEPS Building, MARDI Complex, Jalan MAEPS Perdana, 43400 Serdang, Selangor Darul Ehsan, Malaysia, \textsuperscript{d}Medical Genetics Laboratory, Genetics & Regenerative Medicine Research Centre, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia

Objective: Mesenchymal stem cells (MSCs) are known as immunosuppressant for they can modulate inhibition in various in vitro and in vivo inflammatory conditions. Although several molecular studies were conducted, but the precise signaling molecular pathway that attributes to this phenomenon remains elusive. This study was aimed to further elucidate the underlying molecular pathway that governs umbilical cord derived MSC (UC-MSC) mediated immunosuppression on activated T cells via microarray global gene expression profile.

Material and Methods: The successfully generated and characterised UC-MSC were assessed for their immunomodulatory properties through stimulated thymidine cell proliferation, transwell, apoptosis and cell cycle assays. The microarray data were processed and the transcriptomic changes were further enriched via pathway enrichment analysis.

Results: UC-MSC profoundly exerted a dose-dependent inhibition on T cells proliferation intervened mainly via cell-to-cell contact than soluble factors, (p*< 0.05). This inhibition was not mediated by...
apoptosis but was significantly due to cell cycle arrest of T cells at G0/G1 phase. The expression of many genes in the activated T cells was found to be dysregulated by UC-MSCs. For example, IFNG, CXCL9, IL2, IL2RA and CCND3 were downregulated while IL11, VSIG4, GJA1, TIMP3 and BBC3 were upregulated. Using the Ingenuity Pathway Analysis, 9 canonical pathways were identified as enriched with these dysregulated genes. These pathways include T helper cell differentiation, cyclins and cell cycle regulation as well as gap/tight junction signaling.

**Conclusion:** This study helps in directing specific transcriptomic changes and pathways that might contribute to the inherent ability of UC-MSCs to suppress activated T cells.

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**P099**

**The Effect of Different Concentrations of Poloxamer 188 on the Size of PLGA Nanoparticles**

S.N. Kamarudin, M. Tripathy, I.N. Iezhitsa, G.R. Froemming, R.N. Alyautdin, N.M. Ismail

Faculty of Medicine, Universiti Teknologi MARA, Sungai Buloh Campus, Faculty of Pharmacy, Puncak Alam Campus, Selangor, Malaysia

**Objective:** Surfactant plays an important role in the nanoparticles dispersion system. It acts as surface-lowering agents, minimizes the uptake of nanoparticles by the reticuloendothelial system, able to reduce toxicity level of the particles to the cells, and enhances penetration of the nanoparticles across the blood-brain barrier (BBB). The concentration of surfactant should be optimized in order to formulate an efficient brain-targeting drug delivery system crossing the BBB. The objective of the study was to evaluate the effects of the different concentrations of poloxamer 188 on the size of PLGA nanoparticles.

**Material and Methods:** PLGA nanoparticles were prepared by water/oil/water double emulsion solvent evaporation method. After 72 hours storage in freeze dryer, the lyophilized nanoparticles were dispersed in poloxamer 188 solutions at concentrations ranging from 0.1% to 1.0% respectively. The particle size was measured by ElectroAcoustic Spectrometer Dispersion Technology Inc. (USA).

**Results:** In our study the particle size was increased from 1580 nm to 2090 nm respectively with increasing poloxamer 188 concentrations from 0.1% to 1.0% (w/v), and was significantly higher as to compare with PLGA nanoparticles (27.92 nm) dispersed in water.

**Conclusion:** Surface coating of PLGA nanoparticles with surfactants poloxamer 188 affects the particles size of nanoparticles.

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**P100**

**Knowledge and Attitude of IIUM Final Year Bachelor of Pharmacy Students Regarding Pharmacogenetic Testing**

S. Zaini, M.H. Akkawi

Pharmacy Practice Department, Kulliyah of Pharmacy, International Islamic University Malaysia (IIUM), 25200 Kuantan, Pahang, Malaysia

**Objective:** Knowledge and attitude of pharmacogenetic testing in clinical practice among undergraduate pharmacy students are vital to determine the feasibility of implementation. This cross-sectional study aims to explore IIUM final year Bachelor of Pharmacy students’ knowledge and attitude toward pharmacogenetic (PGx) testing.

**Material and Methods:** A self-reported questionnaire consisting of 21 questions in five parts was adopted, validated and distributed manually to 98 IIUM final year pharmacy students.

**Results:** The response rate was 85.7% (84), with most of the respondents aged 23 years old (85.7%), female (60.7%), Malay (96.4%) and had done community pharmacy posting (60.7%) instead of hospital pharmacy posting (39.3%) during their year three semester three of study. In terms of genetics knowledge, respondents with past elective posting in community pharmacy had higher mean scores compared to hospital pharmacy (3.92±0.77 vs 3.76±0.83). For PGx knowledge, hospital pharmacy had higher mean scores compared to community pharmacy (3.88±0.89 vs 3.75±0.96). Most of them had good understanding of genetics, while only fair understanding of PGx testing. Almost all respondents had positive attitude toward PGx testing except lower score in terms of the effect to the reduced cost of developing new drugs. Regarding the potential subjects to include this PGx testing topic in future, most of the respondents chose Pharmaceutical Biotechnology & Biopharmaceutical subject.

**Conclusion:** Academic review should consider PGx testing topic to be included in suitable subjects so that knowledge and attitude of this future pharmacist will be improved.

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**P101**

**Teaching of Pharmacogenomics in Australian Pharmacy Schools**

N. Matti, S.N. Khoo, M. Wiese, C. King, V. Suppiah

School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, South Australia, Australia

**Objectives:** This exploratory study was designed to assess the extent of pharmacogenomics (PGx) teaching in Australian Pharmacy schools via a web-based survey.

**Material and Methods:** The survey consisted of ‘check boxes’ or ‘fill in the space’ questions with additional space at the end for written comments. Ethics approval for this study was granted by the Human Research Ethics committee at the University of South Australia.

**Results:** A representative from 12 out of 17 (71%) Australian Pharmacy schools provided a valid response to the survey. 11/12 (92%) respondents had a PhD and half of them had more than 10
years of teaching experience. 50% of respondents had PGx research interests. PGx teaching was mainly delivered in the 3rd and 4th years of the degree (8/12, 66.7%). 25% of the respondents were familiar with the Clinical Pharmacogenomics Implementation Consortium (CPIC) dosing guidelines.

**Conclusion:** All respondents indicated that PGx teaching is important to future pharmacists and PGx will have an impact on future practice. However, there appears to be poor awareness of the CPIC guidelines, and not all schools teach their students about the PGx of drugs that have specific PGx testing requirements for access through the PBS. This gap in PGx teaching needs to be addressed at a national level so that future pharmacists are competent in tackling PGx issues in practice.

### P102
**Nutrigenomic Effects of Edible Bird’s Nest on Oxidative Stress and Inflammation in High Fat Diet-Fed Rats**

Z. Yida, M.U. Imam, M. Ismail, H. Zhiping

Laboratory of Molecular Biomedicine, Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia, Cardiology Department, Affiliated Hospital of Chengde Medical University, 067000 Chengde, Hebei, China

**Objective:** The aim of this study was to evaluate the anti-inflammatory and antioxidant effects of edible bird’s nest (EBN) on high fat diet (HFD)-fed rats, and possible mechanistic bases of these effects.

**Material and Methods:** HFD feeding was compared with HFD + Simvastatin or HFD + EBN (2.5% or 20%) for 12 weeks. Weekly weight measurements were recorded during the intervention, while blood and liver tissues samples were collected at the end of the intervention for serum and hepatic markers of oxidative stress (total antioxidant status and TBARS) and inflammation (interleukin 6, chemokine [C-C] motif 2, nuclear factor kappa beta 1 and tumor necrosis factor alpha), and mRNA levels of related hepatic genes (superoxide dismutase, glutathione peroxidase, interleukin 10, C-reactive protein, chemokine [C-C] motif 2, nuclear factor kappa beta 1 and tumor necrosis factor alpha).

**Results:** The results showed that HFD increased weight gain and worsened oxidative stress and inflammation partly through transcriptional regulation of the antioxidant and inflammation-related genes, while EBN attenuated the HFD-induced changes better than simvastatin. The results also suggested that other post-transcriptional changes may be involved in the effects observed for EBN.

**Conclusion:** We propose that EBN may be effective as a supplement in preventing obesity-related inflammation and oxidative stress. The overall mechanisms involved however, need to be further evaluated.

### P103
**Evaluation of the Antihypertensive Effect of *Alstonia Scholaris* Extracts and Elucidation of the Mechanism of Action**

I. Bello, N. Salisu, M.Z. Asmawai, R. Mahmud

Department of Pharmacology, School of Pharmaceutical sciences, University Sains Malaysia (USM), 11800 Pulau Pinang, Malaysia

**Objective:** *Alstonia scholaris* (AS) has been widely studied for its several pharmacological benefits and has been used in folklore medicine to lower blood pressure. The present study was carried out to verify the antihypertensive effect of *Alstonia scholaris* extracts and also to study the possible pharmacological mechanism underlying its action.

**Material and Methods:** Male spontaneously hypertensive rats (SHRs) were given Daily oral administration of the AS extract (1000 mg/kg for 2 weeks). Vasorelaxation effect of AS extract was also evaluated from rat aortic rings preparation. Aortic rings preparation were pre-incubated with various antagonists like 1H-[1,2,4] oxadia- zolo-[4,3-a]quinoloxalin-1-one (ODQ 10 μM), a sCG inhibitor, No-Nitro-L-arginine methyl ester hydrochloride (L-NAME 10 μM) a nitric oxide synthase (NOS) inhibitor, Atropine (10 μM) a cholinergic receptor blocker, indomethacin (1 μM) a cyclooxygenase inhibitor, verapamil (1 μM) a calcium channel blocker and various k+ channel blockers such as Methylene blue (MB 10 μM), Gilbenclamide (10 μM) and Tetra ethyl ammonium (TEA 10 μM) for Mechanism study.

**Results:** Daily oral administration of the methanolic AS extract (100 mg/kg for 2 weeks) exhibited a significant decrease in blood pressure (p < 0.05) in SHR rats compare to aqueous extract and control. All extracts and fractions (0.125–4 mg/ml) gives a dose-dependent vasorelaxation on endothelium-intact and denuded aortic ring pre-contracted with phenylephrine.

**Conclusion:** The antihypertensive mechanism may be associated with blockage of the voltage gated calcium channel and and, in part, involvement of the down-regulation of a1-adrenoceptors and 5-HT2A/1B receptors.

### P104
**Differential Expression of microRNA in Peripheral Blood of Bipolar Disorder I Manic Patient Under Asenapine and Risperidone Treatment**

C.H. Lim, N.Z. Zainal, S. Kanagasundram, Z. Mohamed

Pharmacogenomics Laboratory, Department of Pharmacology, PARADIGM, Department of Psychological Medicine, Faculty of Medicine, University of Malaya, Malaysia

**Objective:** Atypical anti psychotics are used in the treatment of bipolar disorder I patient in manic phrase. The molecular mechanisms underlying the action of atypical anti psychotics remains unclear. microRNA are short non-coding RNA that involves in post transla-
tional regulation of gene expression. The present study examine the changes in microRNA expression following treatment of bipolar disorder I patient under two atypical antipsychotics.

**Material and Methods:** 10 bipolar disorder I patients who are treatment naive and currently in manic phase were recruited into the study. The patients were assign into two groups, 5 vs 5 and given either Asenapine or Risperdone. The patients were follow up in 12 weeks period, and blood are taken in three time point, namely week 0, 4 and 12 to examine the expression of microRNA using Affymetrix® miRNA 4.1 Array. Significant results were validated using real time PCR method.

**Results:** We have identified microRNA that up statistically significant regulated and also microRNA that statistically significant down regulated following 12 weeks of treatment between Asenapine and Risperdal. microRNA target gene prediction and functional annotation analysis combined with pathway analysis reveal the that the microRNA were involved in regulation of several genes that associated with nervous system.

**Conclusion:** We proposed that differential expression of microRNA were associated with different stage of bipolar disorder manic phase, and also with the differential action of asenapine and risperdal.

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**P105**

**Characterisation of Genomic Promoter of Human Properdin Gene**

N. Salehena,b, C. Stoverb

aDepartment of Biomedical Sciences, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia, bDepartment of Infection, Immunity and Inflammation, Maurice Shock Medical Sciences Building, University of Leicester, University Road, Leicester LE1 9HN, UK

**Objective:** Structural analysis of complement gene structures has shown that most complement gene promoters are TATA-less promoters. Properdin, positive regulator in alternative pathway has been shown to be expressed in a variety of immune cells. The study aimed in understanding of properdin expression in term of cell lineage specificity, properdin was examined by characterisation of promoter activity of the human gene for properdin.

**Material and Methods:** By bioinformatics study, about ten probable transcription factors obtained that might be functional during the expression of properdin. Data of predicted structure is used to verify which promoter elements could be essential for properdin expression. Successful constructs from human genomic DNA prepared by PCR were inserted into luciferase reporter plasmid and the expression of properdin promoter activity was measured by dual-luciferase reporter system.

**Results:** The multiple alignments in the study did not show any highly conserved regions that were common to all species and led to finding that the properdin gene control regions are varies across the species. The analysis of properdin gene control elements managed to investigate the functional consequences of allelic variants is crucial to define the importance of CYP2D6 polymorphisms in humans. The aim of this study was to identify which promoter elements could be essential for properdin expression in patients which lead a way to increase production of the protein in combating deficiency of the properdin protein in patients.

**Conclusion:** The promoter mapping can be done upon the complete promoter characterisation will lead to determination of transcription factor of a gene that bind to promoter regulatory. In up-regulation promoter activity in patients will lead to increase production of the protein in combating deficiency of the properdin protein in patients.

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**P106**

**Functional Characterization of Cytochrome P450 2D6 (CYP2D6) Allelic Variant CYP2D6*10**

A.N. Donga, C.E. Ongb, Y. Panb, U.D. Palanisamyb, R. Ismailb

aMonash University Malaysia, Jalan Lagoon Selatan, Bandar Sunway, Selangor, Malaysia, bUniversity of Nottingham Malaysia Campus, Jalan Broga, Semenyih, Selangor, Malaysia, cUniversity of Malaya, Jalan Lembah Pantai, Kuala Lumpur, Malaysia.

**Background:** CYP2D6 is amongst the many human CYP isoforms that exhibits relatively high frequency of polymorphic expression with detrimental effect on drug response and toxicity. Despite its low hepatic content of 2-5%, it has been identified to be responsible for more than 70 different drug oxidations. Hence, functional characterisation of allelic variants is crucial to define the importance of CYP2D6 polymorphisms in humans. The aim of this study was to investigate the functional consequences of allele CYP2D6*10 on the enzyme catalytic activity.

**Material and Methods:** Side-directed mutagenesis was performed to introduce nucleotide changes for the generation of CYP2D6*10. The allele was subsequently expressed in Escherichia coli together with the CYP2D6 wild type. A validated venlafaxine O-demethylase assay was used to examine important kinetic parameters such as Michealis-Menten constant (Km), maximum velocity (Vmax), and IC50 in respect to drug inhibition.

**Results:** CYP2D6*10 showed an intrinsic clearance of only 20% of the wild type value. In the inhibition study, our results indicated that CYP2D6*10 only exhibited 0.16-fold and 0.08-fold susceptibility to quinidine and fluoxetine inhibition respectively when compared to the wild type. However, when paroxetine was used as the inhibitor probe, our results showed that this allele was 1.45-fold more susceptible compared to the wild type.

**Conclusion:** These data suggested that individuals with CYP2D6*10 allele are expected to have much lower rate of venlafaxine oxidation and lower susceptibility to quinidine and fluoxetine inhibition when compared to individuals carrying the wild type allele.
Author Index

Numbers refer to page number

Abd Ghafar, S.A. 39
Abd Rahman, A.K. 14
Abdullah, A. 21, 31
Abdullah, M.A. 23, 24
Abdulla, M.A. 20, 33
Abdul Murad, N.A. 21, 23
Abdul Razak, K.N. 38
Abubakar, A.R. 44
Abu Bakar, M.F. 23, 33
Adlin, N.Y. 37
Agarwal, P. 39
Agarwal, R. 39
Ahmad Aizat, A.A. 16
Ahmad-Annuar, A. 23
Ahmadiani, A. 32, 37
Ahmad, M. 31, 38, 40
Ahmad, W.A.W. 9, 42
Ahmed, R.H. 26, 30
Akkawi, M.H. 47
Aktaruzzaman, M. 35
Al-Hamodi, Z. 26, 30
Al Hindi, B. 38
Al-Hindi, B. 31
Al-Kabsi, A.M. 7
Alrawaiq, N. 31
Alshagga, M. 45
Alshakka, M. 45
Alshawsh, M.A. 20, 35
Alwi, S. 42
Alyautdin, R.N. 47
Al Zarzour, R. 40
Amini, E. 32, 37
Amin, Z.A. 20
Amreen, S. 41
Andee, D.Z. 16
Ankathil, R. 10, 16, 29
Apalasamy, Y.D. 19, 29
Arbaeyah Hussain, F. 20
Ashwini, G. 41
Asif, M. 40
Asmawi, M.Z. 48
Asmawi, M. 40
Asmawi, M.Z. 31, 38
Atangwho, I.J. 31
Atia, A. 31
Azhar, A. 46
Azhar, M.Y.M. 12
Azidin, R.E.R. 18
Aziz, S.H. 42
Aznal, H. 10
Azmi, N.H. 23, 24
Baig, S. 33
Baker, D. 5
Basri, H. 23
Batik, A. 45
Baum, L. 19
Bee, P.C. 28
Bello, I. 37, 48
Bernam, S.P. 21
Bhavaraju, V.M.K. 16
Bingle, C.D. 21
Bingle, L. 21
Borgdorff, V. 24
Brunham, L.R. 6, 15
Bulgiba, A. 29
Cha, E.Y. 15
Chaiyakunapruk, N. 5, 15
Chan, C.L. 7
Chang, K.M. 28
Chantratita, W. 2
Chan, W.K. 28
Chatar Singh, G. 40
Chemi, N. 18
Cherny, S.S. 19
Chew, S.S. 19
Chiewthanakul, P. 13
Chik, Z. 12, 35
Chilian, W.M. 43
Chong, H.Y. 15
Cho, S.A. 19
Choudhary, B. 36
Chow, K.M. 41
Choy, C.S. 46
Chui, P. 18
Chumworthayi, P. 5
Coller, J.K. 16
Dandara, C. 9
Darussalam, B. 12
Denning, C. 24
Diong, S.H. 18
Dong, A.N. 49
Dong, D. 10
Ebid, G.T.A. 8
Elliot, R.A. 44
Eng, H.S. 44
Esa, N.M. 35
Fani, S. 41
Finkelstein, E. 10
Foong, L.C. 33
Froemming, G.A. 39
Froemming, G.R. 47
Gan, C.S. 17
Gan, G.G. 28
Gan, S.H. 6, 10, 16
Ghim, J.L. 15
Goh, S.Y. 46
Golpich, M. 32, 37
Guad, R.M. 12, 44
Gui, H. 19
Gureeva, E. 38
Hadiza, A.A. 33
Haerian, B.S. 1, 8, 12, 19, 30, 44
Hamidi, A. 22, 24
Hanipah, N.F. 38
Haque, M. 44, 45
Hassan, N.B. 14
Hui, W.Y. 15
Husain, Z. 10
Ibrahim, M.A. 30
Ibrahim, N.M. 23
Iberis, A. 34
Iezhitsa, I.N. 47
Igbinadolor, U. 39
Iman, M.U. 23, 24, 34, 37, 39, 40, 48
Imn, S.O. 39
Inunchot, W. 5
Iqbal, S. 33
Ishak, A. 40
Ismail, M.M. 35
Ismail, I.S. 30
Ismail, M. 23, 24, 33, 34, 35, 37, 39, 40, 42, 48
Ismail, M.P. 29
Ismail, N. 23, 24, 26
Ismail, N.M. 39, 47
Ismail, P. 26
Ismail, R. 12, 30, 49
Ismail, S. 20
Jalilah Mohd Ridah, L. 20
Jamalpour, S. 26
Jamal, R. 21, 23
Jamaluddin, J.L. 9
Jarrar, Y.B. 15
Jin, S. 15
Kamalideghhan, B. 41
Kamarudin, S.N. 47
Kanagasundram, S. 21, 48
Kanjawnawart, S. 11
Kanjawnawat, S. 5
Kanterakis, A. 3
Kartsaki, E. 3
Katsila, T. 3
Kavitha, N. 36
Keya, C.A. 36
Khaing, S.L. 41
Khan, M.A. 36
Khan, Y. 43
Khoo, S.N. 47
Khor, A.H.P. 8
Kim, D.H. 15
Kim, H.J. 15
Kim, H.S. 15
Kim Wei, C. 33
Kim, W.Y. 19
King, C. 47
Kondrashov, A. 24
Kongpan, T. 5
Kong, T.C. 46
Kong, W.M. 35
Konyoung, P. 5
Koo, S.H. 18
Krasilnikova, A. 38
Kubo, M. 6
Ku, C.S. 3, 18
Kura, M.N. 35
Kutala, V.K. 14
Kwan, P. 19
Lakiotaki, K. 3
Langmia, I.M. 19
Lau, P.S. 25
Law, K.S. 42
Lee, C.S. 30
Lee, E.J. 18
Lee, M.T.M. 2
Lee, P.C. 17
Lee, S.J. 15, 19
Lee, Y.K. 17
Lee, Y.Y. 30
Leong, K.V.G. 25
Lian, L.H. 28
Lim, C.H. 48
Limenta, M. 7
Lim, K.S. 8, 19
Lim, S.K. 44
Lim, S.Y. 23
Loganadan, N.K. 10
Loke, C. 7
Lo, K.M. 41
Looi, C.Y. 33, 41
Low, L.L. 14
Maddin, N. 10
Mahadeva, S. 28
Mahasirimonkol, S. 3, 5
Mahmud, R. 38, 48
Maillet, A. 6
Malempati, A.R. 14
Manolio, T.A. 1
Matt, N. 47
Maznah, I. 33
Md Dom, Z.I. 16
Md Zamri, N.D. 39
Moghadamtousi, S.Z. 33
Mohamad Fairus, A.K. 36
Mohamad, N. 30
Mohamad Pakurul Razy, N.F. 23
Mohamed Alitheen, N.B. 24
Mohamed, E.H.M. 18
Mohamed, R. 15, 17, 27, 28
Mohamed, Z. 1, 8, 12, 13, 15, 17, 18, 19, 21, 22, 24, 25, 27, 28, 29, 30, 32, 33, 34, 35, 37, 42, 44, 48
Mohl, M.A. 41
Mohd Rana, Z.Z. 21
Mohd Yasin, M.A. 30
Mostafa-Hedeab, G. 8
Movahed, E. 32
Moy, F.M. 29
Muhamed, N.H. 22
Muniandy, S. 26, 30
Mus, N. 30
Mushirida, T. 4
Mustafa, M. 14
Mustafa, N. 9
Mustafa, S. 14
Mustapha, N.R.N. 28
Muttalif, A.R.A. 15
Nafeeza, M.I. 38
Nagooor, N.H. 11, 22
Nakkam, N. 11
Nam, N.A. 21
Narasingam, M. 34, 42
Nasir, M. 12
Naushad, S.M. 14
Ng, C.C. 8
Ng, J.B. 26
Ng, K.P. 12, 44
Norliah, M.I. 32, 37
Nozaka, Y. 30
Nurfadhilma, M. 12
Omar, F. 38
Omar, S.Z. 19, 27, 41
Omar, T.M. 36
Ong, C.E. 25, 49
Ong, T.C. 28
Ooi, D.J. 40
Othman, N. 22
Othman, S. 17
Othman, S.N. 21
Ovwolabi, O.J. 39
Pandy, V. 34, 42, 43
Pan, Y. 25, 49
Pulunisamy, U.D. 49
Parhar, I.S. 13
Patrinos, G.P. 3
Pavani, A. 14
Pawitan, Y. 18
Payne, K. 44
Phuah, N.H. 11
Pirmohamed, M. 2, 27
Poh, R. 30
Poh, R.Y.Y. 26
Poniah, P. 25
Potamias, G. 3
Prabmechechi, N. 5
Pung, Y.F. 43
Rae, N.L. 19
Rafia, M.H. 19
Rajan, P. 18
Ramachandran, V. 26
Ramasamy, R. 46
Rampal, S. 29
Rashid, R.A. 18
Raymond, A.A. 8, 19, 21, 30, 32, 37
Razack, A.H.A. 25
Razali, N. 39
Reynolds, G. 4
Reynolds, G.P. 13
Riazahlosseini, B. 17
Roffeii, S.N. 13
Rohin-Glowacki, K.J. 19
Rouhollahi, E. 33
Ryu, Y.S. 15
Sadananda, S.N. 6
Saif-Ali, R. 30
Saimun, S. 21
Saksit, N. 5
Salehnen, N. 49
Salem, S.D. 26
Salimullah, M. 36
Salsiu, N. 48
Salleh M.S. M.D. 16
Sallustio, B.C. 16
Sangviroon, A. 5
Sarega, N. 40
Sekaran, S.D. 7
Seyydan, A.A. 31
Sha’ari, H.M. 1, 19, 30
Shabaruddin, F.H. 44
Shah Abdul Majid, A. 40
Shahid, I. 33
Sham, P.C. 19
Shatrah, O. 36
Shin, H.J. 19
Shin, J.G. 3, 15, 19
Simbak, N.B. 44
Sim, M.S. 13
Sim, S.M. 46
Sirisawat, P. 13
Sivapalan, V. 15
Soga, T. 13
Somogyi, A.A. 7, 16
Stover, C. 49
Subrayan, V. 26
Sukase, C. 5
Suki, S.Z. 27
Sung, C. 7, 10
Suppiah, V. 47
Suthandiram, S. 28
Swart, M. 9
Syafruddin, S.E. 23
Talib, N.A. 20
Tan, C.H. 15
Tan, C.T. 8, 30
Tan, H.J. 8, 19, 30
Tan, H.L. 27, 28
Tan, J.A.M.A. 17, 26
Tan, K. 6
Tan-Koi, W.C. 7, 10
Tan, L.L. 7
Tan, N.H. 46
Tan, P.T. 46
Tan, S.D. 12, 14, 29, 30
Tappenden, P. 44
Tassaneeyakul, W. 5, 11, 13
Teng, G.G. 10
Teoh, S.L. 15
Then, S.M. 21
Thiruchelvam, A. 17
Tiamkao, S. 5, 11, 13
Toh, D.S.L. 7
Toh, M.T. 6
Tripathy, M. 38, 47
Uma, C.H. 41
Usman, N.S. 37
Vannaprasaht, S. 13
Vannaprasaht, S. 5
Veerakumarasivam, A. 46
Vellasamy, S. 46
Vethakkan, S.R. 9, 10
Vijaya kumar, S. 41
Vijayapandi, P. 13
Vijepallam, K. 34, 42
Wang, S.M. 38
Wan Md Adnan, W.A.H. 12, 44
Wan Yusuf, W.N. 14
Westley, I.S. 16
Wichukchinda, N. 5
Widodo, R.T. 46
Widyawati, T. 38
Wiese, M. 47
Wong, S.W. 8
Wong, W.T. 42
Yam, M. 40
Yam, M.F. 31, 37, 38
Yau, A. 45
Yida, Z. 48
Yi, M. 19
Yuliwulandari, R. 4
Yusoff, N.A. 31, 38
Yusof, M.Y. 7
Yusof, N.A.N.M. 24
Yusof, R. 17
Zaharan, N.L. 12, 22, 24
Zahari, Z. 30
Zainal, N.Z. 13, 21, 48
Zaini, A.M. 37
Zaini, S. 47
Zaini, M.A. 21
Zain, S.M. 24, 25, 27, 28
Zainuddin, N. 20
Zakaria, Z.A. 31
Zaman, S.M.R.U. 36
Zhang, Y.D. 34
Zhipei, N. 48